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*Year : 2016*

## IMPACT OF SIMULTANEOUS (A)BIOTIC STRESSES ON INSECT RESPONSES IN BRASSICA NIGRA

Bonnet Christelle

Bonnet Christelle, 2016, IMPACT OF SIMULTANEOUS (A)BIOTIC STRESSES ON INSECT  
RESPONSES IN BRASSICA NIGRA

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive <http://serval.unil.ch>

Document URN : urn:nbn:ch:serval-BIB\_8A094B371B8E6

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**UNIL** | Université de Lausanne

Faculté de Biologie et de Médecine

Département de Biologie Moléculaire Végétale

# **IMPACT OF SIMULTANEOUS (A)BIOTIC STRESSES ON INSECT RESPONSES IN BRASSICA NIGRA**

**Thèse de doctorat ès sciences de la vie (PhD)**

présentée à la

Faculté de Biologie et de Médecine de  
l'Université de Lausanne

par

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Lausanne, 2016





UNIL | Université de Lausanne

Faculté de biologie  
et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie

# Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

<i>Président · e</i>	Monsieur	Prof. John Pannell
<i>Directeur · rice de thèse</i>	Monsieur	Prof. Philippe Reymond
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le Conseil de Faculté autorise l'impression de la thèse de

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intitulée

**IMPACT OF SIMULTANEOUS (A) BIOTIC STRESSES  
ON INSECT RESPONSES IN BRASSICA NIGRA**

Lausanne, le 7 juillet 2016

pour le Doyen  
de la Faculté de biologie et de médecine

Prof. John Pannell





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# Summary

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To deal with (a)biotic stresses such as herbivores, pathogens or environmental changes, plants have evolved several defense strategies. Currently, most studies are focused on plant defense responses to a single attack or infection and knowledge of the effects of multiple stresses is scarce. Plants defenses are constitutive or inducible. Constitutive defenses are composed of physical barriers such as trichomes or cuticle and of toxic secondary metabolites, such as the glucosinolates (GS). Inducible defenses are triggered specifically after infection or feeding to induce an efficient response. In response to single attacks in *Arabidopsis thaliana*, jasmonic acid (JA) and ethylene (ET) signaling pathways are triggered after necrotroph and herbivorous challenge whereas the salicylic acid (SA) pathway is triggered after biotroph invasion. To create a flexible defense response, notably in case of combined stresses, these different pathways can have a synergistic or an antagonistic effect on each other, a phenomenon named crosstalk. Moreover, plants are known to emit biogenic volatile organic compounds (BVOCs) into the atmosphere for their own protection against herbivores by attracting parasitoids.

The aim of my thesis was to characterize plant defense responses under multiple stresses. I performed whole-genome analyses of *Brassica nigra* plants challenged by *Pieris brassicae* egg extract, the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*), *Brevicoryne brassicae* aphids or ozone followed by *P. brassicae* larval feeding. Surprisingly, results indicated that the primary stress has a weak effect on transcriptional responses to a secondary stress. In addition, expression of genes involved in the biosynthesis of GS and BVOCs were not affected by any treatment.

In the second part, I focused on the effects of *P. brassicae* egg application or *Xcc* infection on performance of the specialist *P. brassicae* and the generalist *Spodoptera littoralis*. Strikingly, insects performed differently depending on the stress applied, the site of feeding and their feeding specialization. Moreover, GS, SA and JA were induced specifically at the site of herbivory or infection. Furthermore, we showed that a SA antagonistic effect on the JA pathway, and inversely, occurred depending of the pretreatment and the insect applied.

In conclusion, this work demonstrated that *B. nigra* defense response is localized to the treatment area and can differ from adjacent tissue. Importantly, the response to combined stresses is highly specific to the history of attacks.

Pour faire face aux nombreux stress (a)biotiques, tels que les herbivores, les pathogènes ou les changements environnementaux, les plantes ont développé diverses stratégies de défense. Actuellement, les recherches sont axées sur la défense des plantes contre un seul ennemi et seules quelques études ont été menées dans le cas de la présence simultanée de plusieurs ennemis. Les défenses des plantes sont constitutives ou induites. Les défenses constitutives sont composées de barrières physiques telles que les trichomes ou la cuticule et de métabolites secondaires toxiques, comme par exemple les glucosinolate (GS). Les défenses induites sont déclenchées spécifiquement après l'invasion par un pathogène ou la présence d'un herbivore pour induire une réponse efficace. Chez *Arabidopsis thaliana*, les voies de signalisation de l'acide jasmonique (JA) et de l'éthylène (ET) sont induites suite à la perception d'un pathogène necrotrophe ou d'un herbivore alors que la voie de l'acide salicylique (SA) est déclenchée après l'invasion d'un pathogène biotrophe. Pour créer une réponse flexible, notamment en cas d'attaques multiples, ces différentes voies peuvent avoir un effet synergique ou un effet antagoniste l'une sur l'autre, un phénomène appelé « crosstalk ». De plus, les plantes sont connues pour émettre des composés organiques biogéniques volatiles (BVOCs) dans l'atmosphère dans le but d'attirer des parasitoïdes lors de la présence d'herbivores.

Le but de ma thèse a été de caractériser la réponse des plantes soumises à des stress multiples. Dans cette étude, j'ai effectué une analyse du génome entier de *Brassica nigra* en réponse à un extrait d'œufs de *Pieris brassicae*, infectée par la bactérie *Xanthomonas campestris* pv. *campestris* (*Xcc*), par le puceron *Brevicoryne brassicae* ou en présence d'un niveau important d'ozone, avant de subir l'attaque de larves de *P. brassicae*. Les résultats surprenants indiquent que le premier stress a un faible effet sur le second au niveau transcriptomique. De plus, l'expression des gènes impliqués dans la biosynthèse des GS et des BVOCs n'est modifiée par aucun traitement.

Dans la deuxième partie, je me suis concentrée sur les effets de la présence d'œufs de *P. brassicae* ou d'une infection par *Xcc* sur la performance du spécialiste *P. brassicae* et du généraliste *Spodoptera littoralis*. Etonnamment, ces insectes ont une performance qui diffère en fonction du stress appliqué, de la localisation de l'insecte sur la feuille et de leur spécialisation. En outre, les GS, le SA et le JA sont induits spécifiquement sur le lieu infecté ou dévoré par les larves. De plus, nous avons montré qu'un effet antagoniste de la voie de signalisation du SA sur la voie du JA, et inversement, a lieu en fonction du prétraitement et l'insecte appliqué.

En résumé, ces travaux ont démontré que la réaction de défense chez *B. nigra* est localisée dans la zone de traitement et peut différer dans les tissus adjacents. Surtout, l'historique des attaques semble être un élément important dans la réponse des plantes contre les stress multiples.

# General Introduction

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In nature, plants are attacked by a multitude of biotic aggressors including viruses, bacteria, fungi, oomycetes and herbivorous insects. During evolution, they have developed diverse strategies to specifically recognize and fend off these enemies. In this General Introduction, I describe examples of plant defenses with a specific focus on responses to herbivory. Part I deals with defenses that directly affect the attacker, Part II contains information on how plant-emitted volatiles attract predators, Part III covers plant responses to multiple (a)biotic stresses, and finally Part IV presents the plant model system and biological factors studied in the thesis.

## Part I: Direct defenses

---

### A- Constitutive defenses

Constitutive defenses are present throughout all plant life, regardless of pathogen invasion or herbivore attack. They are composed of physical barriers such as spines, trichomes or cuticle as well as secondary metabolites.

#### 1- Passive defenses: the cuticle and other physical barriers

Plant surfaces represent the first indirect line of defense against invaders and are covered by the cuticle and cuticular waxes. The cuticle is composed of cutin, a hydrophobic material containing fatty acids and fatty esters (Martin, 1984). Waxes contain long-chain aliphatics and are found at the top of the cuticle. Cuticle and waxes' thickness are species-dependent and have an impact on plant resistance against pathogen penetration or herbivores (Chassot and Métraux, 2006). Beneath the cuticle, cell walls also play an important role against pathogen invasion and especially against fungal attackers. The cell wall has to deal with two types of invaders. Necrotrophic fungi, on the one hand, destroy the integrity of cell walls by degrading enzymes and using dead cells as nutriments, while biotrophic pathogens, on the other hand, have to control degradation to keep the cell alive in order to consume plant nutriments (Walters, 2011; Bellincampi *et al.*, 2014). Another physical barriers are stomata that represent around 2 % of total leaf surface. These natural opening structures offer a potential entrance point for some pathogens like fungi or bacteria (Walters, 2011). Melotto and his coworkers found that *Arabidopsis thaliana* transiently closed their stomata after *Pseudomonas syringae* pv. *tomato*

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DC3000 inoculation (Melotto *et al.*, 2006). Whether they play a role against other aggressors is however unknown.

Finally, spines or trichomes are found on plant surfaces and play a role in plant defense, especially against herbivores, as adults or larvae may be impaled on them. In a lot of species, trichome density and insect feeding are correlated (Levis, 1973). In addition, these structures are part of the plant chemical defense. Some trichomes contain glands which exudate terpenoid, phenolic or alkaloid substances that are repellent for herbivores. Laticifers are elongated secretory cells that contain latex, a complex emulsion consisting of alkaloids and tannins. These toxins are released in large amount when herbivores come into contact with these structures or when pathogens penetrate or propagate in plant tissue (Dussourd and Hoyle, 2000; Wittstock and Gershenzon, 2002).

### 2- Chemical defenses: phytoanticipins

Chemical defenses represent an important part in plant innate immune system. Some of them are produced as part of normal plant development and are called phytoanticipins. Phytoanticipins are low weight molecules with antimicrobial activity that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents. These molecules serve as a basic defense to avoid pathogens' and herbivores' progression and their synthesis can be increased in response to various stresses (VanEtten *et al.*, 1994). Three important classes of plant secondary metabolites make up the phytoanticipin family: terpenoids, phenolic compounds and nitrogen-containing organic compounds (Walters, 2011).

- **Terpenes** are complex mixtures of defense compounds including, for example, essential oils that are essentially toxic for pathogens and herbivores (Wittstock and Gershenzon, 2002). They are synthesized by simple or multiple C<sub>5</sub> units (C = carbon) from the mevalonate (MAV) pathway in the cytosol or from the methylerythritol phosphate (MEP) pathway in plastids (Walters, 2011; Hemmerlin *et al.*, 2012).

- **Phenolic compounds** are synthesized from shikimic acid and malonic acid pathways. They are all composed of an aromatic ring with hydroxyl groups and different other constituents (Walters, 2011). Flavonoids constitute a large proportion of phenolic compounds. More than 10,000 different members make up this chemical group and their role against herbivores is well documented (Maag *et al.*, 2014). For example, *Spodoptera frugiperda* larvae are more susceptible to an artificial diet enriched with the flavonoid compound quercetin and *Pieris*

*brassicae* larval growth is impaired when feeding on kaempferol-3,7-dirhamnoside (Gallo *et al.*, 2006; Onkokesung *et al.*, 2014).

- **Nitrogen-containing organic compounds** include cyanogenic glycosides, alkaloids and glucosinolates. All are synthesized from amino acids (Walters, 2011).

Cyanogenic glycosides are present in more than 2500 species and provide an immediate chemical defense after tissue damage due to pathogens or herbivores. During tissue disruption cyanogenic glycosides come into contact with specific degrading enzymes, causing the plant to have a bitter taste and releasing toxic hydrogen cyanide that interferes with the mitochondrial respiratory pathway. Some specialized herbivores have developed strategies to detoxify these toxic compounds and thus ensure a feeding niche (Zagobelny *et al.*, 2008).

Alkaloids constitute a vast class of phytochemicals important for plant defense that also represent a toxicological, pharmacological or cosmetic interest. Known compounds are found in this class, like caffeine, nicotine, cocaine, epinephrine, and morphin (Iriti *et al.*, 2010). Some of these compounds are ubiquitous, whereas others are restricted to a certain genus. For example, nicotine is specific to the genus *Solanaceae* or cocaine to the genus *Erythroxylum*. Interestingly, they do not have a primary function in plants, but they are toxic to animals, in vertebrates and in arthropods alike. Alkaloids are known to interact with physiological processes important for feeder fitness (Mithöfer *et al.*, 2012).

One of the most studied nitrogen-containing organic compounds are glucosinolates found in Brassicaceae plants. Glucosinolates not only give a distinctive flavor and aroma to cruciferous vegetables, but research has identified biological activities that help in human cancer prevention (Grubb and Abel, 2006).

### 3- Glucosinolate-Myrosinase complex:

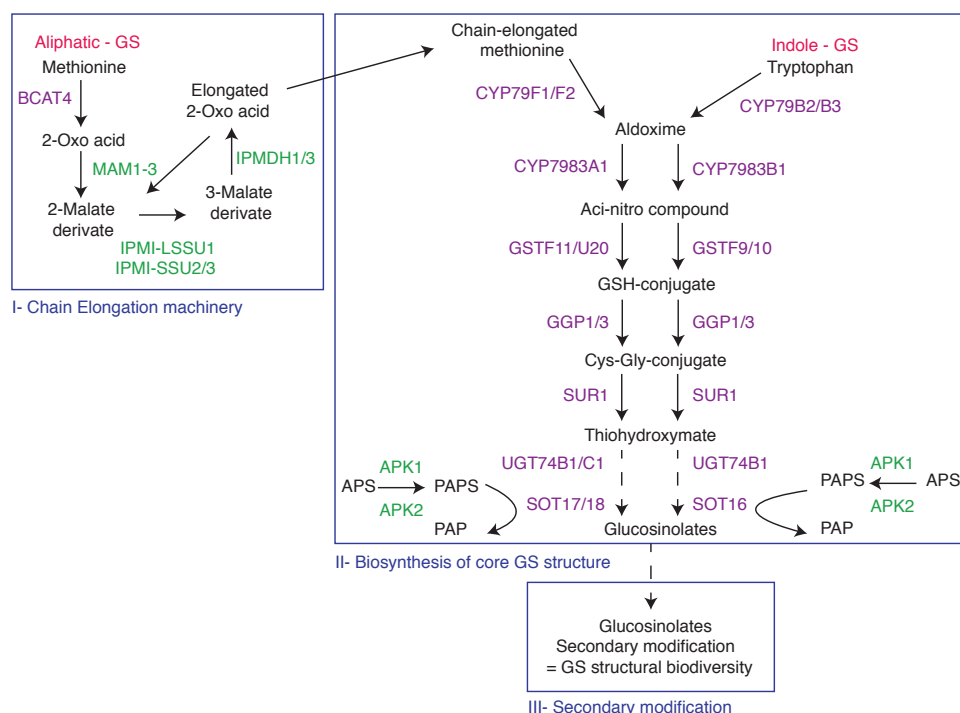
Glucosinolates (GS) are present at a basal level in Brassicaceae species and are known to be deterrents against generalist herbivores and attractants to herbivores specialized to feed on GS-containing plants. Moreover GS have a protective role against certain phytopathogens (Wittstock and Gershenzon, 2002; Walters, 2011; Buxdorf *et al.*, 2013). This secondary metabolite class is derived from nitrogen- and sulfur-containing thioglycosides. Volatile hydrolysis products released after tissue disruption have a strong taste and smell, characteristic of Brassicacea vegetables like cauliflower, Brussels sprouts, broccoli or cabbage (Redovnikovi *et al.*, 2008).

GS work in common with beta-glucosidase enzymes named myrosinases. They are stored in distinct cells to avoid biosynthesis of toxic compounds in healthy plant tissue. GS are localized in vacuoles of sulfur-rich cells and myrosinases in adjacent cells. During plant attack, cell



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compartments are destroyed and GS come into contact with myrosinases, which simultaneously converts GS products into a multitude of physiologically active products including thiocyanates, isothiocyanates or nitrile compounds. GS derive from amino acids and are classified as aliphatic, aromatic and indole GS, depending on the side chain structure. Aliphatic GS that represent 50 % of the known structures are made principally from methionine, alternatively from alanine, leucine, isoleucine, or valine; indolic GS represent 10 % of known GS and derive from tryptophan whereas aromatic GS represent 10 %, and come from phenylalanine or tyrosine. The other 30 % of the known structures are derived from other amino acids or their biosynthetic origin is yet unknown (Redovnikovi *et al.*, 2008; Hopkins *et al.*, 2009; Schweizer *et al.*, 2013b). The overall structure of GS is conserved and is composed of a beta-thioglucose moiety, a sulfonated oxime moiety and a variable side chain. Finally, biosynthesis of the GS is the same throughout these three groups. First, during the amino acid chain elongation, methylene groups are inserted into the side chain. Second, the amino acid fragment is converted into glucosinolate core structure with incorporation of sulfur. Finally, modifications occur in the side chain that creates structural biodiversity. These modifications include oxygenations, hydroxylations, alkenylations and benzoylations for aliphatic GS and hydroxylations and methoxylations for indolic GS (Hopkins *et al.*, 2009; Sønderby *et al.*, 2010; Schweizer *et al.*, 2013b; Figure 1).



**Figure 1: The aliphatic and indolic glucosinolate biosynthesis pathway in *Arabidopsis thaliana*.** The biosynthesis of methionine-derived aliphatic and tryptophan-derived indolic glucosinolates represented by steps. The enzymes are colored according to the cell compartment where the reaction takes place: purple represents the cytosol and green the chloroplast. Adapted from Sønderby *et al.*, 2010 and Schweizer *et al.*, 2013b.

Given the different side chain origins and secondary modifications more than 120 different GS products are found in the plant kingdom, but only a few of these are present in each plant species (Redovnikovi *et al.*, 2008). Around forty of these compounds are found in natural accessions of *Arabidopsis thaliana* and, to the contrary, only five are detected in *Brassica nigra* plants where one compound, sinigrin, represents more than 98 % of the total GS content (16.9  $\mu\text{mol} / \text{g}$  dry weight) throughout the leaf tissue (Gols *et al.*, 2008a; Müller *et al.*, 2010).

Moreover, GS products differ from one species to another, within ecotypes but also within the same individual depending on development stages, tissues, photoperiod or environmental stresses (Redovnikovi *et al.*, 2008). Development stages of *Arabidopsis* were compared for their GS concentration. The highest concentration was found in reproductive organs and young leaves. Authors have also shown that seeds have a distinctive GS composition in comparison to other plant organs like a high amount of methylthioalkyl GS, 26  $\mu\text{mol} / \text{g}$  of dry weight, which is less than 1  $\mu\text{mol} / \text{g}$  dry weight in other tissue (Brown *et al.*, 2003). This allocation difference may be the result of an adaptive trait to protect the most valuable organs for plant fitness from herbivore damage (Hopkins *et al.*, 2009).

After herbivore attack, the concentration of GS is well known to increase locally and systemically throughout all plant tissue (Brown *et al.*, 2003). This increase can appear just after feeding or until eight weeks after the first attack. Also, studies have shown that myrosinase levels can increase after herbivory (Hopkins *et al.*, 2009).

A recurrent trait of GS is its diversity, which seems to be required to limit the growth of different herbivores. In *Arabidopsis*, the generalist *Spodoptera exigua* gained more weight when feeding on a mutant form that was deficient in aliphatic and/or indole GS. For the specialists *Manduca sexta* and *Trichoplusia ni*, it was found that weight was affected only by aliphatic deficient plants (Müller *et al.*, 2010). Further, GS can be totally useless against specialist herbivores. For example, in *B. nigra*, the specialist *P. brassicae* started to feed on leaves during its first and second instar but caterpillars migrated at their late second instar to flowers and buds. Flower and bud feeders had a significant higher growth rate than leaf feeders. This phenomenon appeared to be correlated to the GS content since flowers contained 5 times more GS, like sinigrin, 4-hydroxyglucobrassicin and phenylethylglucosinolate compounds, than leaves (Smallegange *et al.*, 2007). The GS concentration provides a nutritional benefit with a higher growth rate for this specialist. Another example of herbivore adaptation to GS is in *Arabidopsis* choice tests assay with the specialists *Pieris rapae* and *P. brassicae*. Caterpillars always choose the control plant over plants deficient in aliphatic and/or indole GS. In the case of a choice between the plant deficient in aliphatic GS or indole GS, they prefer to feed on the aliphatic deficient plant. The same effect is observed in choice tests for *P. rapae* and *P. brassicae*

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oviposition. Aliphatic and indole GS work in a cumulative manner to stimulate this specialist's feeding and oviposition (Müller *et al.*, 2010; Schweizer *et al.*, 2013b). This difference between generalists and specialists of the Brassicaceae family is explained by the adaptation during evolution to the isothiocyanates released after GS hydrolyzation by myrosinases on tissue disruption. Larvae from the Pieris group have developed aptitudes to avoid toxic isothiocyanate compounds by converting breakdown products into nitriles, which are much less toxic. This conversion is due to a gut protein, named nitrile-specifier protein, which is unrelated to any functional characterized protein (Wittstock *et al.*, 2004).

Other methods were developed by insect herbivores to counteradapt to the glucosinolate-myrosinase system. The first one is to avoid tissue damage and to prevent GS hydrolysis like sucking herbivores do. The other one is to sequester intact GS from the host plants and to use them as their own weapons against other herbivore enemies. For this, the insect has to possess an active GS uptake mechanism and avoid GS breakdown. One amazing illustration of this GS diversion is presented in the genus *Athalia*. *Athalia rosae* sawfly larvae destroy plant cells but despite this, they accumulate intact GS 30 minutes after feeding in their hemolymph, in a concentration 10 to 30 times higher than that found in host plants. When predators attack these caterpillars, a droplet of hemolymph containing GS is released during the defensive phase and serves as a deterrent (Winde, 2011). Finally, we can conclude that GS also have an impact on trophic levels. In his review, Hopkins and his coworkers discussed the fact that GS can reduce the quality of the host as herbivore food or because specialists may sequester GS, as described before, for their own defense and thus reduce prey fitness (Hopkins *et al.*, 2009).

Besides their role in plant defense against herbivores, GS are also effective against phytopathogens. Buxdorf and his coworkers found a two times increase of aliphatic and indolic GS concentration after the invasion of the necrotrophic fungus, *Botrytis cinerea* B05.10 strain but not in the case of the Brassicaceae-specific fungus *Alternaria brassicicola* in comparison to an uninfected plant. They also showed that *A. brassicicola* is more sensitive to indole GS products than to aliphatic GS products (Buxdorf *et al.*, 2013).

In conclusion, all these examples demonstrate the importance of physical and chemical constitutive defenses against a wide range of attackers. However, plants have developed more sophisticated strategies for their own defense, including inducible defenses.

## B- Inducible defenses

Inducible defenses are activated in response to biotic stresses and are specific to each attacker due to the recognition of invaders by different plant receptors. Following recognition, specific phytohormone signaling pathways are involved. The three major hormone pathways are the jasmonic acid (JA) and ethylene (ET) pathways, which are activated after herbivorous and necrotrophic attacks, and the salicylic acid (SA) pathway, which is activated after biotrophic invasion (Figure 2). These different pathways can interact with each other, a phenomenon named crosstalk. Crosstalk can have an antagonistic or synergistic effect. It helps the plant to minimize energy costs and creates a flexible signaling plant defense response for each stress encountered (Pieterse *et al.*, 2009). Also, it is well documented that, during evolution, herbivores or pathogens have developed ways to manipulate signaling crosstalk to their own benefit. An example is *B. cinerea*, which elicits the SA pathway by exopolysaccharides production to inhibit the JA pathway via crosstalk and finally increases its own infection in plants (Oirdi El *et al.*, 2011).

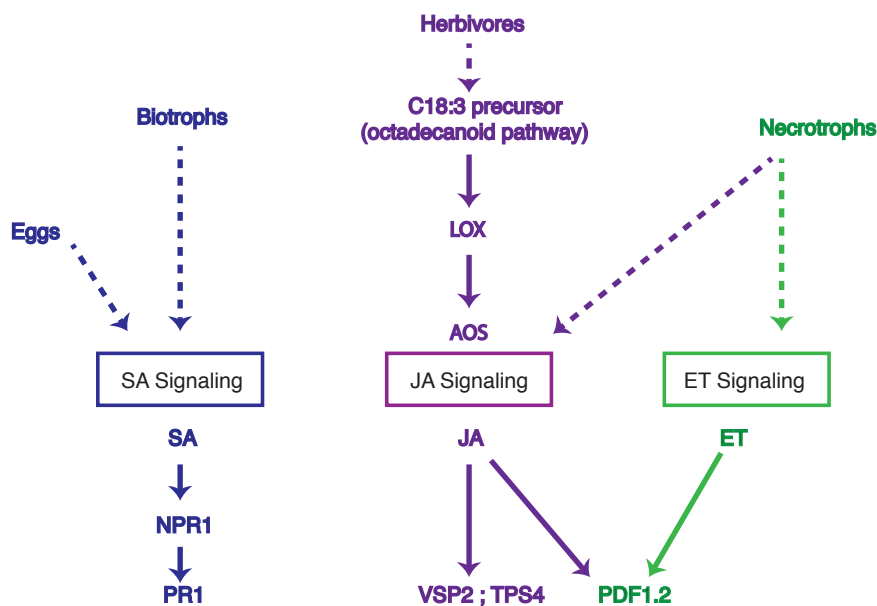


Figure 2: Plant defense signaling pathways and their typical responsive genes.

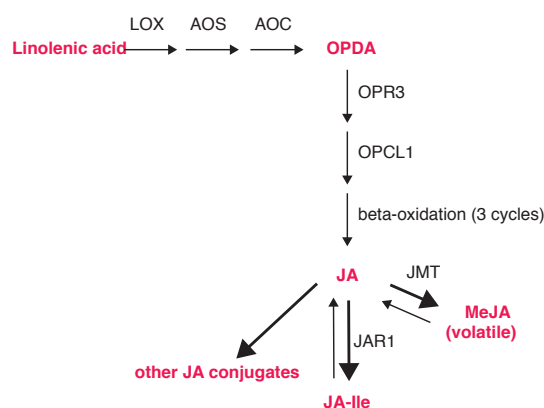
### 1- The jasmonic acid pathway: the MYC branch

JA and its bioactive conjugates are the main molecules responsible of plant defense activation after the detection of herbivore and/or damage-associated molecular patterns (HAMPs and DAMPs respectively) (Ballaré, 2011; Schweizer *et al.*, 2013a; Schweizer *et al.*, 2013b). Moreover, JA signaling is also involved in response to necrotrophic pathogen attacks and some abiotic stresses (Fernandez-Calvo *et al.*, 2011). Actually, it is well described that herbivory triggers a

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massive JA biosynthesis that is used for a large transcriptional reprogramming and expression of defense genes (Reymond *et al.*, 2000; Reymond *et al.*, 2004; Schweizer *et al.*, 2013b).

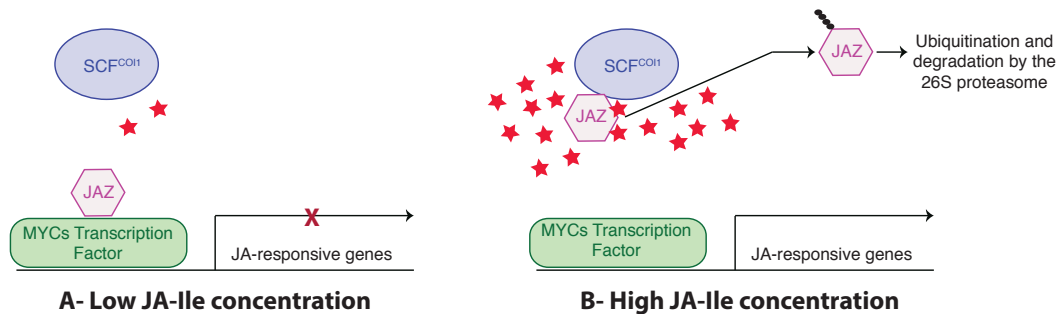
JA biosynthesis occurs through the octadecanoid pathway (Farmer and Ryan, 1992). The first step occurs on chloroplastic membranes where  $\alpha$ -linolenic acid (18:3), a fatty acid released from galactolipids (Santino *et al.*, 2013; Wasternack and Hause, 2013), is converted by the action of lipoxygenases (LOXs), allene oxide synthase (AOS), and allene oxide cyclases (AOCs) in 12-oxo-phytodienoic acid (OPDA). OPDA is then transported to the peroxisome where it is reduced in OPC-8:0 by OPDA reductase 3 (OPR3). A ligase, OPC-8:0 CoA ligase (OPCL1), converts OPC-8:0 to its CoA derivative, which is the starting point of three  $\beta$ -oxidation cycles that produce (+)-7-iso-JA (JA). JA can be metabolized to different derivatives, for example in methyl jasmonate (MeJA), a volatile compound, by the action of the JA carboxyl methyltransferase (JMT) or to jasmonate isoleucine (JA-Ile) by JASMONATE RESISTANT 1 (JAR1) (Howe and Jander, 2008, Figure 3).



**Figure 3: The octadecanoid pathway for the biosynthesis of jasmonic acid and bioactive conjugates.**

JA-Ile is the biologically active JA that promotes the interaction between the F-box protein CORONATINE INSENSITIVE 1 (COI1), a key regulator of the JA signaling pathway, and JASMONATE ZIM-domain (JAZs) transcriptional repressors. COI1 is the F-box component of a multi-protein E3-ubiquitin ligase called SCF<sup>COI1</sup>. In case of an unchallenged plant, intracellular levels of JA-Ile are low and the SCF<sup>COI1</sup> is inactive, allowing JAZs to accumulate and repress MYC2, MYC3 and MYC4, which are JA-dependent transcription factors (Howe and Jander, 2008; Fernandez-Calvo *et al.*, 2011; Figure 4 A). Upon herbivory or necrotrophic attack, the accumulation and binding of JA-Ile to SCF<sup>COI1</sup> leads to ubiquitination and degradation JAZs by the 26S proteasome. This JAZ degradation releases MYC2, MYC3 and MYC4 transcription factors

that can activate the expression of JA-responsive genes (Figure 4 B), such as *VSP2* (*VEGETATIVE STORAGE PROTEIN 2*; Santino *et al.*, 2013; Figure 2).



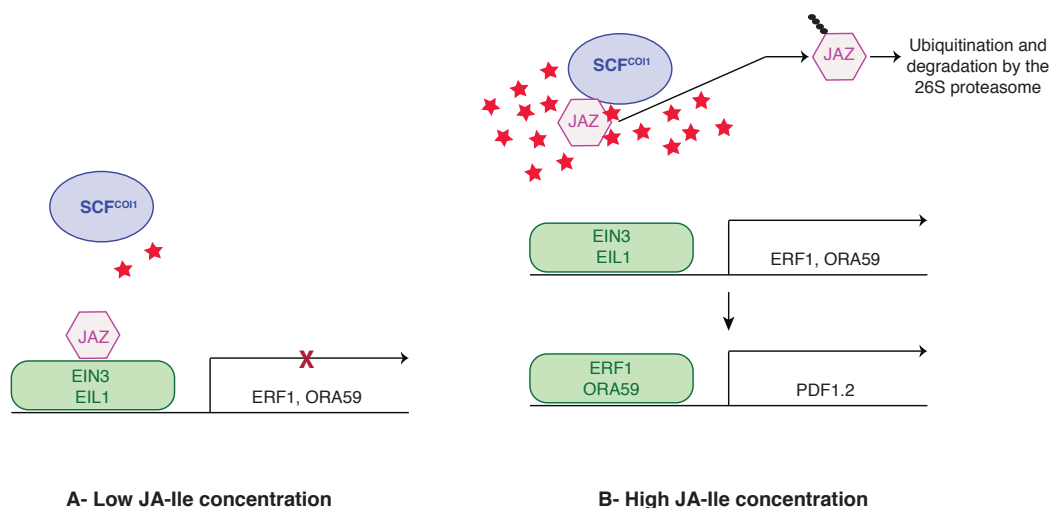
**Figure 4: The JAZ SCF<sup>CO11</sup> model of jasmonate signaling.**

- A- Low jasmonate-isoleucine (JA-Ile) concentration allows jasmonate ZIM domain (JAZ) proteins to bind MYC2, MYC3 or MYC4 transcription factors and repress JA-dependent transcription.
- B- After herbivory, plants accumulate JA-Ile, this high concentration promotes the JA-Ile binding to SCF<sup>CO11</sup> and JAZ proteins are recruited from their initial binding site to the SCF<sup>CO11</sup>-JA-Ile complex. This step permits to polyubiquitinate JAZ proteins and directed them to 26S proteasome for degradation. Finally, MYCs transcription factor are derepressed and can activate JA-responsive genes.

## 2- Jasmonate / ethylene pathways: the ERF branch

The ERF branch requires JA and ET signaling pathways and is triggered after necrotrophic attack. In this case *PDF1.2* (*PLANT DEFENSIN 1.2*) is up-regulated by the ERF branch (Pieterse *et al.*, 2009; Figure 2). Arabidopsis plants deficient in JA or ET pathway show an increased susceptibility to necrotrophic fungi *B. cinerea* or *A. brassicicola*, demonstrating the concomitant activation of JA and ET response pathways for defense against these pathogens (Pré *et al.*, 2008).

The ERF branch is regulated by the large plant-specific APETALA 2 / ETHYLENE RESPONSE FACTOR (AP2 / ERF) superfamily of transcription factors. Main actors of this branch are ETHYLENE RESPONSE FACTOR 1 (ERF1) and OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2 / ERF domain containing protein 59 (ORA59; Lorenzo *et al.*, 2003; Pré *et al.*, 2008). In non-induced plants, ET specific transcription factors, ETHYLENE INSENSITIVE 3 (EIN3) and EIN3-LIKE 1 (EIL1) interact directly with JAZ proteins and recruit a corepressor, the HISTONE DEACETYLASE 6 (HDA6), to inhibit transcriptional activity of EIN3/EIL1. Here also, like in JA signaling, JAZ proteins prevent the activation of ET signaling by inhibiting ET-dependent transcription factors (Figure 5 A).



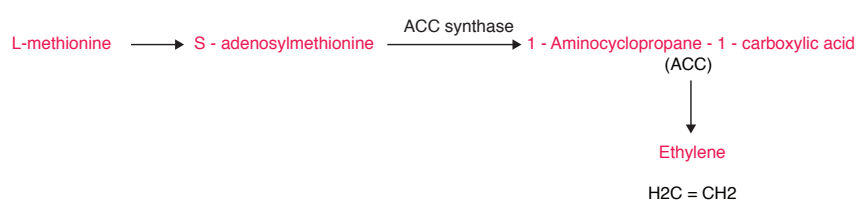
**Figure 5: The ERF branch activation.**

- A- Low jasmonate-isoleucine (JA-Ile) concentration allows jasmonate ZIM domain (JAZ) proteins to bind EIN3/EIL1 transcription factors and repress *ERF1* and *ORA59* transcriptions.
- B- After necrotroph attacks, plants accumulate JA-Ile, this high concentration promotes the JA-Ile binding to SCF<sup>COI1</sup> and JAZ proteins are recruited from their initial binding site to the SCF<sup>COI1</sup> - JA-Ile complex. This step permits to polyubiquitinate the JAZ proteins and directs them to the 26S proteasome for degradation. Then, EIN3/EIL1 transcription factor are derepressed and can activate ERF1 and ORA59 that in turn permit the transcription of *PDF1.2*.

After a necrotrophic invasion, JA / ET accumulate, allowing the degradation of JAZ proteins by COI1-dependent complex and enhancing the transcriptional activity of MYCs and EIN3/EIL1. Finally, ERF1 and ORA59 are activated by EIN3/EIL1 and result in the transcription of JA / ET dependent genes, such as *PDF1.2* (Pieterse *et al.*, 2012; Wasternack and Hause, 2013; Figure 5 B). However ET alone can also play a role in plant defense.

### 3- The ethylene pathway

The increase of the gaseous hormone ET is one of the earliest detectable events after pathogen attack and affects the expression of plant defense-response genes (Ecker and Davis, 1987). ET biosynthesis pathway is tightly regulated, with redundant regulation and post-transcriptional control points involving specific enzymes. This type of regulation suggests that ET is modulated in a dose-dependent manner and not purely by its presence or absence. Methionine is used for the first step of ET production by S-AdoMet synthase. Conversion of S-AdoMet into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) is the second step. This conversion seems to be the rate-limiting step in ET biosynthesis (Adie *et al.*, 2007; Figure 6).



**Figure 6: Ethylene biosynthesis in plants.**

The Arabidopsis genome contains twelve *ACS* genes and studies have shown that 8 *ACS* seem to be involved in ET biosynthesis. Yamagami and his coworkers found that *ACS*s are activated in unique cellular environments, thus allowing ET, by this diversity, to exert a unique effect in tissue or cell-specific manner (Yamagami *et al.*, 2003). For example, in plant defense, ET has been discovered to be important for xylem occlusions after wounding or for phytoalexin production such as isocoumarin in carrot roots. Phytoalexins are low molecular weight compounds produced *de novo* in response to pathogen attacks. Moreover, ET takes part in a complex network of interactions between different signals. Interaction with JA is described in the previous paragraph but ET can also be involved with SA and abscisic acid (ABA) pathways, in PATHOGENESIS-RELATED (PR) protein regulation. PR proteins represent a large class of defense genes expressed locally and systemically in response to biotic stresses (Adie *et al.*, 2007).

#### 4- The salicylic acid pathway

The plant hormone SA plays a major role in plant resistance against biotrophic attacks, eggs laid by butterflies or sucking herbivores (Thaler *et al.*, 2012). SA is a phenolic compound well studied for its role in local and systemic acquired resistance, but also for the accumulation of PR proteins (Pieterse *et al.*, 2009; Boatwright and Pajeroska-Mukhtar, 2013; Kumar *et al.*, 2014). SA biosynthesis is triggered after recognition of pathogen-associated molecular patterns (PAMPs), egg-associated molecular patterns (EAMPs) or effectors of pathogens (Pieterse *et al.*, 2012; Gouhier-Darimont *et al.*, 2012). After plant recognition of this type of invaders, the lipase-like proteins ENHANCED DISEASE SUSCEPTIBILITY (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4) act together to activate SA biosynthesis.

SA is synthesized in plastids from the shikimate-phenylpropanoid pathway by two distinct branches:

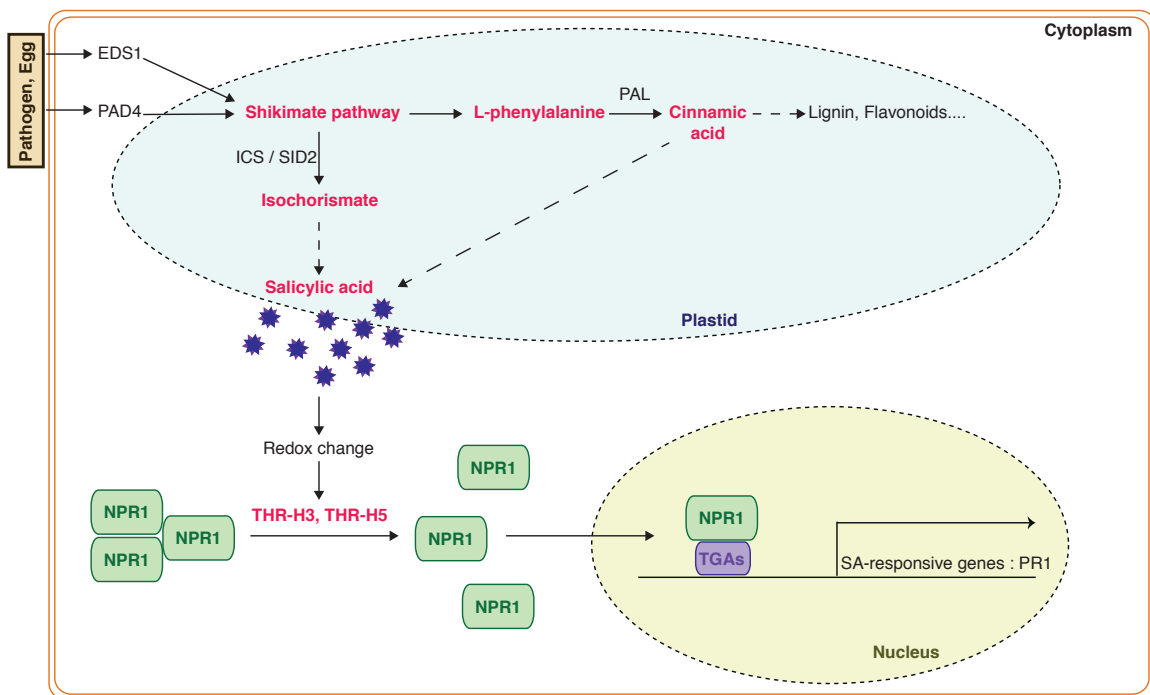
- The most important one converts chorismate to isochorismate by isochorismate synthase (ICS1 / SID2) and then isochorismate pyruvate lyase (IPL) converts isochorismate to SA.



This pathway is responsible for more than 90 % of SA production after plant pathogen attack.

- The second one is through the phenylalanine ammonia lyase (PAL) pathway. PAL converts phenylalanine into cinnamic acid and, in its turn, cinnamic acid can be hydroxylated to *ortho*-coumaric acid. Finally, oxidation of the *ortho*-coumaric side chain produces SA (Boatwright and Pajerowska-Mukhtar, 2013; Kumar *et al.*, 2014; Figure 7).

Downstream of SA biosynthesis, NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) is the major actor of SA signaling regulation. In uninfected plants, NPR1 is sequestered in the cytoplasm as an oligomer by intermolecular disulfide bonds. In SA-induced plants, the cellular redox state is changed and permits monomerization of NPR1 through the activity of thioredoxins (THR-H3 and THR-H5). Monomeric NPR1 can translocate to the nucleus via nuclear pore proteins and binds to the TGA subclass of the basic leucine zipper (bZIP) transcription factors. NPR1 acts as a transcriptional coactivator of a large set of SA-related genes, such as *PR* genes that include *PR1* (Pieterse *et al.*, 2009; Pieterse *et al.*, 2012; Figure 7).



**Figure 7: SA biosynthesis and signaling in plant defense.**

Pathogens or eggs detection elicit SA biosynthesis through EDS1 and PAD4. The shikimate pathway is involved and two branches act in SA production. The phenylalanine ammonia-lyase (PAL) pathway and the isochorismate synthase (ICS / SID2) pathway. Biosynthesis of SA compounds change the redox state of the cytoplasm that allows thioredoxins (THR-H3, THR-H5) to monomerize NPR1. Monomeric NPR1 can enter into the nucleus to coactivate SA-responsive genes like *PR1* with TGAs transcription factors. Solid lines indicate direct interaction, dashed lines represent indirect interaction.

NPR1 is well documented to be one of the modulators in the crosstalk that occurs between SA and JA / ET pathways (Spoel *et al.*, 2003).

### 5- Crosstalk between JA, ET and SA pathways

SA and JA / ET-dependent defense pathways have been shown to interact; this hormonal crosstalk allows plants to be equipped with a sophisticated defense system and to fine-tune their immune response to the encountered attacker (Reymond and Farmer, 1998). In nature, plants often deal with multiple (a)biotic aggressions and / or beneficial organisms. On the other hand, activation of plant defense responses has an energy cost. That is why signaling pathways cross-communicate in an antagonistic or synergistic manner depending on the sequence and type of stress. This strategy helps plants to respond efficiently to the invader and underplays time and energy efforts (Pieterse *et al.*, 2009; Denancé *et al.*, 2013).

- **JA-ET crosstalk.** Microarray experiments in *Arabidopsis* clearly show that JA and ET synergistically regulate many common genes (Bodenhausen and Reymond, 2007). As demonstrated before, the ERF branch is required for the expression of the defense-related gene *PDF1.2* in response to necrotrophs and is activated by JA and ET together. The main transcription factors MYC2, EIN3 / EIL1, activated by JA and ET respectively, play a significant role in this JA / ET synergistic crosstalk. Downstream of EIN3 / EIL1 other transcription factors ERF1 / ORA59 appear to have a role in the antagonistic effect of ET in JA. After necrotrophic attack, ERF1 / ORA59 are activated, leading to the expression of *PDF1.2*, thus antagonizing the MYCs activity. This phenomenon has an impact on herbivory responses. Plants with activated ERF branch seem to be more attractive than plants with activated MYCs branch (Verhage *et al.*, 2011).

- **SA-ET crosstalk.** Interaction between SA and ET exists in plant defense. In *Arabidopsis*, plants treated with *P. syringae*, a pathogen responsible for SA-induced response showed that many SA-responsive genes were significantly affected in the *ein2-1* mutant that is deficient in the ET signaling pathway (Glazebrook *et al.*, 2003).

- **SA-JA crosstalk.** In *Arabidopsis*, several studies have shown that JA-responsive genes *PDF1.2* and *VSP2*, markers of the ERF branch and MYC branch of the JA pathway respectively, are highly suppressed after induction of the SA pathway. SA application also repressed key enzyme genes encoding for the JA biosynthesis, such as LOX2, AOS, AOC2 and OPR3 (Leon-Reyes *et al.*, 2010). The same effect was observed after treatment by *P. brassicae* eggs known to induce the SA pathway. Eggs application reduced JA-induced marker genes and had a positive

impact on larval performance. In plants pretreated by *P. brassicae* eggs, *Spodoptera littoralis* larvae were larger than in untreated plants (Bruessow *et al.*, 2010). The antagonistic effect of SA on JA appears to occur through the regulatory protein NPR1. In *npr1* plants, the negative crosstalk of SA on JA does not occur. More surprisingly, the nuclear localization of NPR1 is not required for SA-mediated suppression of JA-responsive genes that appears to occur in the cytosol (Koornneef and Pieterse, 2008). In addition, other elements seem to be important for SA / JA crosstalk, for example, WRKY transcription factors, including WRKY70. WRKY70 is a transcription factor activated in SA-mediated responses defense. Overexpression of WRKY70 leads to the up-regulation of *PR* genes and a suppression of the JA-induced expression of marker genes (Pieterse *et al.*, 2012). Moreover, JA antagonistic effects on SA have been also characterized using JA-signaling mutants. In *wrky33* plants, the authors showed an increase in SA-regulated genes expression, like *PR1* and an increase in SA levels (Birkenbihl *et al.*, 2012).

Finally in Arabidopsis, synergistic events have been identified between SA and JA pathways. At low concentrations of both hormones, *PDF1.2* and *PR1* are both expressed. However, at high concentrations, the authors observed antagonistic effects, reflecting the importance of the hormone concentration in SA / JA crosstalk (Mur *et al.*, 2006). Also, the time scale appears to have a role in these interactions. When SA is applied more than 30 hours after the JA response, the antagonistic effect of SA on JA is totally absent (Koornneef *et al.*, 2008). All these examples demonstrate that kinetics of phytohormone biosynthesis, concentration and time scale alike are decisive in the final outcome of the defense response to the encountered attacker or pathogen.

Recently, studies tend to conclude that abscisic acid, gibberellin, brassinosteroids, plant hormones involved in growth and development, also interact with JA, SA and ET and the redirecting of plant defense responses (Verhage *et al.*, 2010; Denancé *et al.*, 2013).

### 6- Other secondary hormone pathways: the example of the abscisic acid pathway

Plant defense responses can be induced or interfered by abiotic stresses. For example, environmental stresses can induce ABA accumulation, which itself can trigger physiological and developmental changes that are crucial to plant adaptation. ABA is an important phytohormone for plant growth and development but, during a stress period, ABA can target guard cells, induce stomatal closure and control the expression of various transcription factors that induce stress tolerance (Fujita *et al.*, 2006). Moreover, ABA is known to interfere with plant defense. In Arabidopsis, ABA treatment increases plants' susceptibility to an avirulent strain of *P. syringae* or in tomato ABA addition increases susceptibility to *B. cinerae* and *Erwinia chrysanthemi* (Atkinson *et al.*, 2013). It is postulated that ABA could be a link between the SA and JA / ET pathways.

## 7- Manipulation of hormone pathways by pathogens or herbivores

Some pathogens and herbivores have evolved ways to manipulate plant hormone pathways and hijack plant defenses for their own benefits. For example, in *Arabidopsis*, the lepidopteran herbivores *P. brassicae* and *S. littoralis* are known to inhibit plant defense independently from JA and SA pathways by means of oral secretions (Consales *et al.*, 2012). *P. syringae* bacteria strains secrete coronatine to suppress host defense responses by activating JA signaling. Katsir and coworkers demonstrated that coronatine binds to COI1-JAZ complex to mimic JA-Ile. In this way, bacteria activate the JA pathway that in turn can inhibit the SA pathway, enabling *P. syringae* strains to be more virulent in host tissue (Katsir *et al.*, 2008).

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## Part II: Indirect Defenses

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### *How plants generate airborne information*

Indirect defenses play an invisible but major role in plant defense. Throughout all plant life, a blend of volatiles is released into the atmosphere. These phytochemical molecules are named biogenic volatile organic compounds (BVOCs). BVOCs play different roles in floral and vegetative organs. They can attract pollinators by the release of floral scent or be involved in important steps of plant defense (Pichersky and Gershenzon, 2002). For example, in the cases of wounding or herbivore attack, the BVOCs' blend attracts natural enemies of herbivores, such as parasitic wasps, which may confer protection to the plant. Parasitic wasps and predators recognize the special blends emitted by the damaged plant and come to actively feed larvae (Kessler and Baldwin, 2001). Also, BVOCs are key components in plant communication. BVOCs released after herbivore damage are known to be recognized and to activate herbivore-induced genes in neighboring healthy plants to prevent future attack (Holopainen, 2004; Ton *et al.*, 2007).

#### 1- Biosynthesis of volatiles

BVOCs are lipophilic compounds with high vapor pressure that can cross membranes freely to be released into the atmosphere, if they are not conjugated. In fact, BVOCs comprise more than 1800 molecules, but thanks to the increasing sensitivity of headspace analysis equipment, their number is growing as many new species are being investigated. BVOCs are constitutively expressed in all plant parts, in leaves, flowers and roots, and can be detected at all development stages. Furthermore, stresses may induce blend composition changes of BVOCs constitutively emitted. The BVOCs family contains many compounds but only three major metabolic pathways are involved in their biosynthesis (Loreto and Schnitzler, 2010; Venkatesan, 2015; Figure 8)

- **The terpene (= isoprenoids) pathway** is the most important for plant protection and contributes to more than half of the emitted volatiles.

The investigation of terpene biosynthesis has increased since the complete discovery of pathways responsible for the formation of the common isoprenoids C<sub>5</sub> block by the isopentenyl diphosphate (IDP) and its isomer dimethylallyl diphosphate (DMADP). IDP and DMADP are synthesized by two pathways. The mevalonate (MVA) pathway, coming from acetyl-CoA, is located in the cytosol. The MVA pathway is, normally, involved in the sesquiterpenes (C<sub>15</sub>) formation. The methyl-D-erythritol-4-phosphate (MEP) pathway, coming from pyruvate and glyceraldehyde-3-phosphate, is located in plastids. The MEP pathway generally provides

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hemiterpenes ( $C_5$ ) and monoterpenes ( $C_{10}$ ). Also, recent research appears to show that MEP can be involved in sesquiterpenes formation (Loreto and Schnitzler, 2010; Pichersky *et al.*, 2006). For example, the synthesis of abundant sesquiterpenes in trichomes involves a strong  $C_5$  supply that can be supported by MEP pathway-derived IDP (Paetzold *et al.*, 2010). As a second step, IDP and DMADP are condensed into geranyl diphosphate (GDP) and geranylgeranyl diphosphate (GGDP) in plastids or into farnesyl diphosphate (FDP) in the cytosol by synthases. Finally, a family of terpene synthases (TPS) convert DMADP, GDP, GGDP and FDP to form hemi-, mono- and sesquiterpenes. TPSs have the ability to create all these different terpene structures using a variety of reactions such as cyclization or rearrangements (Loreto and Schnitzler, 2010; Venkatesan, 2015).

In *Arabidopsis*, known as a BVOCs low-emitter plant, 40 *TPS* genes have been characterized and are tissue specific, including fourteen genes that are involved in terpene biosynthesis. This high number, even in a plant that produces a low level of BVOCs, reflects the complex mechanism of terpene production in plant metabolism (Aubourg *et al.*, 2002; Venkatesan, 2015). The *TPS* family is also well studied in response to herbivory. For example, in *Zea mays*, most of the North American varieties have lost their ability to produce (E)- $\beta$ -caryophyllene. This sesquiterpene is known to be synthesized after insect-damaged roots and to be an attractant to nematodes that infect and kill root pests. These varieties were more sensitive to root herbivory than emitter lines. Researchers have shown that (E)- $\beta$ -caryophyllene is synthesized by a terpene synthase gene, *TERPENE SYNTHASE 23* (*TPS23*). *TPS23* is fully present in the North American varieties but no transcript of this gene is present. Finally, by transformation of the North American lines with a *TPS23* gene coming from emitter lines, the (E)- $\beta$ -caryophyllene emission can be restored and maize lines recover their ability to attract nematodes (Degenhardt *et al.*, 2009). Future progress in the understanding of terpene biosynthesis could help to improve pest management in other plant species.

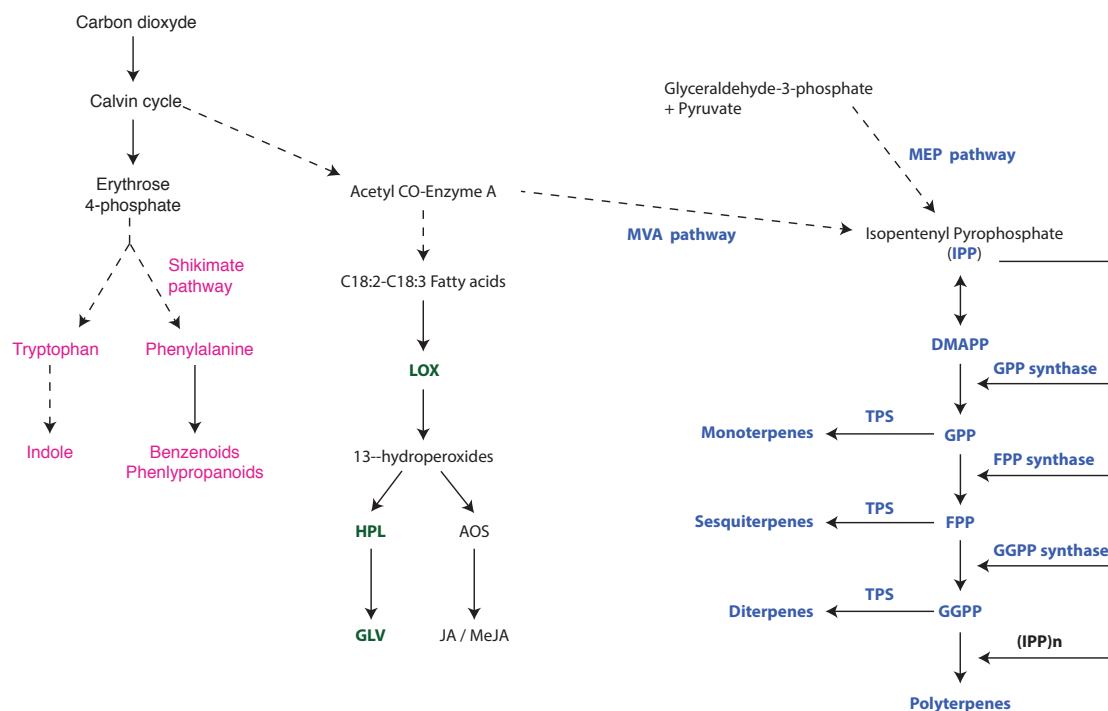
- **The oxylipin pathway** is known to produce **Green Life Volatiles** (GLVs), the characteristic odor of freshly cut grass. GLVs are synthesized locally after wounding or herbivore feeding, but can also be induced and released systemically. Also, GLVs possess antibiotic properties that inhibit invasion in damaged tissue after wounding and can be used to induce or prime defenses in all green plants. Most of GLVs are  $C_6$  aldehydes,  $C_6$  alcohols or esters, and are commonly named  $C_6$  or LOX-products (Engelberth *et al.*, 2004). GLVs are synthesized from 18:2 (linoleic acid) or 18:3 (linolenic acid) fatty acids. Lipoxygenases (LOX) catalyze the first reaction by adding  $O_2$  (dioxygen) to unsaturated fatty acids. LOX genes are the same as those that initiate the octadecanoic pathway involved in JA biosynthesis. The split between the octadecanoic pathway and GLV biosynthesis occurs after the synthesis of hydroperoxides. In the case of GLVs

synthesis, 13-(S) hydroperoxides are cleaved into C12 and C6 by 13-(S)-hydroperoxide lyase (HPL). Depending on the C18-substrate, this cleavage gives birth to n-hexanal and (Z)-3-hexanal, precursors for aldehydes, alcohols and acetylated compounds (Loreto and Schnitzler, 2010; Venkatesan, 2015). HPL is the key enzyme in GLVs biosynthesis as shown in Arabidopsis by manipulating HPL activity. Plants overexpressing *HPL* showed increased GLVs emission after *P. rapae* attacks and attract more parasitic wasps *Cotesia glomerata*. Moreover, this overexpression resulted in a greater inhibition of fungal infection. On the contrary, antisense *HPL* plants were less attractive to wasps, while more susceptible to herbivores and pathogens (Shiojiri *et al.*, 2006). In maize, (Z)-3-hexanal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate have been reported to stimulate JA biosynthesis and BVOCs emission. More interestingly, GLVs have a priming effect on plants. Plants can respond more strongly against subsequent herbivore attacks by increasing JA biosynthesis and BVOCs releasing (Engelberth *et al.*, 2004). GLVs appear thus to be important for herbivore-induced defense and priming.

- **The shikimic and benzoic acid pathways** are responsible for the formation of phenylpropanoids and benzenoids BVOCs, compounds containing an aromatic ring.

Plant benzoic acids are well characterized to be regulators in a lot of plant-environment interactions. BVOCs from those pathways are known to be synthesized directly from shikimate and phenylalanine. However, every step of these pathways has not yet been identified. Research has concluded that the combination of pathways and the variety of final modifications might facilitate precise temporal and spatial controls of active forms (Wildermuth, 2006). An early step of phenylpropanoids biosynthesis, which takes place in the plastids, converts L-phenylalanine, which comes from the shikimate pathway, into *trans*-cinnamic acid by the L-phenylalanine ammonia-lyase (PAL). Following steps are shared with lignin biosynthesis and lead to coumaric acid, ferulic acid and sinapic acid production. Hydroxylation and methylation reactions of these monomers form diverse cinnamic acid volatile derivatives (Pichersky *et al.*, 2006; Venkatesan, 2015). Methyl-salicylate (MeSA) originates from these pathways by O-methylation of SA by a methyltransferase. MeSA is a signaling molecule whose induction is frequent in response to biotic stresses. MeSA may function as an airborne signal that activates SA-responsive genes on distal tissue of attacked plants or in neighboring plants (Kumar, 2014; Shulaev *et al.*, 1997).

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**Figure 8: BVOCs biosynthesis pathways.**

BVOCs molecules and enzymes are colored according to the pathway they are involved: pink represents the shikimate and benzoic acid pathways, green the green leaf volatile biosynthesis pathway and blue the terpenes biosynthesis pathway.

Solid lines indicate direct interaction, dashed lines represent indirect interaction. LOX: lipoxygenases, HPL: 13-(S)-hydroperoxide lyase, GLV: green leaf volatiles, AOS: allene oxide synthase, JA: jasmonate, MeJA: methyl-jasmonate, MVA: mevalonate pathway, MEP: methyl-D-erythritol-4-phosphate, IPP: isopentenyl pyrophosphate, DMAPP: dimethylallyl diphosphate, GPP: geranyl diphosphate, FPP: farnesyl diphosphate, GGPP: geranylgeranyl diphosphate, TPS: terpene synthase.

## 2- Roles of BVOCs in plant-herbivore interactions

Plants are under pressure of a plethora of arthropod herbivores, from aphids to caterpillars and from beetles to spider mites. After recognition of the attacker plants activate a transcriptional program that lead to the expression of numerous defense genes (Reymond *et al.*, 2000; Thomma *et al.*, 2001; Reymond *et al.*, 2004; Verhage *et al.*, 2010), including BVOC biosynthesis genes (Van Poecke *et al.*, 2001; Broekgaarden *et al.*, 2011; Mathur *et al.*, 2013). The phytochemical response has a significant impact on plant defense mechanisms against herbivores. Herbivore-induced plant volatiles (HIPVs) are the most studied volatiles among BVOCs. HIPVs are synthesized *de novo* after an herbivore attack and include terpenoids, phenylpropanoids and GLVs, which are released into the atmosphere (Dicke and Baldwin, 2010). For example, the induction of HIPVs by lima bean plants infected by spider mites comprises several terpenoids as well as methyl salicylate, which are not emitted in undamaged plants (Dicke *et al.*, 2009). Moreover, herbivore attacks can target roots and shoots, and all plant parts have to be able to

defend efficiently. HIPVs induction in shoot has been found in all investigated plant species. Likewise, all arthropods, root or shoot attackers, induce HIPVs (Dicke *et al.*, 2009). Induction of HIPVs also occurs in the root and may attract carnivorous enemies of belowground herbivores. An example of this is previously shown in paragraph one with the sesquiterpene, (E)- $\beta$ -caryophyllene, emitted by maize root after *Diabrotica virgifera virgifera* larvae. This volatile attracts nematodes that kill the rootworm larvae (Degenhardt *et al.*, 2009). HIPVs are described as signals for natural enemies of herbivores to increase predation or parasitism rates. Using HIPVs emitted after feeding damage, parasitoids can find caterpillars and lay eggs inside larvae to kill them. Thus, HIPVs may have evolved to confer protection to the plant. A study on *Nicotiana attenuata* plants demonstrated that plants can reduce herbivory rates by 90 % using HIPVs (Kessler and Baldwin, 2001).

Elicitors present in herbivore oral secretions are recognized by plants and have been shown to induce the release of HIPVs. For example, cabbage treated with  $\beta$ -glucosidase, a molecule found in *P. brassicae* regurgitate, released the same blend of volatiles than plants challenged with feeding caterpillars. Moreover,  $\beta$ -glucosidase-treated plants were highly attractive to parasitic wasps *C. glomerata* (Mattiacci *et al.*, 1995). Another example is an elicitor isolated from *S. exigua* regurgitant, named volicitin. Maize seedlings treated with low concentrations of volicitin released more terpenoids HIPVs and were more attractive to the parasitoid *Microplitis croceipes* (Turlings *et al.*, 2000). The attraction of predators by HIPVs takes place rapidly. In cabbage leaves challenged by *P. brassicae*, HIPVs emission and *C. glomerata* attraction begin 30 to 60 minutes after the first caterpillar damage (Dicke *et al.*, 2009). In maize, the first HIPVs emitted immediately upon damage were GLVs, whereas terpenoids emission appeared to take more time, around 90 to 120 minutes, and increased in the following hours (Turlings *et al.*, 1998). In addition, HIPVs emission has been shown to attract parasitoids to at least several meters from the emitted plant (Dicke *et al.*, 2009). The impact of HIPVs on caterpillar performance has also been reported. Tomato plants, *Solanum lycopersicum*, exposed to *Spodoptera littura* specifically emitted (Z)-3-hexenyl-vicianoside. This volatile compound is used by undamaged neighboring plants to form a glycoside that acts as a defense metabolite against the cutworm. *S. littura* feeding on (Z)-3-hexenyl vicianoside-exposed plants had a lower survival rate and a lower weight than caterpillars feeding on control plants. The same effects were observed on an artificial diet containing the glycoside. Interestingly, this glycoside seems to be accumulated in various plant species after GLV exposition (Sugimoto *et al.*, 2014).

Besides, eggs laid by butterflies on the surface of a leaf are also known to induce the emission of volatiles, named oviposition-induced plant volatiles (OIPVs) (Meiners and Hilker, 2000; Reymond, 2013; Hilker and Fatouros, 2015). Evidence shows that volatiles emitted by an

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oviposited plant are not due to leaf damage associated with egg deposition since no leaf damage was observed when Lepidopteran laid their egg on leaves. The blend of volatiles emitted by plants after egg oviposition is thus specific for this response. For example, *Pinus sylvestris* twigs do not release the same blend of volatiles after feeding or oviposition by *Diprion pini*, which is illustrated by an absence of attraction of the specialized egg parasitoid *Chrysonotomyia ruforum* during herbivory (Mumm *et al.*, 2005). Similarly, Bruce and coworkers (2010) reported evidence for specific OIVPs emission in *Brachiaria brizantha* exposed to *Chilo partellus* oviposition. The African forage grass emitted less (Z)-3-hexenyl acetate after oviposition than herbivory but some other minor compounds, found after feeding, were released in higher abundance (Bruce *et al.*, 2010). Finally, OIVPs, like (E)-ocimene, (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), attract parasitoids that kill herbivores either at the egg stage or at the first-instar larvae (Tamiru *et al.*, 2011).

Finally, some phytohormones are converted into volatiles, such as the SA into MeSA or JA into methyl jasmonate (MeJA) or *cis*-jasmonone (CJ). MeSA is a common volatile derived from SA through methylation by the enzyme BSMT1 (Chen *et al.*, 2003). Volatilization of SA through MeSA synthesis could help plants to reduce SA accumulation, which has the potential to create cell death and be toxic, by excluding it from the cell. Moreover, MeSA is described as a signaling molecule in various physiological and defense responses (Ulland *et al.*, 2008; Kumar *et al.*, 2014). For example, in *Brassica* sp., MeSA has an inhibitory effect on the oviposition of *Mamestra brassicae* (Ulland *et al.*, 2008). Furthermore, in *Arabidopsis*, plants treated with MeSA or plant overexpressing *BSMT1* repel *P. brassicae* butterflies, thus inhibiting oviposition. Interestingly, *BSMT1* is not induced after five days by *P. brassicae* egg extract but is strongly induced after two days of herbivory. Together, these results suggest that the deterring effect of MeSA on gravid butterflies is triggered by larval feeding and could be regulated by the JA pathway (Groux *et al.*, 2014). This hypothesis could be linked to the meta-analysis of Gripenberg and coworkers (2010), who supposed that female insects may have evolved to oviposit on hosts on which their offspring will survive better (Gripenberg *et al.*, 2010). On the other hand, MeJA is biosynthesized from JA by an S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT). MeJA may act as an intracellular regulator because plant overexpressing *JMT* have constitutive expression of JA marker genes such as *AOS*, *LOX2* and *VSP* resulting in an increase of plant resistance against pathogens like *Botrytis cinerea*. Moreover, MeJA could act as a diffusible intercellular signal transducer and an airborne signal mediating intra- and interplant communications (Seo *et al.*, 2001). For example, in cotton plants, MeJA-treated plants emitted the same blend of volatiles, such as (E)- $\beta$ -ocimene, linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene,  $\beta$ -farnesene, than plants challenged by *S. exigua* larvae (Rodriguez-Saona *et al.*, 2000). Also, CJ, a major component in floral volatile bouquet, is known to attract insect pollinators and parasitoids, to repel aphids or to

be released in response to herbivory (Birkett *et al.*, 2000; Matthes *et al.*, 2010). Interestingly, gene expression analysis of CJ- or MeJA-treated *Arabidopsis* plants showed differences in defense responses. For example, *LOX2*, *AOS* and *OPR3* were induced in MeJA-treated plants and not in CJ-treated plants. On the contrary, CJ-treated plants induced specific genes such as *CYP81D11*. Finally, a mutation of CJ-inducible gene *CYP81D11* seems to block CJ-induced attraction of the aphid parasitoid *Aphidius ervi*. In conclusion, CJ and MeJA are two volatiles compounds originating from the JA signaling pathway that seem to control indirect defense by distinct pathways (Matthes *et al.*, 2010). All these examples demonstrate the fine regulation of plant response to herbivory or egg oviposition through the help of volatiles.

### 3- Effect of pathogen and multiple attacks on BVOCs emission

For many years, research has focused on a single biotic or abiotic stress on the same plant, but only few studies have been done using a combination of attacks. In these rare cases, studies focused on pathogen development and fitness, or on BVOC emission. Blend concentration is known to change after different biotic or abiotic stresses. A study on pepper plants, *Capsicum annuum* L., revealed significant differences in BVOCs' concentration after *S. exigua* and / or *Xanthomonas campestris* treatments. Pepper plant volatiles were produced in larger amounts in plants infected by both compatible bacteria and beet armyworm than on plants attacked by the herbivore alone. Thus, BVOCs composition appears to be pathosystem specific (Cardoza and Tumlinson, 2006). Moreover, as shown in maize challenged with both *Setosphaeria turcica* and *Spodoptera littoralis*, BVOC emission was reduced by around 50 %, with no change in larval fitness or parasitoid attraction. This is due to the fact that the SA pathway was triggered by the fungus, which, in turn, inhibited the JA pathway (Rostàs *et al.*, 2006). In an interaction between a plant, an insect and ozone it was shown that BVOCs emitted by *Phaseolus lunalus* infested by *Tetranychus urticae* were degraded by ozone treatment at 80 ppb (parts per billion) in comparison to a control atmosphere without ozone. Also, the authors found that ozone treatment decreased the communication distance by BVOCs between plants from 70 cm (centimeter) to 20 cm (Blande *et al.*, 2010). However, the effect of ozone and herbivory on gene expression was not analyzed. In *Malus domestica* exposed to both water stress and the chewing herbivore *S. littoralis* or the sap-feeding *Aphid pomi*, authors showed a positive effect on larval feeding and growth in plants subjected to a severe stress compared to control plants or plants subjected to an intermediate stress. On the contrary, stressed plants attracted fewer aphids than control plants (Mody *et al.*, 2009). Again, the effect of biotic and abiotic stresses on the same plant was not analyzed at the molecular level. Another study found that a combined stress with *S. exigua* and *X. campestris* changed the BVOCs' blend but the work did not try to understand which genes



were responsible for these differences or which pathways were involved (Cardoza and Tumlinson, 2006).

Moreover, a summary of different studies clearly demonstrated that volatile release from plants is quantitatively related to the severity of (a)biotic stress. Nevertheless, in nature, BVOCs' concentration decreases with distance from the emission source, thus in the case of severe stress, a higher emission implies a greater spread of the signal. Even so, responses cannot be as high as the stress at any time during herbivore or pathogen attacks. A time lag between stress and volatile emission and the combination of local and systemic responses can both be at the origin of this temporal difference (Niinemets *et al.*, 2013). However, when plants are challenged by a diverse community of herbivores, one HIPV can be effective as a repellent against one specie but attractive to another and may deregulate parasitoids' attraction. The tritrophic structure seems to really be dependent on plant volatiles (Xiao *et al.*, 2012).

#### 4- Regulation of BVOCs biosynthesis and emission

The ecological impact and importance of BVOCs is well described, but few studies have actually been conducted on the regulation of BVOCs biosynthesis and emission at molecular or metabolomics levels. Several reports indicate that phytohormone signaling pathways play a role in the control of BVOCs emission, where JA, SA and ET seem to be involved. For example, JA and SA applications on Lima bean revealed that JA / SA crosstalk modulates the spider mite's, *T. urticae*, preference because of the effects of phytohormones on volatile biosynthesis and emission. The application of SA, independently of the dose, suppressed JA-induced repellence to spider mites and the transcription of JA-inducible *PIOS* gene, a gene involved in the (E)- $\beta$ -ocimene emission (Wei *et al.*, 2014). Earlier work by Zhang and coworkers, in 2009, demonstrated that infestation of Lima bean by the whitefly *Bemisia tabaci*, an inducer of the SA signaling pathway, interferes with HIPVs emission in response to the spider mite *T. urticae* through inhibition of the JA signaling pathway. The same phenomenon was mimicked with application of 1 mM SA in *P. lunatus*, which suppressed the emission of the HIPVs, such as (E)- $\beta$ -ocimene and (Z)- $\beta$ -ocimene, normally released after spider mite attack. Authors also reported the down-regulation of *PIOS* in the case of mite, whitefly infestation and SA application. It was concluded that the down-regulation of many JA-induced volatiles seemed to be SA-dependent (Zhang *et al.*, 2009). On the other hand, a negative effect of JA application on SA-induced volatiles was also observed (Wei *et al.*, 2014). These data provide evidence that the SA- and JA-signaling pathways and their reciprocal antagonistic effect have an impact on plant volatiles emission.

Moreover, the effect of JA and SA on volatiles emission in *Populus nigra* clearly showed that 200  $\mu$ M JA application induced terpenes, nitrogen-containing and aromatic volatile compounds but not SA application at the same concentration. A comparison between transcript abundance of volatile biosynthesis genes *TPS2* and *CYP79D6/D7* showed a positive correlation with the concentration of volatile they are linked to, the homoterpenes 4,8-dimethylnona-1,3,7-triene ((E)-DMNT) and nitrogen-containing compounds 2 and 3-methylbutyraldoxime respectively (Calvijo McCormick *et al.*, 2014). Thus, some evidence of JA and SA involvement in BVOCs biosynthesis and emission regulation seems clear. However, mechanisms that control these responses and regulatory genes are not well characterized. Efforts have to be made on this issue to understand BVOCs biosynthesis and emission.

### 5- A new player: the importance of microbial volatile organic compounds in plant defense

In this chemical network, we cannot exclude the production of volatiles by the pathogen itself. Studies demonstrated that volatiles emitted by soil-born microorganisms are known to have an impact on plant growth and pathogen resistance. In maize, 2,3 butanediol released by *Enterobacter aerogenes*, a phyllosphere bacteria, renders plants more resistant to the leaf fungus *Setospharia turcica* but less resistant to *S. littoralis*. Most interestingly, 2,3 butanediol attracts parasitoids when it is released onto soil with all phyllosphere community members. This attraction does not occur when the volatile is sprayed on the headspace. The authors concluded that 2,3 butanediol had an indirect effect on parasitoids (D'Alessandro *et al.*, 2014). This work illustrates that microbial volatiles play an important role in multitrophic interactions. Little work has been conducted to elucidate the biological and ecological importance of bacterial and fungal volatiles in a dynamic environment. This research may help enable the discovery and development of novel agricultural practices (Piechulla and Degenhardt, 2014).

### 6- Impacts of BVOCs in the atmosphere

HIPVs and other volatiles are emitted in large amounts in the response to (a)biotic stresses, but BVOCs are also released in lower quantities by undamaged plants throughout their entire life. When BVOCs are produced, undamaged plants can prime and activate their own defense. Nevertheless, many BVOCs are highly reactive with atmospheric oxidants and take part in various functions in atmospheric processes. BVOCs have a role in the formation of ozone in  $\text{NO}_x$  (nitrite oxide) polluted atmosphere, of OH-radicals (hydroxide radicals), of organic nitrates and secondary aerosols (SOA). BVOCs emission is the major source of SOA formation. On the other hand, UV-radiation (UV: ultra-violet) and various reactive gases can dramatically alter BVOCs

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emitted by plants. These phenomena can reduce BVOCs' lifetime, distance of emission or change the composition of the emitted blend and have a consequence on parasitoids' or pollinators' attraction (Amin *et al.*, 2013; Holopainen and Blande, 2013).

In an atmosphere with high NO and NO<sub>2</sub> (nitrogen dioxide) levels, as in a smoky air for instance, BVOCs are oxidized and increase ozone levels. On the contrary, in cleaner air with a low level of NO<sub>x</sub>, oxidation of BVOCs decreases ozone in the troposphere but promotes the formation of SOA by ozonolysis reactions. SOA are nano-scale aerosol particles typically formed during late morning with an increasing incidence throughout the day. The chemistry of SOA biosynthesis is very complex because, for example, an undamaged pine emits 20 different monoterpenes and only one of these monoterpenes alone can form nearly 1200 different SOA. Finally, SOA could grow by nucleation and condensate until it forms cloud condensation nuclei. This formation leads to two dramatic consequences; SOA have an impact on air quality and the formation of a cloud cover leading, in turn, to enhanced albedo and reduced solar radiation at ground level (Holopainen and Blande, 2013). BVOCs and atmospheric compounds connection lay out questions for the future, within the context of climate change. Increases in temperature can have an impact in (a)biotic stresses with an increase in predator and pathogen rates. This pressure will result in an increase in BVOCs emission, which in turn can affect the plant-attacker-predator tritrophic level and SOA formation. Researchers have to focus on consequences of these different interactions, which at their final step could have dramatic effects on plant fitness and air quality.

In summary, BVOCs are a complex family of molecules, which play a prominent role in plant defense; one volatile may attract some parasitoids or predators but on the other hand this volatile renders the plant visible for other herbivores or pathogens. Likewise, BVOCs can have a noxious impact on plant fitness because of their implication in the production of atmospheric aerosols, which can decrease light intensity and, in the end, photosynthesis. Finally, BVOCs regulation in the case of one or multi-attackers is not well defined; hence efforts should be made to increase our knowledge on this important part of plant defense in the field.

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## Part III: How plants deal with multiple stresses

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Biotic and abiotic stresses are always present in nature and limit agricultural yields. Combinations of stresses such as heat, drought or high light intensity have profound effects on crop performance and yields (Chew and Halliday, 2011). Because of these, research has aimed at understanding the responses of model plants such as *Arabidopsis*, and crops such as rice or wheat, to single stresses, but further work is needed in the case of multiple stresses (Chew and Halliday, 2011; Rasmussen *et al.*, 2013). Plants have developed specific mechanisms that allow them to detect precise environmental changes and respond to complex stress conditions with the objective of minimizing damages and conserving sufficient resources for growth and reproduction (Rasmussen *et al.*, 2013). Clearly understanding these mechanisms as well as an in-depth comprehension of plant defense evolution are crucial in our future context of climate change, to know how plants will be able to adapt to this new environment (Ahuja *et al.*, 2010; Figure 9).

### 1- Interactions between herbivores and plant pathogens

Induced defenses are controlled by phytohormones, as described in the previous part of this introduction. As indicated, biotrophs activate SA-induced defenses, while necrotrophs and herbivores activate JA- and ET-induced pathways (Pieterse *et al.*, 2009; Figure 2). They are important mediators for interactions between herbivores and pathogens. The immune response starts by the recognition of organisms that interact with the plant through mechanical- or pathogens-associated molecular patterns (MAMPs or PAMPs). This recognition leads to defense response targeted especially against the attacker (Pieterse and Dicke, 2007). As biotrophs and necrotrophs have a distinct life style, they induce different plant responses. Their effects on plants will influence the phytochemical environment of the insect attacker in different ways. Moreover, effects on insects will depend on the kind of herbivore, phloem feeding or chewing herbivores (Lazebnick *et al.*, 2014). For example, on *Vicia faba*, the necrotroph pathogen *B. cinerea* had an inhibitory effect on the development, fecundity and survival rate of the aphid *Aphis fabae*, whereas the biotrophic fungus, *Uromyces viciae-fabae* enhanced the performance of the aphid. These opposing effects were explained by diverse activations of phytohormones. Biotrophs and aphids are known to trigger the SA pathway, but necrotrophs stimulate the JA pathway that could be beneficial to aphids. Most interestingly, the impact on aphid performance by dual *B. cinerea* and *U. viciae-fabae* stress seems to really fluctuate between sequential infections and illustrates the complexity of dual infection and herbivory on plant defense responses (Al-Naemi and Hatcher, 2013). Another example of positive interaction can be found in *N. attenuate*. *M. sexta* larvae have a higher performance, with a mass weight 4.1 fold heavier, on plants treated by the



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bacterial quorum-sensing compound *N*-acylhomoserine lactone than on untreated plants. This chemical signal, which is responsible for communication between bacteria, seems to have an effect on JA-mediated defenses (Heidel *et al.*, 2009). To the contrary, interactions can be negative, as demonstrated by Hilfiker and coworkers (2014). They showed that oviposition by *P. brassicae* inhibits the growth of *P. syringae* strains in Arabidopsis, similarly to the systemic acquired resistance (SAR), which confers early systemic resistance to a number of pathogens after a primary infection. Furthermore, Arabidopsis challenged by *P. syringae* decreased the performance of *P. brassicae* larvae. During this interaction, bacterial growth inhibition by *P. brassicae* oviposition is significant enough to have an impact on larval performance (Hilfiker *et al.*, 2014).

Some other studies show that multiple plant pathogens / herbivores-interaction may not so easy to estimate. Several studies have been done on the effect of *P. rapae* against diverse pathogens in Arabidopsis. For pathogens that trigger JA-dependent defenses, pretreatment by *P. rapae* feeding during 24 hours before the second stress has different consequences. On *A. brassicicola* infection development, larval pretreatment has no effect. But, on *P. rapae*, the authors observed a decrease of larval performance. Moreover, *P. rapae* feeding damage significantly reduced infection of pathogens that activated SA pathways. The reduction can be local against *P. syringae* and *X. campestris* or also systemic against *Turnip crinkle virus*. All this contrasting effects seem not to be dependent only of SA, JA and ET phytohormones. Arabidopsis mutants in these different pathways showed wild-type levels of *P. rapae*-induced protection against *P. syringae* (De Vos *et al.*, 2006). Another work has been done on interactions between pathogens and herbivores that highlight the importance on the SA pathway. Tomato plants challenged by *P. syringae* reduced the *S. exigua* growth and induced the JA pathway, both on wild-type and on SA-deficient plants. On the contrary, tomato mosaic virus, TMV, increased caterpillar performance on wild-type plants but not in the SA-deficient mutant. Also, TMV did not induce the JA pathway in both wild-type and SA-deficient plants. Finally, they observed that aphid colonization is reduced after TMV infection on wild-type plants only. The authors conclude that tomato defense in case of multiple attacks is dependent of the type of insect and pathogens applied to the plants. Some pathogens induced the JA pathway independently of SA, whereas some other induced both SA and JA. In this latter case, SA can have an impact on insect performance depending of his feeding style. SA has a negative impact on chewing insects that induce only the JA pathway and inversely, a positive effect on sucking feeders that induce both SA and JA pathways (Thaler *et al.*, 2010).

In conclusion, the effect of interactions between herbivores and pathogens on the same plant is really complex and depends primarily on the severity, duration of the infection, lifestyle of pathogens, herbivores involved, and the plant species considered (Figure 9).

## 2- Interactions between diverse (a)biotic stresses

Biotic and abiotic stresses are present on a daily basis throughout all plant life and are at the origin of the reduction in harvest yields. Efforts in research have been made for the past few years to understand the impact of these combinations of stresses on plant defense(s). A recent study in *Alnus glutinosa* induced by *Monstera pulveratun* and drought-stressed conditions demonstrated that BVOCs emission is stronger and reached a maximum value earlier due to dual stress conditions than feeding alone. In addition, herbivores seemed to be more attracted to well-watered plants and consumed four-fold more tissue. Overall, *A. glutinosa* was more resistant against herbivores under drought and herbivory stresses (Copolovici *et al.*, 2014). Moreover, Rasmussen and coworkers analyzed transcriptomic differences in ten ecotypes of *Arabidopsis* challenged by (a)biotic stresses in single or double combinations. They concluded that 61 % of gene regulation changes in response to dual stresses were not predictable using single treatment results. Also, 28 % of transcripts regulated by either of the individual stresses were unaffected by combined stresses. Plants seem thus to activate specific sets of genes in response to combined stresses (Rasmussen *et al.*, 2013). Moreover, a transcriptome analysis of *Arabidopsis* plants challenged by drought and heat stress also revealed a new pattern of defense response with a specific regulation of 454 transcripts in dual stresses, not expressed in plant stress by a single treatment (Rizhsky *et al.*, 2004). Recently, Suzuki and coworkers summarized results of 33 different combined stresses and demonstrated that few common molecular responses were shared. Each stress treatment seemed to result in unique responses and reflected the need to specifically adapt to a changing and complex environment (Suzuki *et al.*, 2014). The same conclusion was reached for the impact of combined stresses in metabolomics and proteomics, with several unique metabolites and proteins accumulating after multiple stresses and not present under single stress (Rizhsky *et al.*, 2004; Suzuki *et al.*, 2014). On the contrary, a study demonstrated a prioritization of one stress over another. Transcriptome analysis of *Arabidopsis* challenged by drought, nematode infection or both stresses simultaneously showed that the majority of transcripts regulated by the dual stress was also regulated by drought alone but not by nematode infection. Plants might thus prioritize against the most damaging stress (Atkinson *et al.*, 2013).

Soil composition also plays a role in the impact of (a)biotic stresses on plant defense. For example, four commonly hyperaccumulated metals in plants, the four elemental chemical defenses Co (carbon monoxide), Cu (copper), Ni (nickel) and Zn (zinc), combined with others metals or organic compounds, have enhanced defensive effects on *S. exigua* performance, higher than after treatment with single metals. A plant with a better access to metals and with a hyperaccumulator capacity may be more resistant against herbivores (Cheruiyot *et al.*, 2015).

In conclusion, plant responses to multiple stresses seem difficult to predict and the plant defense against (a)biotic stresses, like in field conditions, appears to be very complex. Each type of interactions needs to be analyzed separately, and no conclusion can be drawn on the evolution of future plant defense mechanisms (Figure 9).

### 3- Interactions between multiple attackers and beneficial organisms

Plants have to recognize and discriminate an enemy from a beneficial organism. Beneficial organisms are frequent throughout the plant community, improving nutrition or helping the plant overcome (a)biotic stresses. These beneficial organisms can be fungi such as mycorrhizal symbionts, bacteria such as plant growth-promoting rhizobacteria, natural enemies of insects such as predators and parasitoid wasps, or pollinators such as bees, moths and ants (Pieterse and Dicke, 2007). In the case of mycorrhizal symbiosis, plant phytohormones may affect symbiosis with the fungi either positively or negatively. For example, SA and ET seem to have inhibitory effects on arbuscular mycorrhizal symbiosis, whereas JA and ABA, to the contrary, have positive effects on this beneficial organism (Miransari *et al.*, 2014). Moreover, in rice, transcriptomic analysis revealed an overlap of 40 % between mycorrhiza-responsive genes and fungal pathogens-responsive genes (Van Wees *et al.*, 2008). Finally, the involvement of phytohormones could have an impact either on symbiosis, plant defense or in the end, on plant nutrition.

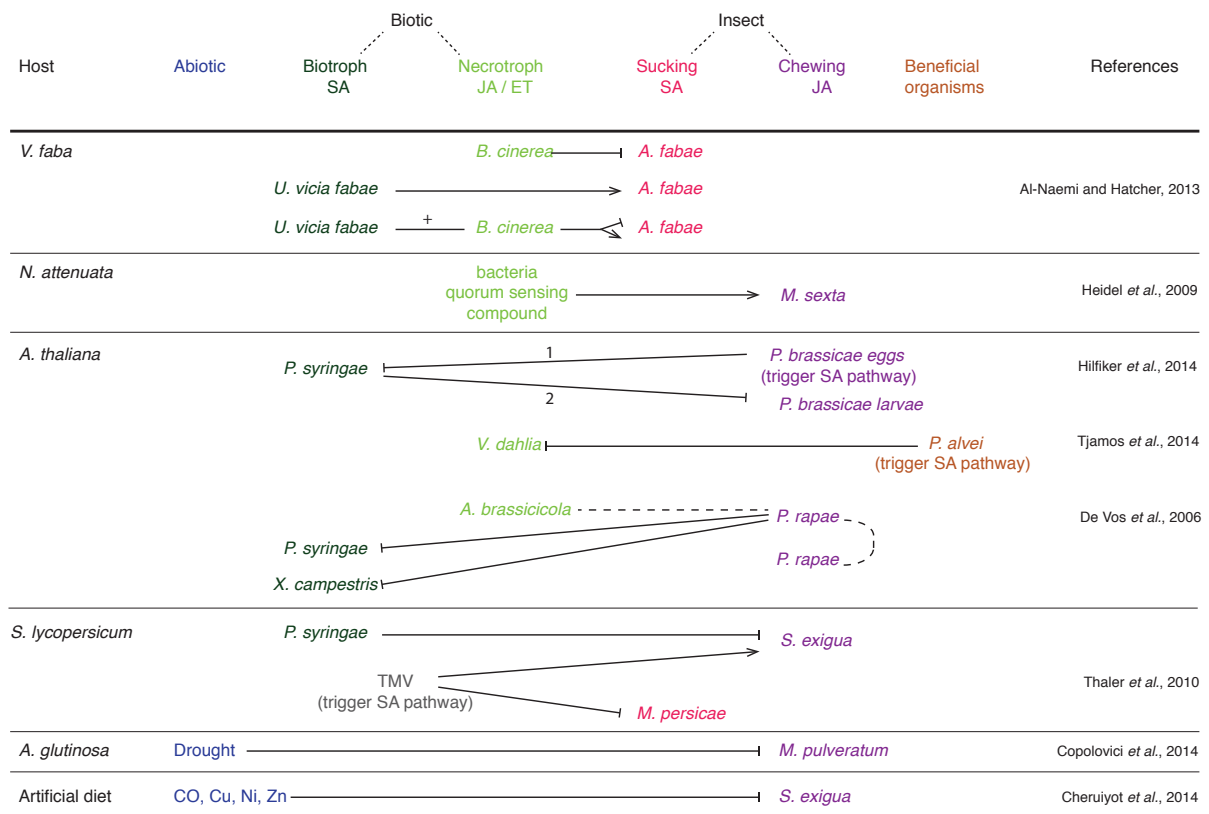
Furthermore, some plant-beneficial organism interactions are known to enhance plant defense. In *Arabidopsis*, a root beneficial fungus *Trichoderma asperellum* T34 induced plant defense through a defense gene *MYB72* and the ET collaboration (Van Wees *et al.*, 2008). Another example is the beneficial rhizobacteria *Paenibacillus alvei* K165 that primes for enhanced SA-dependent defenses and increased the resistance of plants against the destructive vascular wilt soil fungus *Verticillium dahlia* (Tjamos *et al.*, 2005). Interestingly, endophytic actinobacteria can prime both SA and JA / ET pathways. Inoculation of *Arabidopsis* plants with these endophytic strains induced defense mechanisms, with higher abundance of defense gene expression, such as *PR1* or *PDF1.2*, compared to non-inoculated plants (Conn *et al.*, 2008). Nonpathogenic rhizobacteria can induce resistance in plants similarly to SAR. In this case, the priming for enhanced defense gene expression from rhizobacteria is called induced systemic resistance (ISR). Beneficial organisms, such as this kind of rhizobacteria, can induce ISR in the roots and protect against diverse pathogens in aboveground foliar tissues (Pieterse *et al.*, 2002). Moreover, *Cordia nodosa* is a tree that is a host for ants. Plant provides food, such as extrafloral nectar or food bodies, or housing, called domatia, to attract ants that in turn will protect plants against

herbivores. *C. nodosa* has a life average of 77 years, is colonized by bodyguard ants, which live around 8-14 years, depending of ant species. As a consequence, these plants sometimes live without protection by ants, between losing one ant colony or when the colony is not the best for herbivory protection, because the colony is small or some ants species are most effective as bodyguards than other. Study demonstrated that both direct and indirect defenses are inducible in *C. nodosa* and are activated when ants colony of *Allomerus octoarticulatus* where not present on tree or too smaller to have a good protection (Frederickson *et al.*, 2013).

Finally, BVOCs also play this dual role, as they have to attract pollinators and defend plants against enemies that rob nectar or consume floral parts alike. In this context of defense or attraction dilemma, Kessler and coworkers analyzed the floral volatiles bouquet from lines of *Petunia x hybrida*. Florivores seemed to be attracted to a single compound of the bouquet, methyl benzoate. Otherwise, more compounds were used in host location for pollinators (Kessler *et al.*, 2013). In *B. nigra*, JA treatment results in multiple responses at the different trophic level. Herbivores, *P. rapae* prefer to oviposit on untreated plants but the parasitoid *C. glomerata* is more attracted by JA-treated plants. Pollinators are not influenced by JA treatment, but plants excreted less nectar than control ones (Bruinsma *et al.*, 2008). Few studies have been conducted on the influence of induced defenses on flower nectar chemistry and pollinator behavior and the impact of stress on the floral bouquets. Moreover, Pineda and coworkers (2012) demonstrated that beneficial rhizobacterial colonization modifies the blend composition of HIPVs. Volatiles emitted by plants treated both by the rhizobacteria, *Pseudomonas fluorescens* and aphids, *Myzus persicae*, are less attractive for parasitoids, *Diaetetiella rapae*, than plants without rhizobacteria. This lost of attractiveness is absent in an Arabidopsis double mutant impaired in JA biosynthesis, *aos / dde2-2*. Authors hypothesized that *P. fluorescens* seems to modify the HIPVs blend through JA-signaling pathways and interfered with parasitoids attraction against aphids (Pineda *et al.*, 2012).

In conclusion, plant defense and more specifically BVOCs or phytohormones involved can have an impact on the development of beneficial organisms of the phyllosphere and *vice-versa* (Figure 9). Plant defense and its evolution in multiple stress contexts is very difficult to predict and seems more complex than expected, and as such each stress has to be analyzed separately (Figure 9). The growing knowledge on this topic may help us to conclude and find out if plants respond totally differently to combined stresses or if they prioritize some patterns in function of stress severity.

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**Figure 9: Complexity of plant defense in multiple stresses context.**

Each (a)biotic stresses are represented by colour: abiotic stress in blue, biotroph stress in dark green, necrotroph in green, virus in dark grey, sucking herbivore in pink, chewing herbivore in purple and beneficial organisms in brown. Hormone pathway involved by each group of stresses are indicated on the top or specifically under the organism: SA, salicylic acid, JA, jasmonic acid, ET, ethylene. Dash lines represent a pathogen / herbivore without effect on the growth or performance of the other.

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## Part IV: The experimental system:

*Brassica nigra* plants challenged with *Pieris brassicae* larvae or eggs, by *Xanthomonas campestris* pv. *campestris*, by *Brevicoryne brassicae*, or by ozone

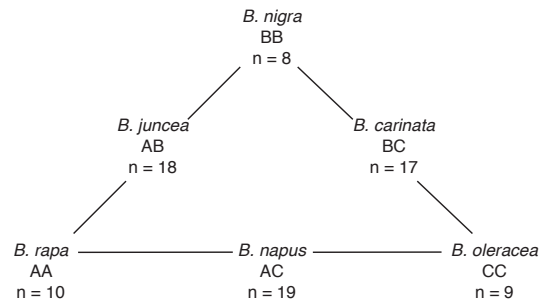
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### **Information about the plant used in this study as well as its enemies.**

#### 1- The plant: *Brassica nigra*

The black mustard, *B. nigra*, belongs to the *Brassica* genus, which contains economically important crop species, for human and animal foods, such as *Brassica juncea*, the green mustard, *Brassica napus*, the rapeseed, *Brassica oleracea*, which includes cabbage, Brussels sprouts, cauliflower, broccoli and *Brassica rapa*, the turnip. In total, the crucifer family comprises 3500 species (Westman and Kresovich, 1999; Westman *et al.*, 1999). *B. nigra* (L.) Koch. is an annual herb, native of the Mediterranean region in Europe and North Africa, cultivated for oil and flour that is used mostly for pharmaceutical products (FAO, <http://www.fao.org/economic/ess/ess-standards/comm-items/details-items/zh/c/1479/>; Odour *et al.*, 2011). Their medicinal properties are well known from as far back as the Ancient Greek civilization, 480 years before Christ. The ancestor of the black mustard could be *Sinapis arvensis*, which shows high homology of genetic sequences and protein fractions, or *Hirshfeldia incana*, which shares nuclear DNA. The *Brassicaceae* species' diversification appears to come from three plants species, which constitute the basis, named the U triangle: *B. nigra* (n = 8) named the BB-genome, *B. rapa* (n = 10) the AA-genome and *B. oleracea* (n = 9) the CC-genome. For example, *B. napus* share genomes from *B. nigra* and *B. oleracea* with an AC genome. This explains the origin of the amphidiploid species of *B. napus* but also of *Brassica carinata* and *B. juncea* (Figure 10). Furthermore, phylogenetic studies explain the evolution of *Brassica* from a common ancestor with a genome n = 6, from which the number of chromosomes and the partial homology of U triangle genomes came (Branca and Cartea, 2011).





**Figure 10: The U triangle.**

Adapted from Branca and Cartea, 2011.

Most specifically, *B. nigra* genome analysis of 32 individuals coming from gene bank accessions and weed populations of Europe, North Africa - the two native regions - India, Ethiopia and North America show a considerable variation across regions and individuals alike. Half of the variations are observed between plants within entries. Ethiopian entries compose the most distinct group while European and North American entries are close together, reflecting the species' agricultural history (Westman and Kresovich, 1999). Moreover, analysis of disease resistance to *X. campestris* pv. *campestris* and *A. brassicicola* between *B. nigra* entries plants from diverse parts of the world demonstrated a regional variation. North American plants had the highest percentage of entries resistant to both infections (Westman *et al.*, 1999). Furthermore, native plants coming from Africa, Europe and Asia, were compared to invasive *B. nigra* populations coming from North America. Under native herbivory levels and range exposition, resistance traits are always lower in native plants, such as trichome density or sinigrin concentration (Odour *et al.*, 2011). As a reminder and as mentioned in part I, all plants of the *Brassica* genus produce GS: in *B. nigra*, more than 95 % of the total GS concentration is sinigrin (Gols *et al.*, 2008; Müller *et al.*, 2010). Likewise, the growth performance traits, height of mature plants and biomass yield of plants are lower in native plants in comparison to invasive populations. Only individual seed biomass is higher in native *B. nigra*. Interestingly, in spite of lower resistance and growth traits, the tolerance towards herbivore damage is higher in native *versus* invasive plants. In conclusion, invasive populations possess a higher resistance to herbivore leaf damage than native plants, but a lower tolerance level. This is illustrated by a rapid post-introduction evolution of *B. nigra* traits leading to invasion success, because invasive and native populations were separated by less than 200 years (Westman *et al.*, 1999; Odour *et al.*, 2011).

Brassicaceous crops can be infected by several pathogens such as *Alternaria* sp., the black leaf spot, *B. cinerea*, the famous downy mildew, *Plasmodiophora brassicae* that caused

clubroot disease. Moreover, numerous nematodes can colonize cruciferous roots, like, for example, *Heterodera* sp. or *Pratylenchus* sp. Finally, insects cause most of the damage on Brassicaceae production, despite GS contents; specialized insects from Lepidoptera, Diptera, Coleoptera and Homoptera are able to feed on these plants (Mennan and Handoo, 2006; Björkman *et al.*, 2011). However, *B. nigra* plants respond specifically to each invader. For example, transcriptional analyses demonstrated that the black mustard triggered different phytohormone signaling pathways and not the same expression ratios for specific defense genes in function of the feeding mode of the insects. Infestation by ten first instar *P. rapae* larvae induced expression of JA marker genes, such as *LOX2*, and the repression of SA marker genes such as *PR1*. However, application of twenty *Brevicoryne brassicae* aphids activated *CTR1* suggesting a repression of the ET pathway and no expression changes in JA or SA marker genes. Moreover, defense genes such as the terpene synthase gene *TPS04* are induced by a chewing herbivore only and not by a sucking feeder (Broekgaarden *et al.*, 2011).

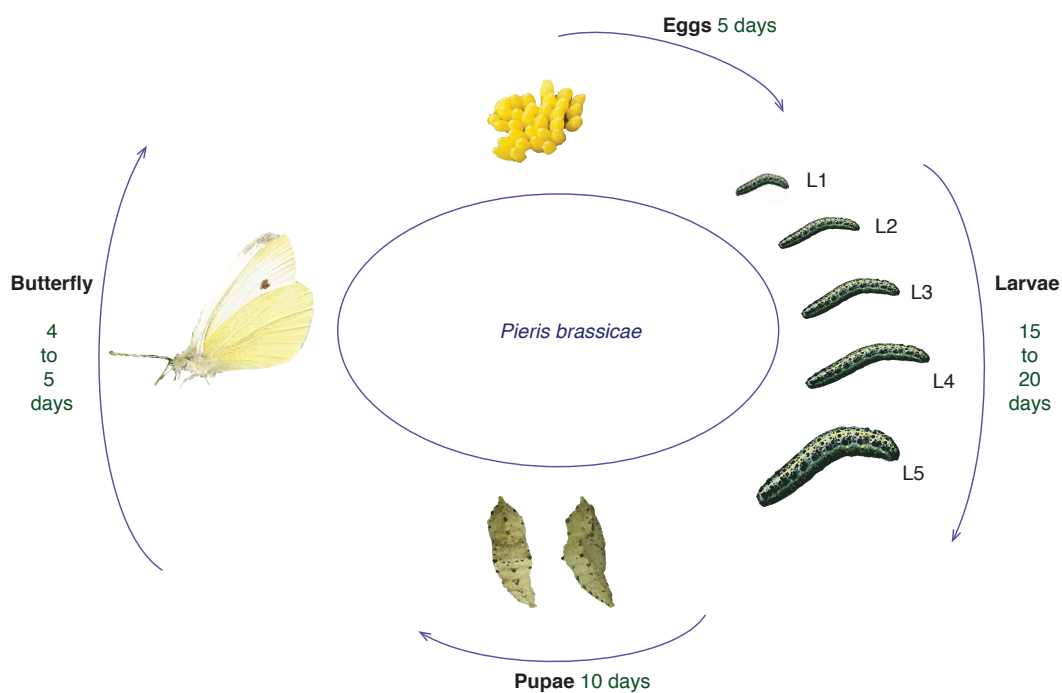
Finally, a study on *B. nigra* showed that plants increase and accelerate their seed production in response to egg oviposition by *P. brassicae* and not BVOCs emission. The black mustard seems to invest in reproduction to successfully prevent herbivory consumption of flowers (Lucas-Barbosa *et al.*, 2013). This finding can be correlated with the fact that *B. nigra* flowers contain more GS than green tissue and are more nutritious because *P. brassicae* flower feeders have a higher performance than leaf tissue feeders (Smallegange *et al.*, 2007). In *B. nigra*, acceleration and increase in seed production in response to herbivory may be a consequence of flower protection, as they are rich in GS and therefore known to attract specialists.

## 2- The herbivore: *Pieris brassicae*

*Pieris brassicae* is an insect of the Lepidoptera order belonging to the Ditrysia suborder. The Large White butterfly is placed on the true butterflies in the superfamily Papilionoidea, in the family Pieridae. According to Talbot (1939), *P. brassicae* is distributed throughout all Eurasian continents from Europe to Tibet and Himalayas. In 1974, the Large White butterfly reached Chile, by importation (Feltwell, 1982). The specialist *P. brassicae* is a gregarious feeder of several species from the Brassica family (Held and Spieth, 1999). The Large White Butterfly is one of the most destructive pests at all growing stages of cruciferous plants, from seedling to the vegetative and flowering stage (Ali and Rizvi, 2007). Butterflies' mating can occur around eighteen hours after pupae eclosion (Feltwell, 1982). During mating, *P. brassicae* males have been shown to synthesize and transfer the antiaphrodisiac benzyl cyanide to females. This antiaphrodisiac molecule is characteristic of male odor, suggesting that chemicals from mated females mimic the male odor, thus decreasing the attractiveness of mated females (Andersson *et al.*, 2003). After maturation of ova, female recognizes Brassicaceae as food plants by BVOCs and will lay eggs on

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a suitable leaf, i.e. a leaf with few batches already oviposited. Normally, a batch comprises an average number of 26 eggs. Eggs are glued onto the underside of the leaf surface (Feltwell, 1982). The orange-yellow eggshell changes to a darker color at the top before larvae hatching, five days after oviposition. After larvae emergence, they start to eat the eggshell, source of vital nutrients for growth. Larvae have five instars that take between three and four days each before going through the next ecdysis, irrespective of the host plant (Feltwell, 1982; Ali and Rizvi, 2007). The sensitivity of light – dark cycles during larval development, and more specially during the second larval stage to the last one, have an impact on the pupation. Indeed, pupae can enter in diapause if larvae were exposed to short-days coupled with temperatures above 21 °C. The “required day number” and a “daylength threshold” determine the photoperiodic response of *P. brassicae*. This sensitivity is an adaptation to minimize the risk of hatching at the wrong period or during winter (Spieth and Sauer, 1991; Spieth, 1995; Held and Spieth, 1999). Finally, at the end of the fifth larval instar, *P. brassicae* is able to walk great distances to find a suitable place for pupation. Caterpillars start to pupae only when they have enough materials left to make the silk girdle and cremaster. Moreover, they usually start to pupate in an environment protected against weather and predators (Feltwell, 1982). The period of wandering can take up to 2 days and the pupation time takes around 10 days before butterfly hatching. Female butterflies live older than males, with 5 and 4 days respectively (Figure 11; Ali and Rizvi, 2007).



**Figure 11: *Pieris brassicae* life cycle.**

Development stages of *Pieris brassicae* from eggs to butterfly. L1, First larval instar; L2, Second larval instar; L3, Third larval instar; L4, Fourth larval instar; L5, Fifth larval instar.

*P. brassicae* has a lot of predators, such as birds, parasitoid wasps, ants, beetles, spiders and also fungal, viral and bacterial infections (Philips *et al.*, 2014). Predation could have a large impact on life development and finally on herbivory.

Actually, most of the studies on plant defense responses against *Pieris* sp. have been done with another closely related species, *Pieris rapae*. In *Arabidopsis* and *B. nigra*, herbivory by *P. rapae* induced JA-marker genes such as *VSP2* or *LOX3* (Reymond *et al.*, 2004; Broekgaarden *et al.*, 2011). Inversely, eggs of *P. brassicae* induced SA-marker genes such as *PR1* and an accumulation of SA, which then suppresses expression of caterpillar-induced JA-dependent defense genes by the SA / JA crosstalk and will provide an advantage to future larvae (Bruessow *et al.*, 2010).

In conclusion, *P. brassicae* seems to activate different hormone pathways during its life cycle. However, eggs and larvae are both recognized by plants as attackers.

### 3- The bacterial pathogen: *Xanthomonas campestris* pv. *campestris*

The biotroph *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the causal agent of black rot disease of Brassica genus, which is one of the most destructive pathogens in the family (Williams, 1980; Gabriel, 1999). *Xcc* is a gram-negative bacterium that develops faster in warm, humid climates and is more damaging in tropical or humid continental regions. *Xcc* enters plants through water pores of leaf margins or wounds and colonizes the vascular system, where bacteria produce extracellular polysaccharide, named xanthan. Bacteria and xanthan collapse xylem vessels during the infection, resulting in characteristic V-shaped chlorotic lesions on leaves originating from the point of bacterial entry and black rot of the vascular tissue. Under ideal conditions, symptoms appear 10 to 14 days after bacterial entry into the plant; however, this pathogen may survive within the vascular system without producing symptoms if temperature is not optimal, (Williams, 1980). Simpson and Johnson showed that *Arabidopsis* is a host for *Xcc*. They obtained clear infection by spray, wound or infiltration inoculation (Simpson and Johnson, 1990). The successful infection of *Xcc* requires type III protein secretion system to deliver virulence factors into host cells. Moreover, the type III secretion system injects effector proteins into host cells to interfere with host immunity responses and ensure bacterial multiplication and infection. *Xanthomonas* sp. can inject more than 25 different effector proteins with different functions, such as enzymatic activity or manipulators of host transcription (Kay and Bonas, 2009). For example, in *Arabidopsis*, one of the *Xcc* manipulator of host transcription is an avirulent factor, *AvrXccC<sub>8004</sub>*, responsible for the induction of ETI and disease resistance, which are observed by a hypersensitive response. Further, *AvrXccC<sub>8004</sub>* possesses the capacity to manipulate ABA signaling pathway, leading to an accumulation of ABA, through an increase in

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*NCED5* gene expression. *NCED5* encodes for a key enzyme in ABA biosynthesis and plays an important role in plant defense. For example, exogenous application of ABA results in an increase of Arabidopsis sensitivity to *Xcc*. *AvrXccC<sub>8004</sub>* seems to attenuate host defense response during Arabidopsis – *Xcc* interaction and ABA may act as a negative signaling molecule to suppress host immunity and enhance bacterial development (Ho *et al.*, 2013). Another example of *Xanthomonas* sp. type III effector with an enzymatic function, *AvrBsT*, is known to induce hypersensitive cell death and defense responses in pepper and tobacco plants. *AvrBsT* is a member of the YopJ/*AvrRxv* family in *Xanthomonas campestris* pv. *vesicatoria*, *Xcv*. This family contains a putative catalytic triad, formed by histidine, glutamate and cysteine, which are required for R-gene mediated recognition (Kay and Bonas, 2009; Kim and Hwang, 2015). Kim and Hwang demonstrated that *AvrBsT* binds to *CaALDH1*, *C. annuum* Aldehyde deshydrogenase 1, in the cytoplasm and involves ROS (reactive oxygen species) burst and activation of the SA signaling pathway, which together trigger cell death and defense responses against *Xcv*. Interestingly, overexpression of *CaALDH1* in Arabidopsis reduced susceptibility to *P. syringae* pv. *tomato* and *Hyaloperonospora arabidopsis* infection (Kim and Hwang, 2015).

Inversely, plants activate phytohormone pathways to fight against *X. campestris* infection. For example, *Xcv* triggers both the ET and SA signaling pathways. After infection of tomato plants by *Xcv*, SA accumulates in the tissue and necrosis appears, but does not in ET-deficient plants. Only an exogenous application of SA on ET-deficient plants can restore necrosis and plant response to the pathogen (O'Donnell *et al.*, 2001). In Arabidopsis, after 3 days of inoculation, different SA-, JA-, ET-deficient plants showed three- to fivefold higher growth of *X. campestris* pv. *armoraciae* than wild-type plants. This result indicates a basal resistance to *X. campestris* through a combination of SA-, JA- and ET-dependent defenses (Ton *et al.*, 2002).

Like for *Pseudomonas* species, analyses of coronatine production, which can activate the JA pathway, have been done in *Xanthomonas*. Interestingly, out of 12 strains from 10 pathovars of *X. campestris*, only the pathovar of *X. campestris* pv. *phormiicola* synthesized coronatine analogues (Mitchell, 1991). Moreover, *X. campestris* infection can impact plant defense against herbivores. Cardoza and Tumlinson demonstrated that pepper plants infected by *X. campestris* pv. *vesivatoria*, pepper race 3, a compatible strain, or tomato race 1, an incompatible strain, do not have the same BVOCs profiles in the case of both bacterial infection and *S. exigua* feeding, depending on the strain of *X. campestris* used. In the case of infection by the compatible strain and larvae, the mean of total BVOCs released after 4 days is higher than in control, bacterial infection or herbivore treated plants. In the case of incompatible infection, dual stress has a lower mean of total BVOCs released than in the case of dual stress by the compatible strain and herbivore treated plants. More interestingly, *S. exigua* eats more tissue and gains more weight in plants previously pretreated with *X. campestris* than control plants. The authors suggest that *X.*

*campestris* infection may change the plant's biochemical properties, which increases insect feeding and performance (Cardoza and Tumlinson, 2006). Furthermore, in *B. nigra*, *Xcc* infection induced the emission of a blend of volatiles that is clearly different than the one emitted by *P. brassicae* feeding or control plants. Interestingly, *Xcc* and *P. brassicae* combined stresses emit a blend of volatiles that cannot be distinguished from the one emitted by herbivory alone (Ponzio *et al.*, 2014).

In conclusion, *X. campestris* seems to activate SA, JA and ET pathways in Arabidopsis and can have a significant impact on plant defense against other attackers.

#### 4- The aphid: *Brevicoryne brassicae*

Aphids are phloem feeders, distributed throughout the world and causing devastating losses to cultivated plants (Hughes, 1963; Giordanengo *et al.*, 2010). *B. brassicae*, Sternorrhyncha: Aphididae, is a specialist phloem feeder of *Brassica* crops and prefers feeding on younger plant tissues. It can move into floral buds in development, which makes it the worst economic loss in Brassicaceae, rendering the head unmarketable (Costello and Altieri, 1995). This effectiveness is related to a highly efficient colonization (Giordanengo *et al.*, 2010). This colonization power is due to important factors in their life style. Firstly, their parthenogenesis capacity increases the number of individual and decreases the pre-reproduction time. Secondly, a high aphid density leads to an increased nutrient loss, but also in the number of stylets that wound plants, thus creating an open door to pathogens. Thirdly, two kinds of adults can be present simultaneously on plants; winged adults that can colonize new neighbor host plants and wingless adults that invest more energy in reproduction. The wingless adults are viviparous females, who exploit the food supply to the limit and produce young as fast as possible. Moreover, aphid survival is due to their ability to access phloem in avoiding or sabotaging plant defense responses, while keeping phloem cell alive to feed enough. For this, they insert a long and flexible stylet through the cell wall apoplasm between cells (Hughes, 1963; Giordanengo *et al.*, 2010). The success of this feeding method is due to the fact that aphids inject watery saliva into the phloem to avoid protein clogging and always proceed to phloem sap ingestion. Also, another watery saliva is added to the ingestion sap to prevent clogging inside the capillary food canal. This inhibition of protein coagulation by aphid saliva may explain the resistance of sucking feeders in some plants (Tjallingii, 2006). Moreover, in Arabidopsis, Mewis and coworkers demonstrated in 2006 that *B. brassicae* or the generalist aphids *M. persicae* feeding on SA-insensitive *npr1* mutant have a lower performance than aphids feeding on control plants. On the contrary, their performance is improved when they grow on JA-insensitive *coi1* mutant plants. Arabidopsis triggers the SA-signaling pathway against generalist and specialist aphids (Mewis *et al.*, 2006). Interestingly, in Arabidopsis, aphids feeding

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on plants constitutively activating the JA signaling, have a strong reduction in performance compared to insects feeding on control plants (Ellis *et al.*, 2002). Likewise, in *Medicago truncatula*, treatment by MeJA reduced infestation by the bluegreen aphid, *Acyrtosiphon kondoi* (Gao *et al.*, 2007). In 2010, Giordanengo and coworkers proposed the hypothesis that activation by the plant of not the most efficient defense pathway can be a decoy strategy. Activation of SA-regulated genes against aphid attacks may prevent future pathogens invasion due to the stylet wounding by phloem feeders (Giordanengo *et al.*, 2010).

Furthermore, the role JA-, SA- and ET-signaling pathways seem to differ between *Arabidopsis* and other plant species. For example, in *M. truncatula*, bluegreen aphids activate most of the octadecanoid pathway genes studied and all of the SA and ET-responsive genes analyzed (Gao *et al.*, 2007). In sorghum, *Sorghum bicolor*, greenbug aphid, *Schizaphis graminum*, induced SA-regulated genes and marginally and temporarily JA-regulated genes, such as *LOX* or a protease inhibitor, *BBPI2*. Authors assumed that the SA signaling pathway elicited by greenbug aphid suppressed MeJA-responding genes or that the tissue damage caused by aphids may not generate sufficiently high endogenous JA levels, which in turn results in an entire activation of the JA-responsive genes (Zhu-Salzman *et al.*, 2004).

However, *B. brassicae* infestation can impact the perception of BVOCs by wasps. At a density of 50 aphids on *B. nigra*, wasps did not discriminate more against control plants than they did against infested plants. At a density of 100 aphids however, wasps avoid plants damaged by the phloem feeder. Surprisingly, in the case of dual infestation by *P. brassicae* larvae and *B. brassicae*, the aphid density-dependent effect disappeared. Wasps were always more attracted to BVOCs of plants feeding by larvae both with 50 or 100 aphids, than to healthy control plants (Ponzio *et al.*, 2014).

In conclusion, aphids are rapidly spreading pests, which lead to considerable damages and reduction in plant yield. Actually, analysis of transcriptome responses to phloem feeders on different plants does not allow to identify a specific hormone-signaling pathway that is triggered for plant defense. Moreover, plants BVOCs emission in case of herbivory seems to not be differentially regulated by aphid infestation. Further research has to be conducted in order to understand the contribution of phytohormones in aphid infestation.

## 5- The abiotic stress: ozone

Plants are not only challenged by biotic stresses, but a number of environmental changes can have an impact on plant life cycle. Ozone, O<sub>3</sub>, is one of these abiotic factors that is perceived as a stress by the plant. Moreover, global climate change and air pollution are closely linked. Knowing that the chemical composition of the troposphere plays an essential role in the health of living organisms, researches have to focus on impacts of air pollutants, such as O<sub>3</sub>, in plant defense to predict the outcome in the future global climate (Baier *et al.*, 2005; Unger, 2012). Two different pools of O<sub>3</sub> are present in the atmosphere. The beneficial part of O<sub>3</sub> is found in the stratosphere, the layer of atmosphere ranging from around 15 to 40 kilometers in altitude, which absorbs the UV-B and UV-C radiations. In the troposphere, however, from 10 to 12 kilometers in altitude from the Earth's surface, O<sub>3</sub> is considered a pollutant. Ozone is produced by reactions between photochemical oxidation of carbon monoxide, methane and nonmethane volatile organic compounds and hydroxyl radical in the presence of sunlight and nitrogen oxides (Iriti and Faoro, 2009; Unger, 2012). Actual models estimate the amount of ozone to exceed, over vast areas, 70 parts per billion (ppb) by the year 2100. Currently, some ozone peak concentrations are higher than 100 ppb and occasionally reach a level of 200 ppb (Blande *et al.*, 2010). O<sub>3</sub> enters into the leaf through the stomata and forms ROS, like superoxide anion and hydrogen peroxide in the apoplast (Ahlfors *et al.*, 2004; Iriti and Faoro, 2009). O<sub>3</sub> is known to trigger visible symptoms: changes in pigmentation, bleaching of mesophyll cells, necrotic lesions and / or accelerated foliar senescence (Sharma and Davis, 1994; Pell *et al.*, 1997; Baier *et al.*, 2005). The severity of damage depends on the ozone concentration and time of exposure. Under short but high concentrations of ozone, more than 200 ppb, damages are visible. Whereas after chronic exposure to low ozone concentration, the symptoms are not invisible while a growth reduction is observed. For example, in *Arabidopsis*, plants treated with either 150 or 300 ppb daily during 6 hours have a reduction in plant biomass and curling leaves (Sharma and Davis, 1994). O<sub>3</sub> clearly has an impact on photosynthesis, with a detrimental effect on carbon fixation through a decrease of the Rubisco activity and on electron transport. In case of high light, the impact of ozone on photosynthesis is increased because high light alone is already a photoinhibitor (Fiscus *et al.*, 2005).

O<sub>3</sub> is known to affect both direct and indirect plant defense mechanisms. For the direct defense, O<sub>3</sub> can cause phototoxicity and triggers a hypersensitive response similarly to pathogen infection (Blande *et al.*, 2010). Analysis intracellular signal transduction clearly demonstrated that O<sub>3</sub>, like other oxidative stress, induced a two-step response. First, ozone changes the calcium signature and protein phosphorylation status and, secondly, it activates SA, JA / ET and ABA signaling pathways. Most of the time, ET and SA are important in amplifying the oxidative signal, while JA constricts ozone-induced damage (Baier *et al.*, 2005). The fastest response is the



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activation of ET by the induction of ACS gene family and ACC synthases (Figure 6). Also, microarray analysis revealed O<sub>3</sub> marker genes for each phytohormone pathway involved in the response, such as *JMT*, *OPCL1*, *AOC1* and *AOC3* for the JA pathway (Figure 3) or *ICS1*, *PAL*, *EDS5*, *PAD4*, *SID2* and *EDS4* for the SA pathway (Figure 7). Moreover, late O<sub>3</sub> stress triggered few genes involved in the regulation of ABA biosynthesis and more specifically the crucial gene for the ABA synthesis, the 9-cis-epoxycarotenoid dioxygenase gene, *NCED3* (Ludwikow and Sadowski, 2008). A recent work has shown crosstalk between ABA and ET under O<sub>3</sub> stress through posttranslational regulation. A protein phosphatase type 2C, ABI1, a core component in the ABA signaling, seems to interact with ACS6 and dephosphorylates its C-terminal fragment, a target for the stress-responsive mitogen activated protein MPK6. In Arabidopsis, MPK6 is important in ET production because phosphorylation of ACSs by MPKs decreases the protein turnover, leading to an increase in ET. Inversely, dephosphorylation of ACS results in their degradation by the proteasome. ABI1 appears to be important under ozone stress to restrict ET production and avoid the detrimental effect of ethylene overproduction (Ludwikow *et al.*, 2014).

Moreover, as already mentioned on part II, O<sub>3</sub> can affect BVOCs emission. In *P. lunatus*, ozone treatment at 80 ppb reduced the distance over which signaling occurs through BVOCs from 70 to 20 cm. Moreover, nectar secretion of Lima bean under high O<sub>3</sub> exposure, 120 ppb, increased significantly. More interestingly, some BVOCs compounds are altered by ozone treatment at 80 ppb, (E)- $\beta$ -ocimene, and TMTT are not present anymore and (E)-DMNT is drastically decreased (Blande *et al.*, 2010). Surprisingly, in *B. oleracea* and lima bean plants, O<sub>3</sub> exposure at 60 nl / l and 120 nl / l, clearly demonstrated a decomposition of HIPVs after herbivore attacks, in comparison to plants growing in ambient ozone air. Predators and parasitoids were still well able to detect herbivore-damaged plants though. In this case, *C. glomerata* and *Phytoseiulus persimilis* are not disoriented by the volatiles' degradation and may use stable HIPVs under ozone exposure to find their prey (Pinto *et al.*, 2007). Moreover, in *B. napus* ssp *oleifera*, O<sub>3</sub> at 100 ppb decreased BVOCs and terpene emission. For example, (E)-DMNT are not emitted anymore, such as (E,E)- $\alpha$ - Farnesene and Limonene is reduced by half.

Finally, ozone has a real impact on plant defense as described above, with a multitude of phytohormone pathways activated and a deregulation of BVOCs emission. Khaling and coworkers demonstrated that ozone pretreatment, at 80 and 120 ppb, in *B. nigra*, reduces *P. brassicae* performance; larval weight and pupal mass were smaller than that of caterpillars feeding on ambient ozone atmosphere (Khaling *et al.*, 2015). On the contrary, after O<sub>3</sub> treatment, *Malacosoma distria* larvae had a better performance in aspen than caterpillars feeding on ambient ozone, mostly explained by differences in foliar quality with an increase in early season nitrogen and a decrease in a phenolic glycoside, tremulacin (Kopper and Lindroth, 2003). Notable

insect performance was seen on *P. sylvestris* under ozone doses 1.5 to 1.7 times of the ambient level. The performance of *Lygus rugulipennis* is smaller after O<sub>3</sub> treatment, inversely, *Gilpinia pallida* grew better than on ambient ozone. Larvae of *Neodiprion sertifer*, *Schizolachnus pineti* and *Cinara pinea* are not affected by ozone exposure. Also, authors demonstrated that total phenolics, total terpenes, total free amino acid, nutrient or sugars concentrations are not affected by O<sub>3</sub> in comparison to ambient ozone in pine. Only serine and proline concentrations are significantly increased after exposure. Low-levels of ozone treatment have a very small impact on plant metabolism and seem to have different effects on performance depending on insect species (Manninen *et al.*, 2000).

In conclusion, ozone seems to activate different phytohormone pathways and effects depending on the doses and the pathosystem studied. Clearly understanding ozone effects can help us to protect plants in the future context of global climate change.

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## Aims of the PhD thesis

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The main goal of my thesis is to determine the impact of (a)biotic stress pretreatment on transcriptomic and biological responses to herbivory. For this purpose, we decided to work with the wild black mustard *Brassica nigra*, a plant from the Brassicaceae family, in presence of different stresses. *B. nigra* is well studied for its responses to individual stresses in an ecological context. Also, the black mustard is more attractive than *Arabidopsis* for plant defense studies from an agricultural point of view (Broekgaarden *et al.*, 2010). To evaluate the transcriptomic response to *Pieris brassicae*, plants are challenged with larvae known to trigger the JA / ET pathway. Different pretreatments are applied before larval deposition, all chosen on the phytohormone pathway that they activate. Plants are first exposed to *P. brassicae* eggs (Bruessow *et al.*, 2010) and the piercing-sucking herbivore *Brevicoryne brassicae*, which mainly activate the SA pathway (Broekgaarden *et al.*, 2008). The necrotrophic bacteria *Xanthomonas campestris pv. campestris* are infiltrated on leaves to trigger SA, JA and ET pathways (Kunkel and Brooks, 2002). Finally, ozone, the most important atmospheric pollutant known to trigger foliar lesions such as chlorosis and necrosis, is used as a stress to induce both SA and JA / ET pathways (Baier *et al.*, 2005). Plants are pretreated by one of these stresses and then challenged with *P. brassicae* larval feeding. The aim of this experimental design is to analyze the impact of a first stress on further transcriptomic responses to *P. brassicae*. For that, whole-genome *Arabidopsis* CATMA microarrays (Sclep *et al.*, 2006) are used. Previous studies have shown that *Arabidopsis* microarrays can be successfully used to monitor transcriptional responses of *Brassica oleracea* or *B. nigra* (Broekgaarden *et al.*, 2011). Moreover, analyses of larval performance, JA and SA concentration, and glucosinolate content complement the transcriptomic profiles after each combination of dual stress. The long-term aim of this work is to identify specific genes involved in response to single or multiple attacks and to improve our knowledge on how plants modulate pathway crosstalk and BVOC biosynthesis and emission in the complex situation of contrasting attacks in nature.

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# Chapter I:

## Transcriptome analysis of *Brassica nigra* after single or simultaneous (a)biotic stresses

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**Abstract:** In nature and fields, plants have often to deal with multiple (a)biotic stresses at the same time. Currently, studies are focused on single (a)biotic stress responses and it is not well known how plants react to a combination of stresses at the molecular level. For example, in response to single attacks in *Arabidopsis thaliana*, jasmonic acid (JA) and ethylene (ET) signaling pathways are triggered after herbivorous and necrotrophic challenges whereas the salicylic acid (SA) pathway is triggered after biotrophic invasion. It is also known that these different pathways can interact with each other, a phenomenon named crosstalk, to create a flexible defense response to encountered stresses. We performed transcriptome analyses of the black mustard *Brassica nigra* to test the effect of different pretreatments on further responses to *Pieris brassicae* insect feeding. Our results show that plants pretreated by *P. brassicae* eggs, the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*), the aphid *Brevicoryne brassicae* or ozone before larval feeding have a transcription profile that is very similar to the profile observed during larval feeding alone. This combination of stresses appears thus to have a weak effect on the molecular responses to *P. brassicae* feeding. Moreover, SA / JA crosstalk does not seem to be active during combined stresses, except in case of the dual stress *Xcc* followed by *P. brassicae*. Furthermore, expression of genes involved in biosynthesis of glucosinolates (GS) and biogenic volatile organic compounds (BVOCs) were not affected by any treatments. Thus, our data indicate that a primary stress has little effect on transcriptional responses to a secondary stress in *B. nigra* and that defense compounds such as BVOCs and GS do not seem to be regulated at the transcriptional level.





## Introduction

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In nature, plants are constantly the target of numerous attackers such as herbivores and pathogens and, at the same time, they are exposed to many environmental changes. Plants possess direct and indirect defenses (Walters, 2011). Direct defenses are composed of constitutive and inducible defenses (Walters, 2011). Constitutive defenses are present throughout plant life, such as cuticle (Chassot and Métraux, 2006), trichomes (Levin, 1973) or toxic secondary metabolites (Walters, 2011), like for example glucosinolates in Brassicaceae (GS; Wittstock and Gershenzon, 2002; Walters, 2011; Buxdorf *et al.*, 2013). GS are nitrogen- and sulfur-containing thioglycosides released after tissue disruption and are toxic for insects (Redovnikovi *et al.*, 2008). Moreover, it is well documented that GS levels increase just after herbivory in all plant tissues, until eight weeks after the attacks (Brown *et al.*, 2003; Hopkins *et al.*, 2009). GS limit the performance of herbivores (Müller *et al.*, 2010) but also the growth of phytopathogens (Buxdorf *et al.*, 2013). Inducible defenses are specifically activated in response to invaders. Following recognition, specific phytohormone signaling pathways are involved. In *Arabidopsis thaliana*, jasmonic acid (JA) and ethylene (ET) signaling pathways are triggered after single attack by herbivorous and necrotrophic challenges, whereas the salicylic acid (SA) pathway is triggered after biotrophic invasion (Pieterse *et al.*, 2009) or *P. brassicae* oviposition (Bruessow *et al.*, 2010). Different defense signaling pathways trigger specific genes known to control herbivore damage and pathogen invasion (Table 1). These genes can be useful to detect the activation of specific responses. Three lipoxygenase genes *LOX2*, *LOX3*, and *LOX4* have been associated with the activation of the JA pathway. In addition, AOS protein is a key enzyme along this pathway. AOS is induced after JA application or wounding. At the final step, known JA-responsive genes are *VSP2* and *TERPENE SYNTHASE 4 (TPS4)*. For JA / ET signaling, *PDF1.2* represents a good marker for the simultaneous activation of these pathways. On the other hand, transduction of the SA signal requires *NPR1* for the activation of the main characterized SA-responsive gene *PR1* (Spoel *et al.*, 2003; Koornneef & Pieterse, 2008; Pieterse *et al.*, 2009; Leon-Reyes *et al.*, 2010; Walters, 2011; Santino *et al.*, 2013).

**Table 1: Examples of marker genes expressed as a result of JA, JA / ET and SA signaling pathways.**

Pathway	Genes
<b>JA</b>	Lipoxygenase (LOX2, LOX3, LOX4) Allene oxide synthase (AOS) Vegetative storage protein (VSP2) Terpene Synthase 4 (TPS4)
<b>JA / ET</b>	Plant Defensin 1.2 (PDF1.2)
<b>SA</b>	NonExpresser of PR genes 1 (NPR1) Pathogenesis-Related protein 1 (PR1)

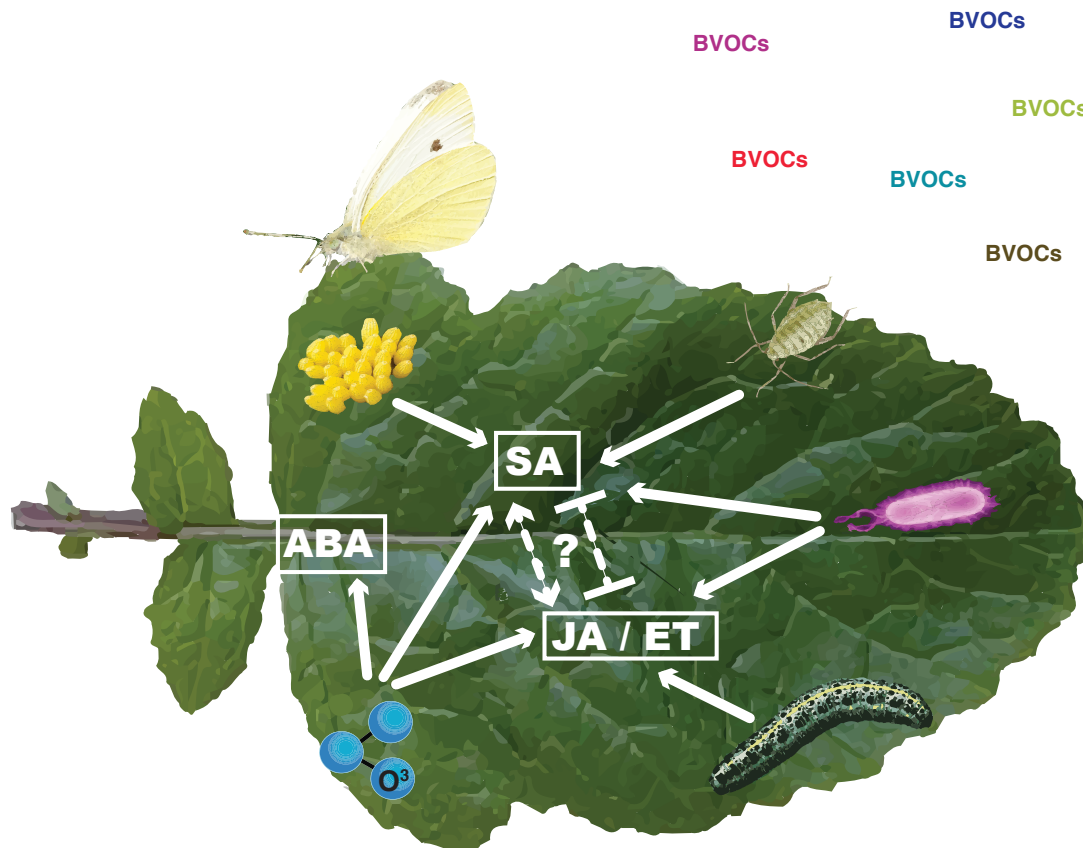
Furthermore, it is also known that these different pathways can interact with each other, a phenomenon called crosstalk, to create a flexible defense response to encountered stresses and, also, minimize time and energy costs (Reymond and Farmer, 1998; Pieterse *et al.*, 2009; Denancé *et al.*, 2013). Some pathogens and herbivores have evolved ways to manipulate SA and JA pathways and hijack plant defenses for their own benefits (Pieterse *et al.*, 2012). In addition, in *Arabidopsis*, the specialist *P. brassicae* and the generalist *Spodoptera littoralis* inhibit plant defenses independently from JA and SA pathways through their oral secretions (Consales *et al.*, 2012).

Indirect defenses are known to play a major, yet invisible, role in plant defense. Throughout all plant life, phytochemical molecules are emitted into the atmosphere for their own protection. These volatile molecules are named biogenic volatile organic compounds (BVOCs). For example, parasitic wasps specifically recognized the BVOCs blend emitted by attacked plants in nature and came to actively feed on larvae (Kessler and Baldwin, 2001). BVOCs regroup around 1800 molecules synthesized by three major pathways, the terpene, the oxylipin and the shikimic and benzoic acid pathways (Loreto and Schnitzler, 2010; Venkatesan, 2015). Currently, research focuses on the ecological impact and importance of BVOCs, but few studies have been conducted on the regulation of BVOC biosynthesis at molecular levels. Several reports indicate that SA, JA and ET signaling pathways play a role in the control of BVOC emission (Zhang *et al.*, 2009; Clavijo McCormick *et al.*, 2014; Wei *et al.*, 2014). However, mechanisms that control these responses and regulatory genes are not well characterized. Efforts have to be made on this issue to understand BVOC biosynthesis in the case of single and combined stresses.

Currently, studies are focused on single (a)biotic stress responses and it is not well known how plants react, on a molecular level, to a combination of stresses. Preliminary studies have shown that plant defenses are unpredictable in multiple stress contexts (Rizsky *et al.*, 2004; Suzuki *et al.*, 2014). For example, in ten *Arabidopsis* ecotypes challenged by multiple (a)biotic

stresses, 61 % of differentially genes were not affected in response to the respective single stress (Rasmussen *et al.*, 2013), whereas other studies have shown that plants prioritize against the most damaging stress. Arabidopsis plants challenged by both nematodes and drought responded mostly to drought (Atkinson *et al.*, 2013). Moreover, the effect of a previous exposure to *Botrytis cinerea* or to drought lightly changed the transcriptional response against *Pieris rapae* (Davila Olivas *et al.*, 2016; Coolen *et al.*, 2016). Plant defense against multiple stresses seems thus really complex and more studies are needed to understand factors that modulate these responses.

*Brassica nigra*, the black mustard, is an economically important crop species belonging to the *Brassica* genus, like Arabidopsis. *B. nigra* is well studied for its responses to individual stresses in an ecological context. Also, the black mustard is more attractive than Arabidopsis for plant defense studies from an agricultural point of view (Broekgaarden *et al.*, 2011). Here, we used whole-genome Arabidopsis CATMA microarrays (Sclep *et al.*, 2007) to assess plant defense expression changes in *B. nigra*. Previous studies have shown that whole-genome Arabidopsis microarrays can be successfully used to study transcriptional responses of *Brassica oleracea* or *B. nigra* (Broekgaarden *et al.*, 2011). Plants were exposed to herbivory by *P. brassicae* larvae, either alone or combined with one pretreatment, including *P. brassicae* egg extract, *Xcc*, *B. brassicae* or ozone. These stresses were chosen because they are known to activate diverse phytohormone pathways in Arabidopsis. Whereas larvae are known to activate JA and ET pathways, *Xcc* are shown to trigger all JA, ET and SA pathways; *B. brassicae* and eggs activate the SA pathway and finally, ozone is known to induce all JA, ET and SA but also ABA pathways (Figure 1; Kunkel and Brooks, 2002; Baier *et al.*, 2005; Broekgaarden *et al.*; 2008, Bruessow *et al.*, 2010).



**Figure 1: The different (a)biotic stresses used on *Brassica nigra* plants.**

Eggs of *Pieris brassicae* and *Brevicoryne brassicae* aphids trigger the SA pathway, *Pieris brassicae* larvae trigger JA / ET pathways, *Xanthomonas campestris* pv. *campestris* triggers both and ozone which triggers SA, JA / ET and ABA.

Dashed lines represented the crosstalk between SA and JA / ET pathways. BVOCs symbols represent volatile compounds emitted by the plant into the atmosphere.

The main goals of this study were to characterize the effect of pretreatment on *B. nigra* transcriptional responses to *P. brassicae* herbivory, to investigate how plants deal with multiple attackers and, furthermore, to increase our knowledge of how plants regulate genes important for BVOC biosynthesis.

# Materials and Methods

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## 1- Biological material

Seeds of *Brassica nigra* were collected from a wild population near Wageningen University in The Netherlands. Plants were grown in growth chambers in L : D 16 h : 8 h 22 - 25 °C, 60 % relative humidity, under white fluorescent light ( $170 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ ). Seeds were always stratified for 3 days at 4°C after sowing.

Bacterial strains of *Xanthomonas campestris* pv. *campestris* were obtained from the Plant-Microbe Interactions group of Utrecht University (Netherlands) in -80 °C glycerol stocks containing King B liquid medium: 20 g / l peptone bacterio, 1.5 g / l dipotassium hydrogen phosphate, 1.5 g / l magnesium sulphate heptahydrate, 12 g / l agar, at a final pH of 7.2. Antibiotic used was rifampycine at 25  $\mu\text{g}$  / ml. Bacteria were transferred into 10 ml of liquid culture in King B with antibiotic and grown in a shaker at 28 °C, 200 rpm, during 48 h. For infection by infiltration, *Xcc* culture was centrifuged at 7000 rpm during 2 min. The supernatant was discarded and the pellet washed in 10 mM  $\text{MgCl}_2$  before centrifugation at 7000 rpm during 2 min. The supernatant was discarded and the pellet diluted in 10 mM  $\text{MgCl}_2$  to an OD 600 of 0.07 to obtain a concentration of  $10^7$  cfu / ml in the leaf.

*Pieris brassicae* colony was reared in 1 m<sup>3</sup> cages in the greenhouse. Larvae were fed with *Brassica oleracea* and butterflies with sugar/water. Eggs were collected from the plants and crushed with a pestle in Eppendorf tubes. After centrifugation (15 000 g, 3 min), the supernatant (egg extract) was stored at -20 °C.

*Brevicoryne brassicae* aphids were reared in Wageningen University (Netherlands) and experiments were performed in Marcel Dicke's laboratory.

## 2- Plant treatments

Plants were five weeks old at the time of the treatment. For *P. brassicae* feeding, egg treatment *Xcc* infection, and combined stresses, plants were placed in transparent plastic boxes and kept in the same growth chambers than for growth. For *P. brassicae* feeding, 10, 20 or 30 first-instar larvae were placed evenly, just after hatching, on the three highest fully developed leaves during 24 h or 48 h. For measurements of mean eaten area, leaves were collected after 24 h or 48 h of insect feeding. Eaten areas were measured with ImageJ on photographs. During the same experiment, leaves of other plants, treated with the same conditions, were frozen in liquid

nitrogen for RNA extraction to determine the relative expression of herbivore-induced genes by qPCR. As controls, plants grown in the same conditions but without larvae were used.

For insect oviposition, plants were transferred to the greenhouse for 2 to 4 h during which butterflies were allowed to lay eggs. On average, *P. brassicae* butterflies laid 3 batches of approximately 30 eggs on the three highest leaves of the plant, the other leaves were covered by cloth during the oviposition to avoid egg deposition. Egg batches were kept for three days. As controls, plants grown in the same conditions but with no contact with butterflies were used. For egg extract treatment, we added different numbers of egg extract droplets (2  $\mu$ l) per leaf on the three highest leaves of each plant. Egg extract droplets were kept for three days. Untreated plants were used as controls.

For infection with *Xcc*, the three highest leaves were treated by three infiltrations per leaf of  $10^6$ ,  $10^7$  or  $10^8$  cfu / ml using a 1 ml needleless syringe. Each infiltration zone represented a circle of 1.5 cm<sup>2</sup>. Samples were harvested three days after infection. As controls, the same number of MgCl<sub>2</sub> infiltrations was done on the three highest leaves of each plant.

For natural oviposition or egg extract pretreatment followed by *P. brassicae* larval feeding, plants and exposed to three eggs batches or 12 x 2  $\mu$ l of egg extract on the three highest leaves of each plant during three days. A total of thirty freshly hatched larvae were then placed on treated leaves for 24 h. For *Xcc* pretreatment followed by *P. brassicae* larvae dual stress, plants were infected on the three highest leaves by three spots of  $10^7$  cfu/ml of bacteria during 3 days. A total of thirty freshly hatched larvae were then placed on treated leaves for 24 h.

All pretreatments with ozone followed by *P. brassicae* larvae were done in Kuppio University (Finland) as described previously by Khaling and coworkers (Khaling *et al.*, 2015). Each treatment was done on three different plants for each biological replicate.

Aphid infestation with *B. brassicae*, followed by *P. brassicae* larval treatments was done in a greenhouse in Wageningen University (Netherlands). Fifty or one hundred aphids were applied onto one fully developed leaf during two days. A total of thirty freshly hatched larvae were then placed on the treated leaves for 24 h. Each treatment was done on nine plants for each biological replicate.

All experiments were repeated a minimum of five times independently at intervals of several weeks. Treated and control leaves were frozen in liquid nitrogen for RNA extraction.

### 3- Transcriptomics - RNA Extraction

*B. nigra* leaves (3-6 g) were ground in liquid N<sub>2</sub> with mortar and pestle and transferred into 50 ml Falcon tube adding 6 ml of solution for precipitation at room temperature, prepared as follow: one volume of 2M Tris-HCL (pH 8.2), two volumes of 0.5M EDTA pH 8.0 (DEPC treated), one volume of 20 % SDS. 3 ml of phenol (saturated with 0.1M Tris-HCL, pH 8.2) were added and the solution

was stirred for 15 min. 3 ml of chloroform were added and the solution stirred for 10 min. Samples were centrifuged (4500 rpm, 15 min) and the upper phase was treated twice with 3 ml chloroform and centrifuged (4500 rpm, 15 min). 4 ml of aqueous phase were transferred to 30 ml disposable polypropylene tube, adding equal volume of ice-cold 6M LiCl (DEPC treated) and the solution precipitated overnight on ice at 4 °C. Samples were then centrifuged (9000 rpm, 20 min, 4 °C) and the pellet was re-suspended in 1 ml of DEPC-treated H<sub>2</sub>O, adding 0.1 ml 3M NaAcetate (pH 5.2) and 3.3 ml EtOH 100 %. The solution was left precipitating at room temperature for 60 min and later centrifuged (9000 rpm, 20 min at 16 °C). Samples were washed with 2 ml 70 % EtOH and centrifuged (9000 rpm, 5 min at 16 °C). The pellet was centrifuged again (9000 rpm, 2 min at 16 °C), and after removing the supernatant, the pellet was left drying 15 min and finally re-suspended in 100 µl DEPC-treated H<sub>2</sub>O. 150 µg of total RNA, in a volume of 100 µl of RNase-free H<sub>2</sub>O, were purified using RNeasy Mini Kit (Qiagen, N° 74104). Purified RNA was concentrated using Microcon centrifugal devices (Life Sciences, Nanosep, 30 K, Omega).

#### 4- Transcriptomics – Hybridization, Analysis and Data processing

For microarray analysis, 100 µg of total RNA was mixed with 2 µg of oligo dT21 (Microsynth) in a final volume of 13.4 µl. The solution was heated 5 min at 70 °C in a PCR machine and left 5 min at room temperature. RNAs were reverse transcribed using 13.6 µl of the following mix: 6 µl of SuperScript II buffer (Invitrogen), 3 µl of DTT 0.1 M, 0.6 µl of dNTPs (25 mM dATP, dGTP, dTTP; 10 mM dCTP; Promega), 2 µl of SuperScript II Reverse Transcriptase (Invitrogen) and 2 µl of RNase Inhibitor (Invitrogen), 3 µl of Cy3-dCTP or Cy5-dCTP (100 µM, GE Healthcare UK). After incubation at 42 °C for 1 h, sample tubes containing Cy3 and Cy5 labelling were mixed with 2.65 µl of EDTA 25 mM and 3.3 µl NaOH 1 M and incubated during 10 min at 65 °C. After addition of 3.3 µl of HCl 1 M and 5 µl of Tris-HCl pH 6.8 at 1 M, labeled probes were purified using a MinElute PCR purification kit (Qiagen, N°28004). Fluorophores incorporation was controlled using nanodrop (30 pmoles of each dyes were needed in total). Labeled probes were hybridized onto CATMAv4 microarrays containing 32,998 Arabidopsis gene-specific tags and gene-family tags (Sclep *et al.*, 2007) using the following steps. The slide with coverslip was prepared in chamber with 10-13 µl of SSC 3X per groove. Solutions were prepared for hybridization using the following mix: 18 µl of labeled probe, 45.35 µl of H<sub>2</sub>O, 8 µl SSC 20X, 8 µl of tRNA (2 µg / µl; Life Technology) and 0.65 µl of SDS 10X (fresh solution). The mix was heated 5 min at 65 °C, centrifuged for 30 s and quickly added to the slide. Slides were immersed overnight in a 50 °C bath. In an ozone-free room, slides were washed 5 min with 2X SSC + 0.5 % SDS at 50 °C, then twice 2 min in 0.5X SSC, and twice 2 min in 0.05X SSC. Slides were dried by centrifugation during 3 min at 2600 rpm. Microarrays scanning was done with an Agilent DNA microarray scanner (Agilent Technologies). Data normalization and statistical analyses were carried-out



using an interface developed at the University of Lausanne [Gene Expression Data Analysis Interface, GEDAI (Liechti *et al.*, 2010)]. To address the issue of multiple comparisons, we calculated an FDR (q-value) using the method developed by (Storey and Tibshirani, 2003). Because we employed Arabidopsis whole-genome microarrays to probe expression of *B. nigra* genes, the number of genes that produced hybridization signals was clearly low and overall hybridization signal intensity was also weaker than with Arabidopsis samples. We thus noticed that high FDR values are estimated when the number of induced genes is small. This is because the q-value is computed from the distribution of all P-values (Storey and Tibshirani, 2003). However, by comparing gene expression between experiments, genes with small P-value in response to one treatment often had a small P-value in another treatment. Thus comparing expression changes between experiments helps data interpretation, and FDR calculations might be too conservative in some cases. For data analysis, we thus used an unadjusted P-value of 0.05. FDR values are included in supplementary data for further evaluation.

### 5- Quantitative PCR

Total RNA was extracted from plant tissue using the ReliaPrep RNA tissue protocol (Promega). Five hundred nanograms of total RNA were transcribed to cDNA using M-MLV reverse transcriptase (Invitrogen) and oligo dT primers according to commercial instructions. cDNA synthesis was done in triplicates. RT-qPCR analysis was performed in a final volume of 25  $\mu$ l according to the Brilliant III Fast SYBR Green instruction manual (Agilent). Specific primers (Table 2) were designed on conserved sequences identified by multiple alignments of genes from different species of Brassica family. Sequences were found using the Brassica database ([www.brassicadb.org](http://www.brassicadb.org)). Each primer has a T<sub>m</sub> (melting temperature) of 60 °C and gives an amplicons length between 100 and 250 bp (base pairs) in the conserved part of the cDNA strand. The efficiency of each primer pair was calculated using a PCR product from a cDNA as a template. Five dilutions (0.1 pg /  $\mu$ l, 0.01 pg /  $\mu$ l, 1fg /  $\mu$ l, 0.1 fg /  $\mu$ l, 0.01 fg /  $\mu$ l) of the PCR product were done and a qPCR was performed. For each primer pair, a standard curve was designed from qPCR data in order to calculate the slope of the curve, and the efficiency was calculated with this formula:  $E=10^{(-1/\text{slope})}$ . For normalization, *SAND* gene was used as housekeeping gene.

**Table 2: Primers used for gene expression analysis by qPCR.**

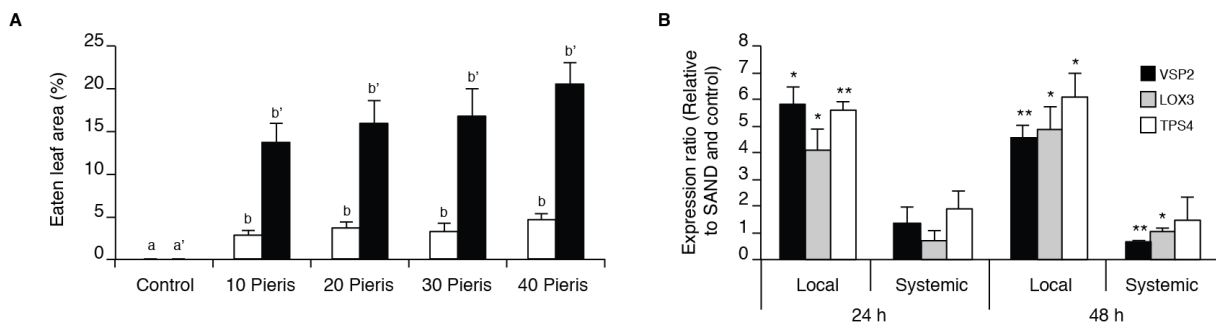
Involved in	Description	Gene Name	AGI	Efficacity	Primer seq 5'- 3'	Amplicon size
BVOCs GLV	Hydroperoxide lyase 1	HPL1	At4g15440	2.03	FW: TGGTGATGAGAGACGCTAAC RV: GTACCGGTTTGTGGACCATT	123 bp
BVOCs GLV	Terpene synthase 4	TPS04	At1g61120	2.00	FW: TTCCGTGCTGCTCAACATGA RV: GCCTTCTTAATGTCTTGAAGC	227 bp
JA	Jasmonate insensitive 1	MYC2	At1g32640	1.69	FW: GTGGAATCGAGCAAGAGGAA RV: ATCGTTAACCACCGACATACT	102 bp
JA	Vegetative storage protein	VSP2	At5g24770	1.85	FW: GGGAACGTAGCCGAACCTCTT RV: CGAAGTCCTTTGGCATAGAAA	200 bp
JA	Lipoxygenase	LOX3	At1g17420	1.97	FW: GCTCTCAACTTCGGACAGTA RV: ACCGCCATAAAGTTTCGACGT	156 bp
SA	Pathogenesis related gene 1	PR1	At2g14610	1.96	FW: ATGCAGTGAATGAGACGCT RV: TACACCTCGCTTTGCCACAT	249 bp
Housekeeping gene	SAND family protein	SAND	At2g28390	1.90	FW: TGCTTGGAGGGACAGATGC RV: AACCTTGTGCTGCACATTAG	247 bp

## Results

### 1- Set-up conditions

#### a. Set-up conditions for *Pieris brassicae* feeding on *Brassica nigra*.

First, we needed to establish the experimental set-up for insect challenge. First-instar larvae were placed on the three biggest leaves of 5 week-old plants during 24 and 48 hours (h). Half of the treated plants were used for measurement of mean area consumption. Leaves from other treated and control plants were frozen in liquid nitrogen for RNA extraction and qPCR analysis of herbivore-induced genes. Different numbers of first-instar larvae per plant were tested (Figure 2 A). With 10, 20, 30 or 40 larvae per plant, the mean area consumption was not significantly different, a little less than 5 % at 24 h and around 10 % at 48 h. At 30 larvae per plant, selected herbivore-induced genes (*VSP2*, *LOX3* and *TPS4*; Bruessow *et al.*, 2010) were significantly up-regulated in comparison to control plants at 24 and 48 h (Figure 2 B). Thus, the damage caused by 30 first instar larvae on *B. nigra* is sufficient to induce known marker genes for herbivory already after 24 h and *P. brassicae* damage was evenly distributed at this density.



**Figure 2: Effect of *P. brassicae* larvae density on plant defense.**

A- Eaten leaf area (%) after 24 (white bars) or 48 hours (black bars) of 10, 20, 30 or 40 1<sup>st</sup> instar larvae. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test)

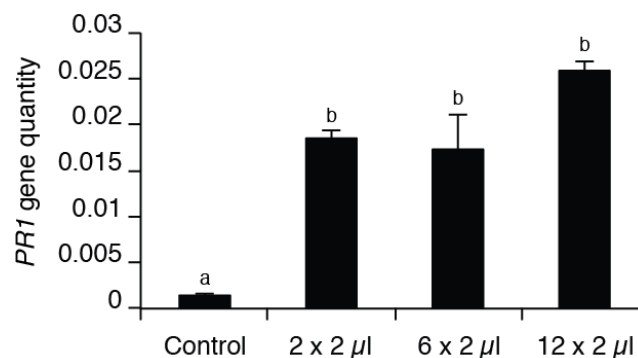
B- Relative expression ratio of herbivore-induced genes, *VSP2* (At5g24770), *LOX3* (AT1g17420) and *TPS4* (AT1g61120) by quantitative PCR. Leaves were treated with 30 1<sup>st</sup> instar larvae during 24 or 48 hours before RNA extraction. Expression levels were normalized with respect to the housekeeping gene *SAND* (At2g28390). Statistical differences between control and herbivore challenged plants are indicated (Student's *t*-test, \* P-value < 0.05; \*\* P-value < 0.01).

For all figures, data bars represent the mean ( $\pm$  SE, standard error) of three biological replicates.

For the following experiments, plants were always treated with 30 first-instar larvae during 24 hours on the three biggest developed leaves.

### b. Set-up conditions for *P. brassicae* egg extract treatment.

To test the effect of oviposition, different amounts of egg extract were applied onto *B. nigra* leaves. The plant response was followed by measuring the expression of *PR1* after 72 h of treatment. It is well established in Arabidopsis that plant response to egg treatment is at the maximum after 72 h for the marker gene *PR1* (Gouhier-Darimont *et al.*, 2013). *PR1* expression was measured by qPCR in treated and untreated plants. In five week-old *B. nigra* plants, *PR1* was clearly induced after application of 2 to 12 droplets of 2  $\mu$ l of *P. brassicae* egg extract per leaf (Figure 3). In order to apply a treatment that mimicked natural oviposition, we added 12 x 2  $\mu$ l of egg extract during 72 hours for all subsequent experiments.

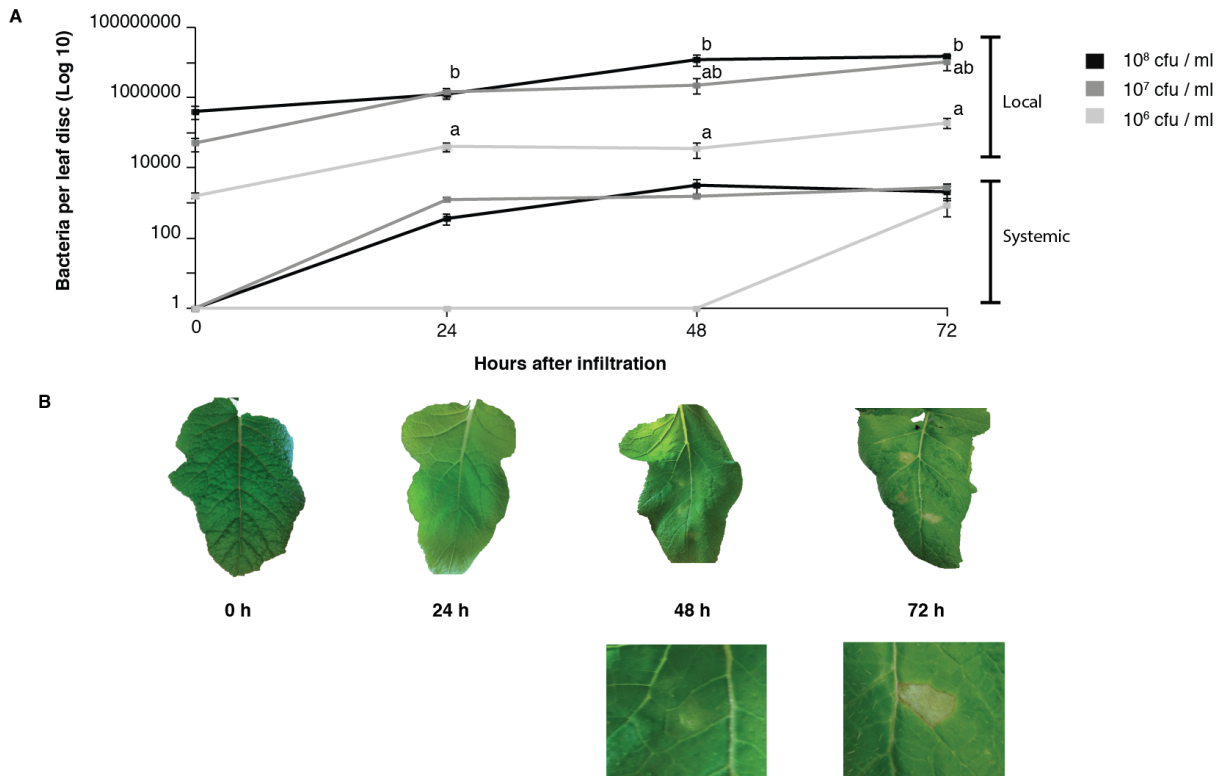


**Figure 3: Effect of egg extract amounts on defense gene expression.**

*PR1* (At2g14610) expression was measured by quantitative PCR. Leaves were treated with different volumes of *P. brassicae* egg extract during 72 hours before RNA extraction. Expression levels were normalized with respect to the housekeeping gene *SAND* (At2g28390). Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). Data bars represent the mean ( $\pm$  SE) of three biological replicates.

### c. Set-up conditions for *Xcc* treatment

To test the effect of *Xcc* on *B. nigra*, different concentrations of infiltrated bacteria were tested per leaf, from  $10^6$  to  $10^8$  cfu / ml. Concentrations of  $10^7$  and  $10^8$  cfu / ml were really close in terms of bacterial growth in local and systemic tissue. On the contrary, at  $10^6$  cfu / ml the bacterial growth was slower especially in systemic leaves (Figure 4 A). Due to a higher variability between samples in systemic tissue, no statistical difference was observed between concentrations. At  $10^7$  cfu / ml symptoms were visible from 48 h after infiltration (Figure 4 B). To be consistent between experiments, we performed 3 infiltrations of *Xcc* at  $10^7$  cfu / ml per leaf to obtain the same treated surface than with egg extract, in the total of 3 leaves per plant.



**Figure 4: Growth of *Xanthomonas campestris* pv. *campestris*.**

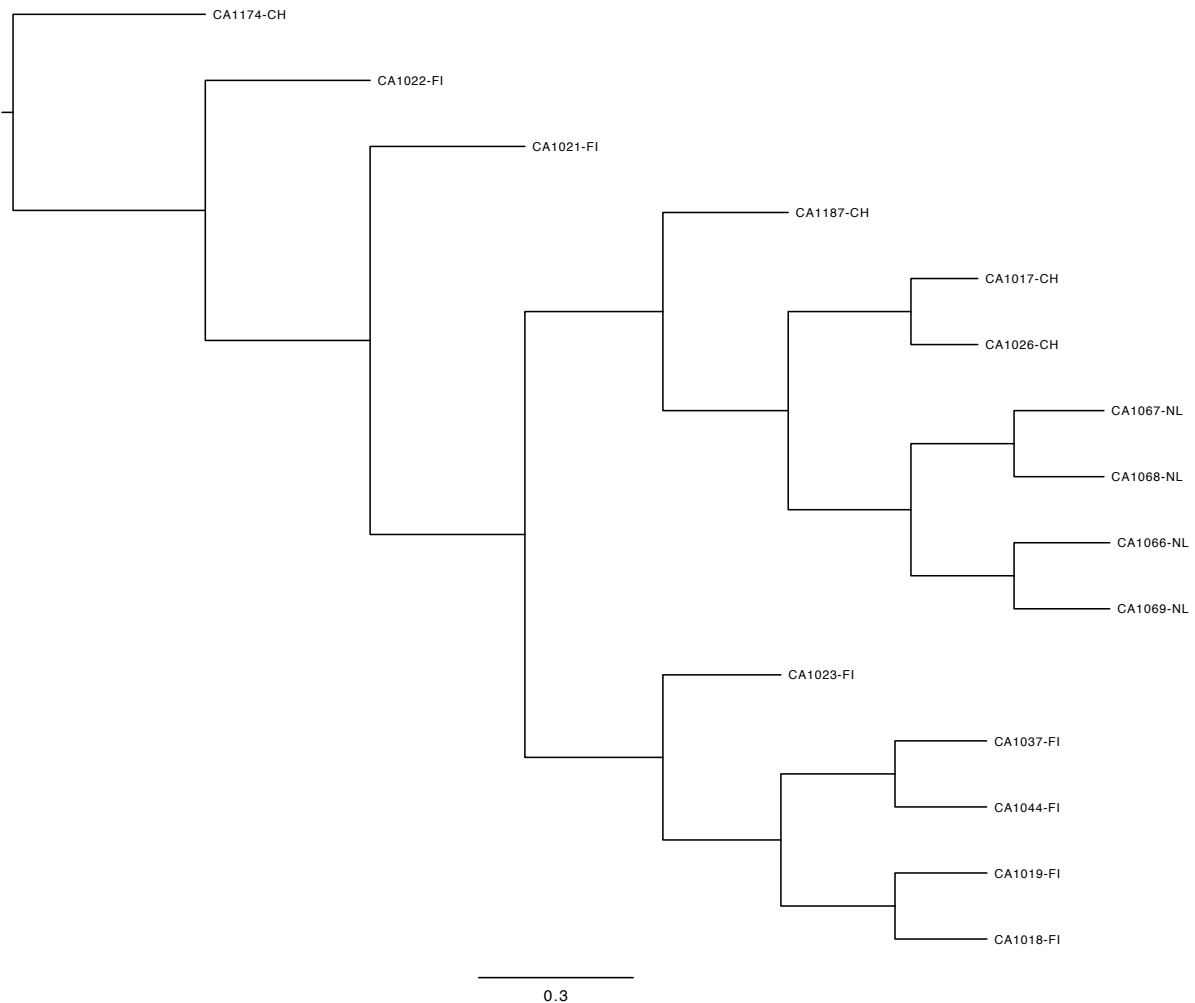
- A- Quantification of bacterial growth after infiltration of  $10^6$  (grey lines),  $10^7$  (dark-grey lines) and  $10^8$  (black lines) cfu / ml, in local and systemic leaves. Significant differences were visualized by different letters at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). Data bars represent the mean ( $\pm$  SE) of three biological replicates.
- B- Pictures at different hours after infiltration of bacteria at  $10^7$  cfu / ml.

## 2- Transcriptome analysis after a single stress

### a. Transcriptome analysis of plants challenged with *P. brassicae* larvae.

Using CATMAv4 Arabidopsis full-genome microarrays, we analyzed the transcriptional response of *B. nigra* plants to 30 first-instar *P. brassicae* larvae feeding during 24 h. We collected and analyzed *P. brassicae* feeding samples from three different laboratories, from Wageningen University, The Netherlands (NL), from Kuppio University, Finland (FI) and from our place, in Switzerland (CH). Experiments were realized with exactly the same set-up conditions. A clustering analysis was performed on all replicates from the three teams for the single herbivory treatment. Each group performed at least 5 biological replicates. Inside each team, outlier experiments were discarded. Then, a transcriptome clustering, using Pearson correlation, was done by MultiExperiment Viewer software, with 4 replicates from Switzerland, 4 replicates from The Netherlands and 7 replicates from Finland. The clustering was represented by FigTree software using a rooted tree (Figure 5). Five replicates from Finland created a distinct group from

other replicates. In order to create a robust list of genes affected by *P. brassicae* feeding we decided to pool only replicates from The Netherlands and Switzerland.



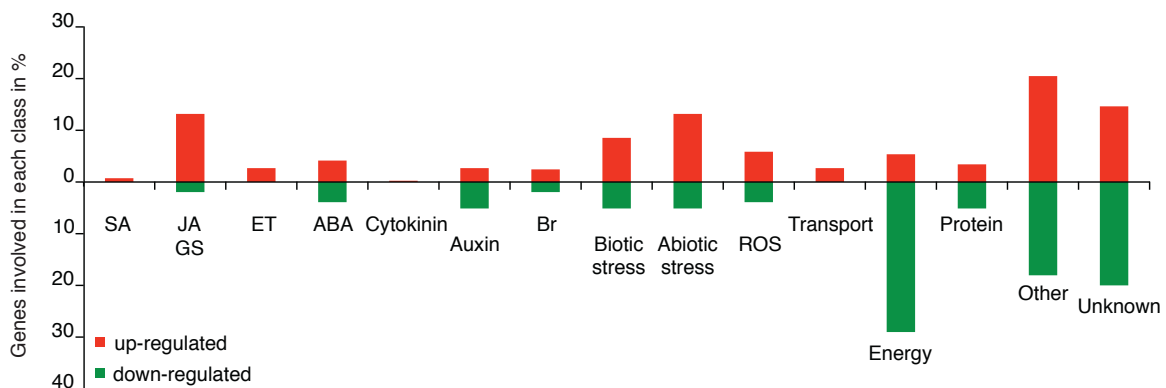
**Figure 5: Transcriptome clustering of all *P. brassicae* feeding experiments.**

Correspondance analysis of expression profiles including all induced or repressed genes ( $\log_2$  ratio  $> 0.585$ , P-value  $< 0.05$ ;  $\log_2 < -0.585$ , P-value  $< 0.05$ ). Clustering and node length calculations were performed with MultiExperiment Viewer 4.8.1 and represented as tree in FigTree 1.4.2. The CA number represent the name of each microarray slide used for each replicate, following by the country abbreviations, CH : Switzerland, FI : Finland, NL : Netherlands.

From this list of replicates, we identified 210 induced and 48 repressed genes ( $\log_2 > 0.585$ , P-value  $< 0.05$ ;  $\log_2 < -0.585$ , P-value  $< 0.05$ ) after 24 hours of herbivory. Using gene ontology (GO) biological processes, we defined different classes in which genes are involved: the SA pathway, JA pathway and GS biosynthesis, ET, ABA, Cytokinin, Auxin and Brassinosteroids (Br) pathways, general plant defense against biotic or abiotic stress with no specific pathway triggered, reactive oxygen species response, transport, energy which regroups photosynthesis,

calvin cycle and growth activity, a class for genes important for protein biosynthesis and activity, unknown function or others that regroup metabolism processes, DNA regulation, cell modification or growth. For each gene analyzed a category was chose depending on information retrieved from the literature.

After 24 hours of feeding by *P. brassicae* on *B. nigra*, more than 50 % of the up-regulated genes are specific to plant defense. Among these 210 up-regulated genes, 18 % are known to be specific to insect feeding and are linked to the JA pathway or glucosinolates (GS) biosynthesis. It is well known that GS are secondary metabolites that accumulate after wounding or herbivory attack (Reymond *et al.*, 2004). These JA and GS classes include the highest number of genes compared to other classes of phytohormone pathways (Figure 6) and most have the highest expression ratio. For example, *LOX3* (At1g17420) and *VSP2* (At5g24770), which are typical JA marker genes, have an expression ratio ( $\log_2$ ) of 2.31 and 1.88 respectively and *BCAT4* (At3g19710), a gene important in GS biosynthesis, has an expression ratio of 1.47 (Annex 1). Moreover, most of the down-regulated genes, 30 %, are related to the GO class "energy". Many genes involved in photosynthesis are down-regulated by herbivory.



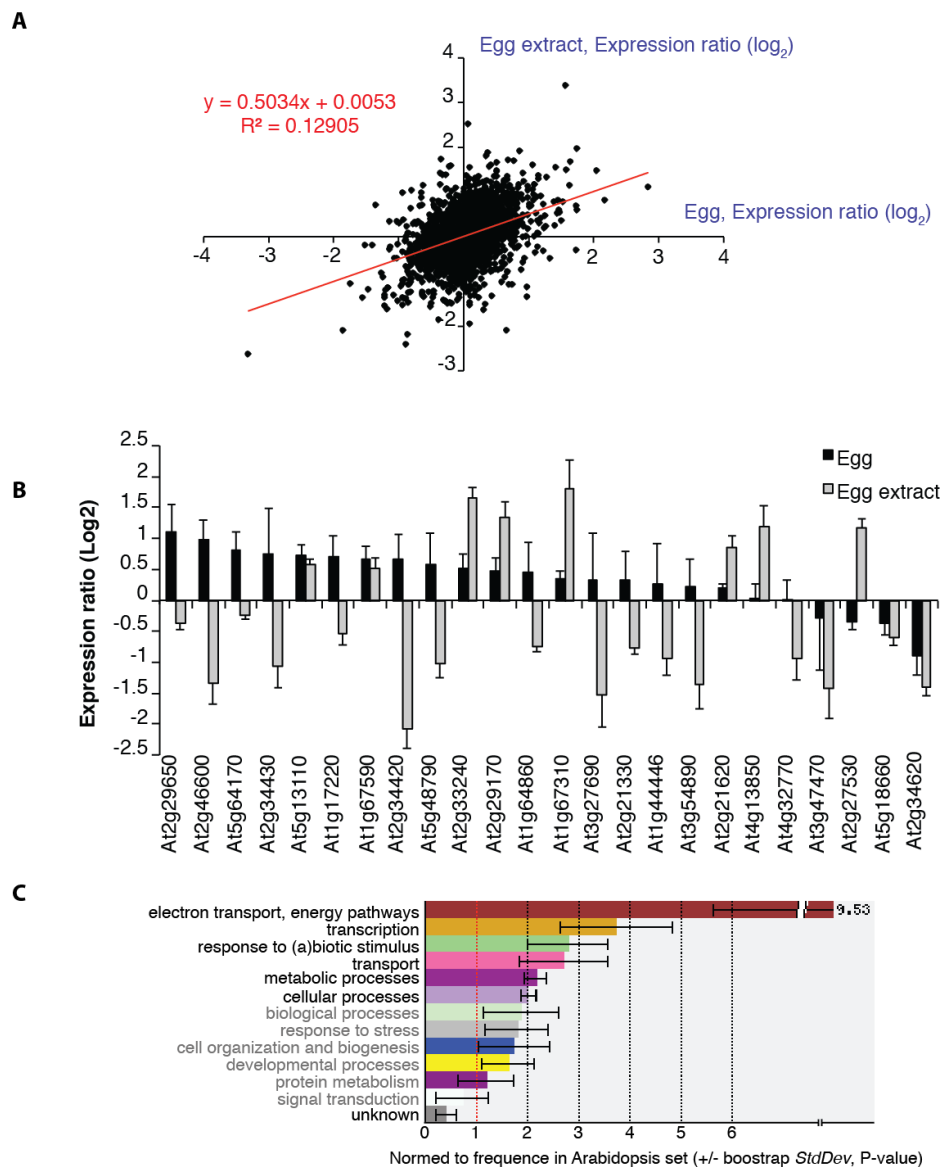
**Figure 6: Repartition of up- and down-regulated genes after *P. brassicae* feeding.**

Transcriptome analysis of *B. nigra* after *P. brassicae* feeding during 24 hours comprised 210 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 48 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

#### **b. Transcriptome analysis of plants treated with *P. brassicae* eggs.**

Transcriptional responses of *B. nigra* plants to eggs of *P. brassicae* during 72 hours were analyzed. We compared two types of egg treatment: natural oviposition or egg extract droplets. For both experiments, treatments were done on the three highest leaves of at least three plants. Gene expression ratios for the transcriptome were plotted for these two experiments (Figure 7 A).

The correlation coefficient is low, 0.13, but only 24 genes are significantly different between oviposition and egg extract treatment (Figure 7 B). Analysis of the GO class of these 24 genes by Classification SuperViewer website, revealed that most of them are important for electron transport and energy pathways ([http://bar.utoronto.ca/ntools/cgi-bin/ntools\\_classification\\_superviewer.cgi](http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi)). Most of them are not involved in plant defense (Figure 7 C).



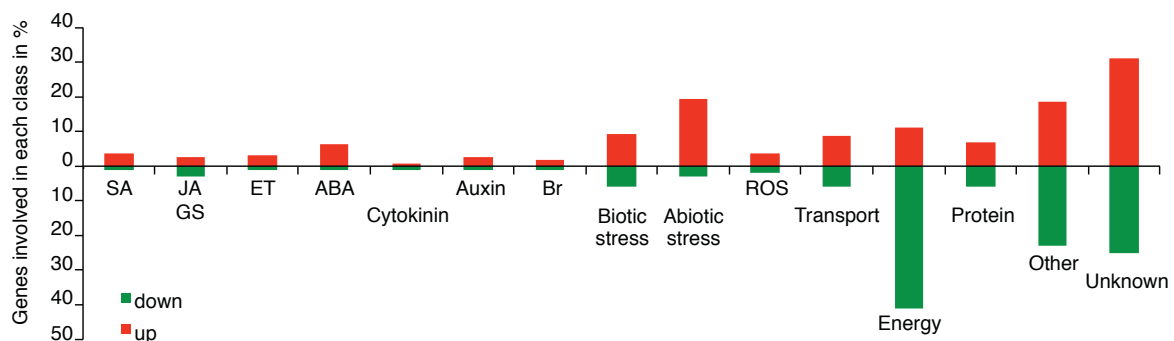
**Figure 7: Transcriptome analysis of plants treated by *P. brassicae* eggs or egg extract during 3 days.**

- A- Comparison of mean expression ratio between egg extract and eggs treatments. Each experiment was replicated five times.
- B- Genes with expression ratios that are significantly different (Student's *t*-test, P-value < 0.05) between eggs and egg extract treatments.
- C- Classification by GO of the 24 genes significantly different between eggs and egg extract treatments, using Classification SuperViewer of the Bio-Analytic Resource for Plant Biology. Biological process class with a P-value > 0.05 are represented in grey or in black with a P-value < 0.05.



Transcriptomic signature between natural oviposition or egg extract treatment is thus close. For example, *PR1* (At2g14610) expression ratios ( $\log_2$ ) were equal to 1.64 after oviposition and to 1.68 after egg extract deposition. We conclude that *B. nigra* response to oviposition or egg extract deposition is comparable at transcriptional level. For the following experiments, we used egg extract treatment as a surrogate for real oviposition method.

Transcriptome analysis of egg extract treatment during 3 days on 5 week-old plants revealed that 149 genes were up-regulated and 138 down-regulated. Analysis of the GO class of induced genes after egg extract deposition indicated that SA and ABA pathways are the most represented phytohormone pathways groups, with respectively 6 % and 10 % of all up-regulated genes. Many genes are also involved in plant defense, mostly against abiotic stress that represented 19 % of all induced genes. On the contrary 41 % of all repressed genes are important for photosynthesis and electron transport (Figure 8). Like after herbivory, eggs of *P. brassicae* have an impact on photosynthesis. Surprisingly, SA-marker genes were lightly induced in our set-up conditions. We expected a stronger response triggered by this pathway, known to be important in *Arabidopsis* against eggs of the Large White butterfly (Bruessow *et al.*, 2010).

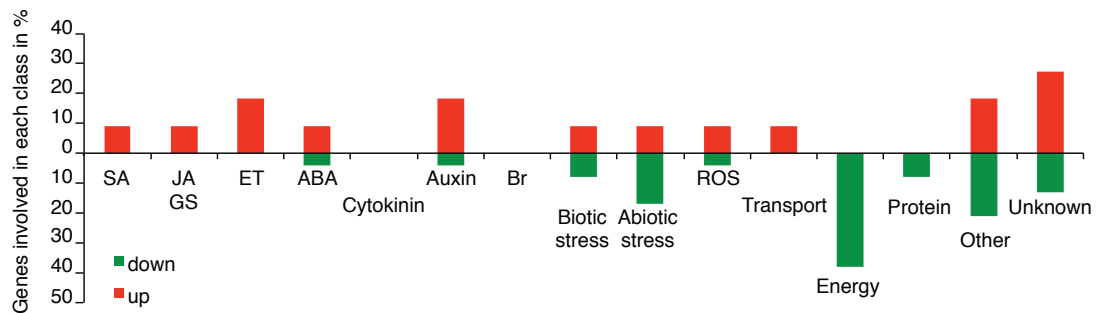


**Figure 8: Repartition of up and down-regulated genes after *P. brassicae* egg extract treatment.** Transcriptome analysis of *B. nigra* after *P. brassicae* egg extract treatment during three days comprised 149 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 138 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

### c. Transcriptome analysis of plants treated with *Xcc*.

Five week-old *B. nigra* plants were infiltrated with *Xcc* during 72 hours. We identified 11 induced genes and 21 repressed genes. Investigation of GO class from each of these genes revealed that together SA, JA and ET pathways are involved in plant defense against *Xcc* in the black mustard, similar to *Arabidopsis* challenged with *X. campestris* pv. *armoraciae* (Ton *et al.*, 2002). Indeed, SA represented 9 % of the total of induced genes, JA 9 %, no gene involved on GS biosynthesis were activated after this treatment and ET 18 %. Interestingly, bacteria treatment activated the

auxin pathway and regrouped 18 % of induced genes. Moreover, genes important for ROS production (9 %) and transport (9 %) were induced after 3 days of *Xcc* treatment. Finally, like for the other stresses, photosynthesis and electron transport were repressed and represented around 40 % of the down-regulated genes (Figure 9).

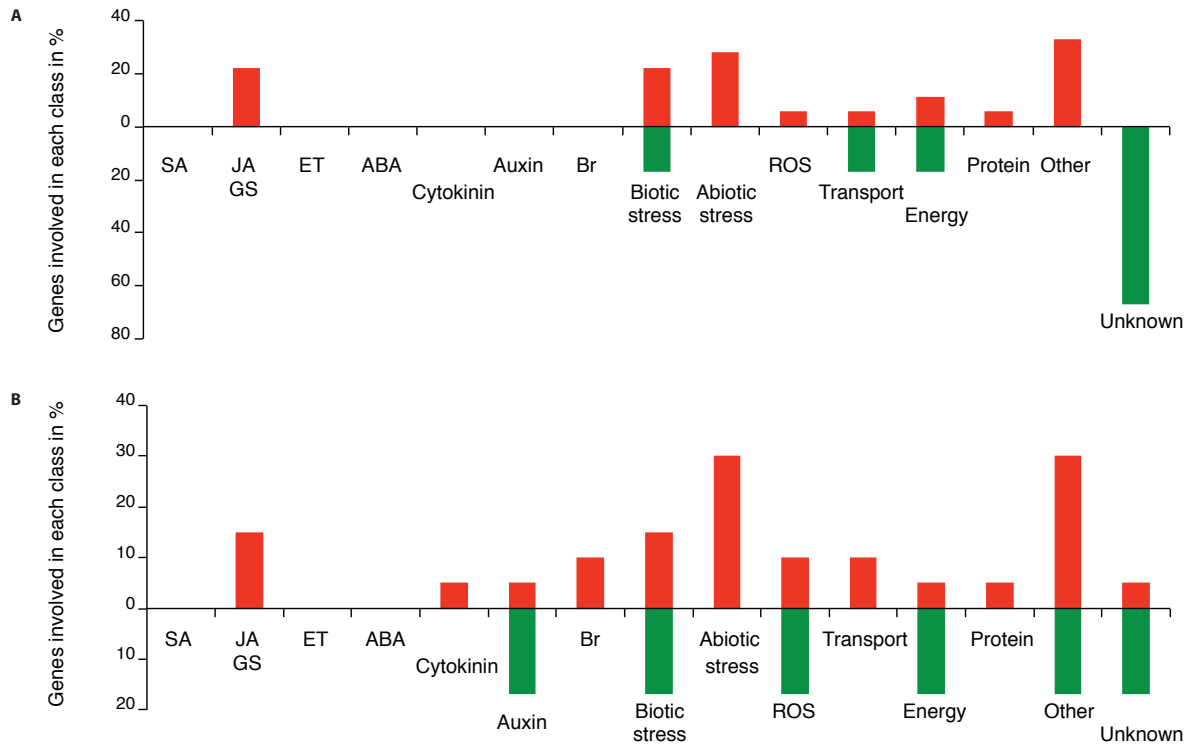


**Figure 9: Repartition of up- and down-regulated genes after infection with *Xcc*.**

Transcriptome analysis of *B. nigra* after *Xantomonas campestris* pv. *campestris* treatment during three days comprised 11 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 21 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

#### d. Transcriptome analysis of plants treated with *B. brassicae*.

*B. nigra* plants were challenged with the aphid *B. brassicae* during 2 days. Surprisingly, few genes were differentially expressed. We identified 18 up-regulated genes and 6 down-regulated genes after a treatment with 50 aphids and 19 up-regulated genes and 6 down-regulated genes with 100 aphids. Analysis of the GO class of the induced and repressed genes revealed that the JA pathway was the major pathway triggered by aphid feeding on *B. nigra* plants with 22 % and 15 % of the genes in this category after challenge with 50 or 100 aphids, respectively (Figure 10 A and B). Unexpectedly, we did not see an increase of SA-signaling genes by *B. brassicae*, as demonstrated previously by Mewis and coworkers (Mewis *et al.*, 2006).

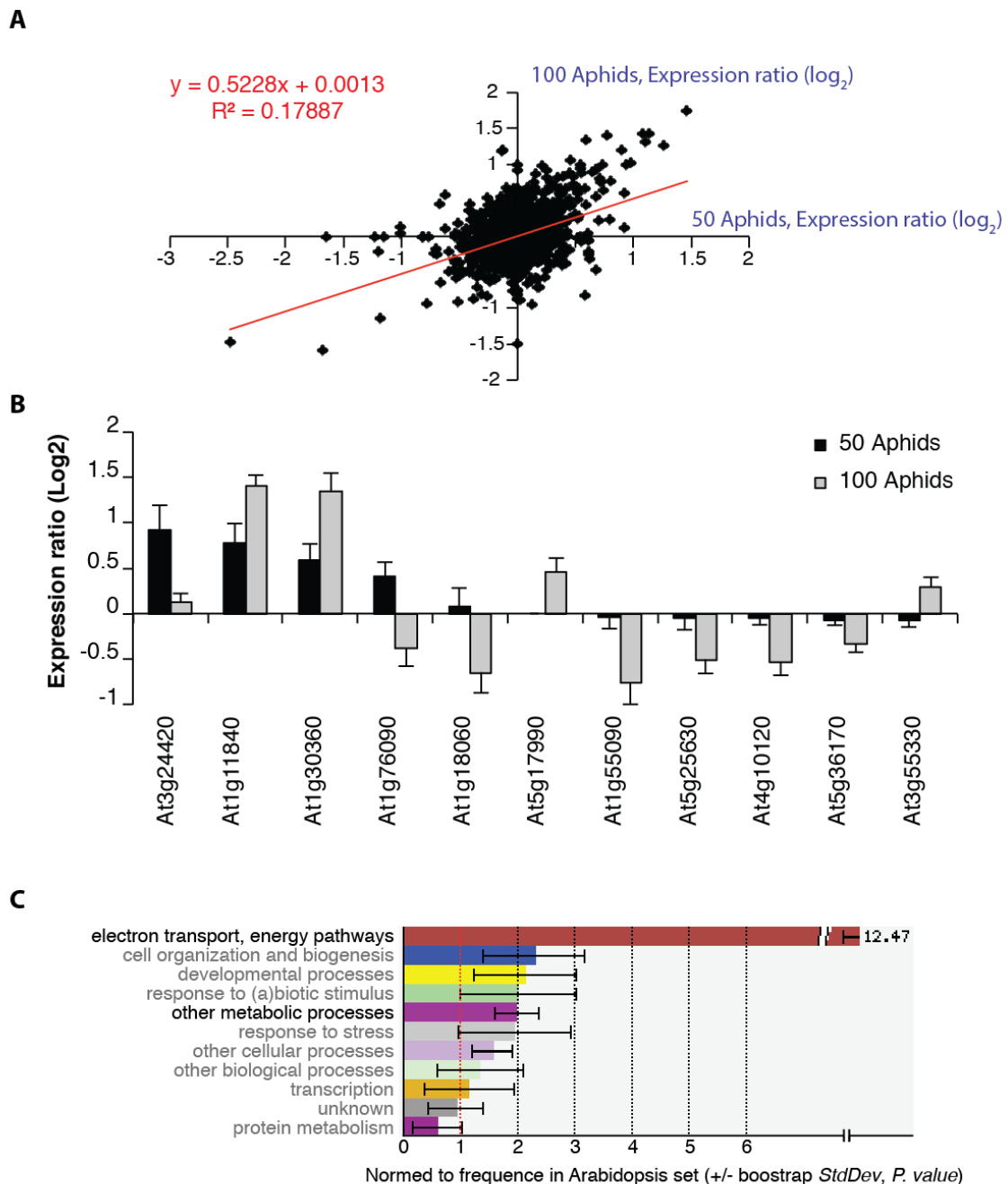


**Figure 10: Repartition of up- and down-regulated genes after *B. brassicae* treatment.**

- A- Transcriptome analysis of *B. nigra* after 50 *B. brassicae* aphids feeding during two days comprised 18 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 6 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ .
- B- Transcriptome analysis of *B. nigra* after 100 *B. brassicae* aphids feeding during two days comprised 19 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 6 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ .

Each gene was classified in a GO class based on information retrieved from the literature.

Gene expression ratios for the transcriptome were plotted for the two aphid density experiments (Figure 11 A). The correlation coefficient was low, 0.18, but only 11 genes were significantly different between 50 or 100 *B. brassicae* treatments (Figure 11 B). Analysis of the GO class of these 11 genes by Classification SuperViewer revealed that most of them are important for electron transport and energy pathways or for not specified metabolic processes (Figure 11 C). Furthermore, gene expression ratios ( $\log_2$ ) between both experiments were equal for the highest JA-induced marker genes: *LOX3* (At1g17420) ratio was 1.47 after treatment of 50 aphids and 1.74 after 100 *B. brassicae* and *LOX2* (At3g45140) ratio was 0.94 and 1.00, respectively (Annex 1). Thus, the application of 50 or 100 aphids in *B. nigra* affected the expression of a small number of genes, but the patterns were similar with, surprisingly, mainly an increase of the JA pathway.

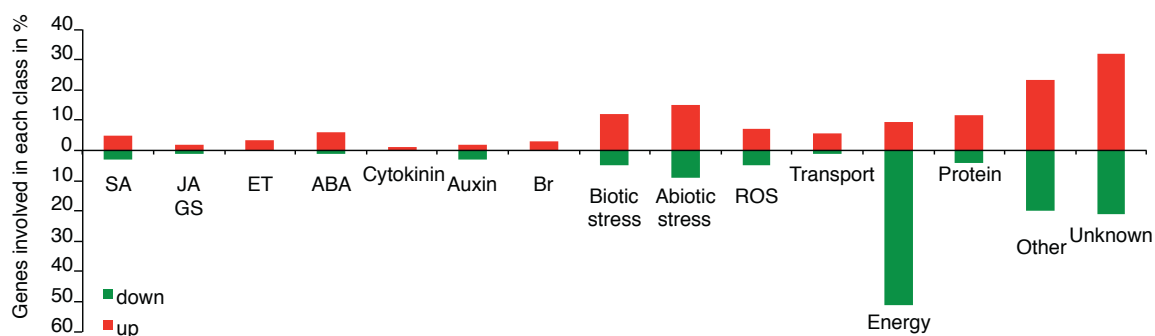


**Figure 11: Transcriptome analysis of plants treated by 50 or 100 *B. brassicae* during 2 days.**

- A- Comparison of gene expression ratio means between 50 and 100 aphids treatments. Each experiment was reproduced five times.
- B- Genes with expression ratios that are significantly different (Student's *t*-test, P-value < 0.05) between 50 and 100 aphids treatments.
- C- Classification by GO of the 11 genes significantly different between 50 and 100 aphids treatments, using classification SuperViewer of the Bio-Analytic Resource for Plant Biology. Biological process class with a P-value > 0.05 are represented in grey or in black with a P-value < 0.05.

### e. Transcriptome analysis of plants treated with ozone.

Plants were subjected to ozone exposure at 70 ppb during 5 days. We identified 154 up-regulated genes and 59 down-regulated genes. Our GO classification revealed that ozone indistinctly activated all SA, JA, ET, and ABA pathways. However, SA and ABA contained the largest group of induced genes with 5 % and 6 % respectively of all up-regulated genes. These observations validate studies that demonstrated that plant defense to ozone involve SA, JA, ET and ABA pathways together (Baier *et al.*, 2005). Moreover, the class regrouping genes important for protein biosynthesis contained 12 % of all induced genes. Furthermore, the GO class analysis revealed that genes important for ROS production (7 %) and transport (6 %) were also induced by ozone exposure. Finally, the class named energy was the one that contained 50 % of the repressed genes (Figure 12; Annex 2).



**Figure 12: Repartition of up- and down-regulated genes after ozone exposure.**

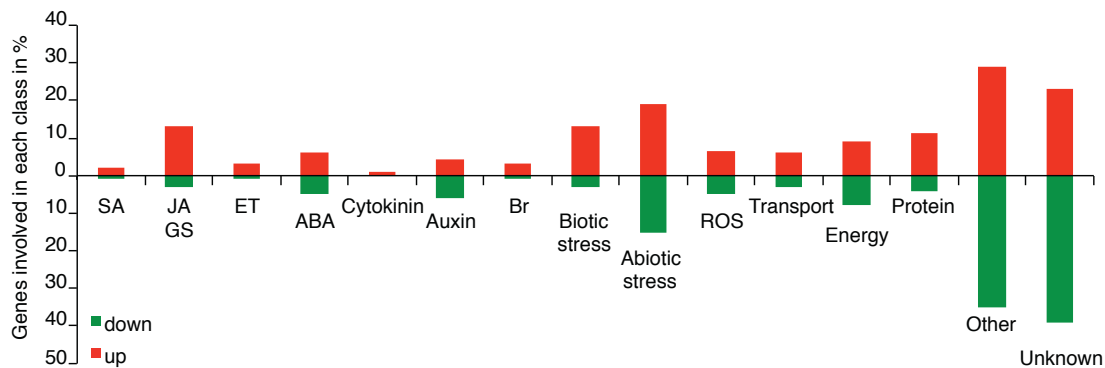
Transcriptome analysis of *B. nigra* after ozone treatment during five days comprised 154 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 59 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

### 3- Transcriptome analysis after a dual stress

#### a. Transcriptome analysis of plants treated with *P. brassicae* egg extract and *P. brassicae* larvae

The transcriptome of *B. nigra* pretreated with egg extract during 3 days followed by *P. brassicae* larval feeding during 24 hours was analyzed. We identified 167 up-regulated genes and 75 down-regulated genes. From all induced genes, 13 % belonged to JA and GS GO classes, including 21 up-regulated genes specific to the JA pathway and 2 involved in GS biosynthesis. After a dual stress, JA was the main induced pathway as the SA group contained only 2 % of the total of up-regulated genes. Genes important for biotic and abiotic responses were also induced, with 13 and 19 % respectively. Interestingly, genes important for photosynthesis or electron transport were

not predominantly represented in down-regulated genes, contrary to what was observed for single stresses. Most down-regulated genes were grouped in abiotic stress (15 %), unknown (23 %) and other (30 %) GO classes (Figure 13).

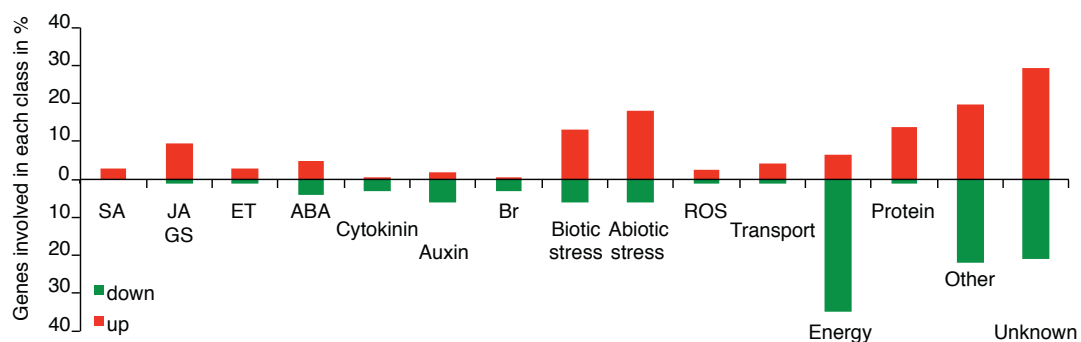


**Figure 13: Repartition of up- and down-regulated genes after the dual stress, egg extract pretreatment followed by *P. brassicae* larvae.**

Transcriptome analysis of *B. nigra* after the dual stress egg extract / *P. brassicae* comprised 167 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 75 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

#### b. Transcriptome analysis of plants treated with *Xcc* and *P. brassicae* larvae.

Next, we analyzed *B. nigra* transcriptome after pretreatment with *Xcc* during 3 days followed by one day of feeding by *P. brassicae*.



**Figure 14: Repartition of up- and down-regulated genes after the dual stress, *Xcc* pretreatment followed by *P. brassicae* feeding.**

Transcriptome analysis of *B. nigra* after the dual stress *Xcc* / *P. brassicae* comprised 164 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 68 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

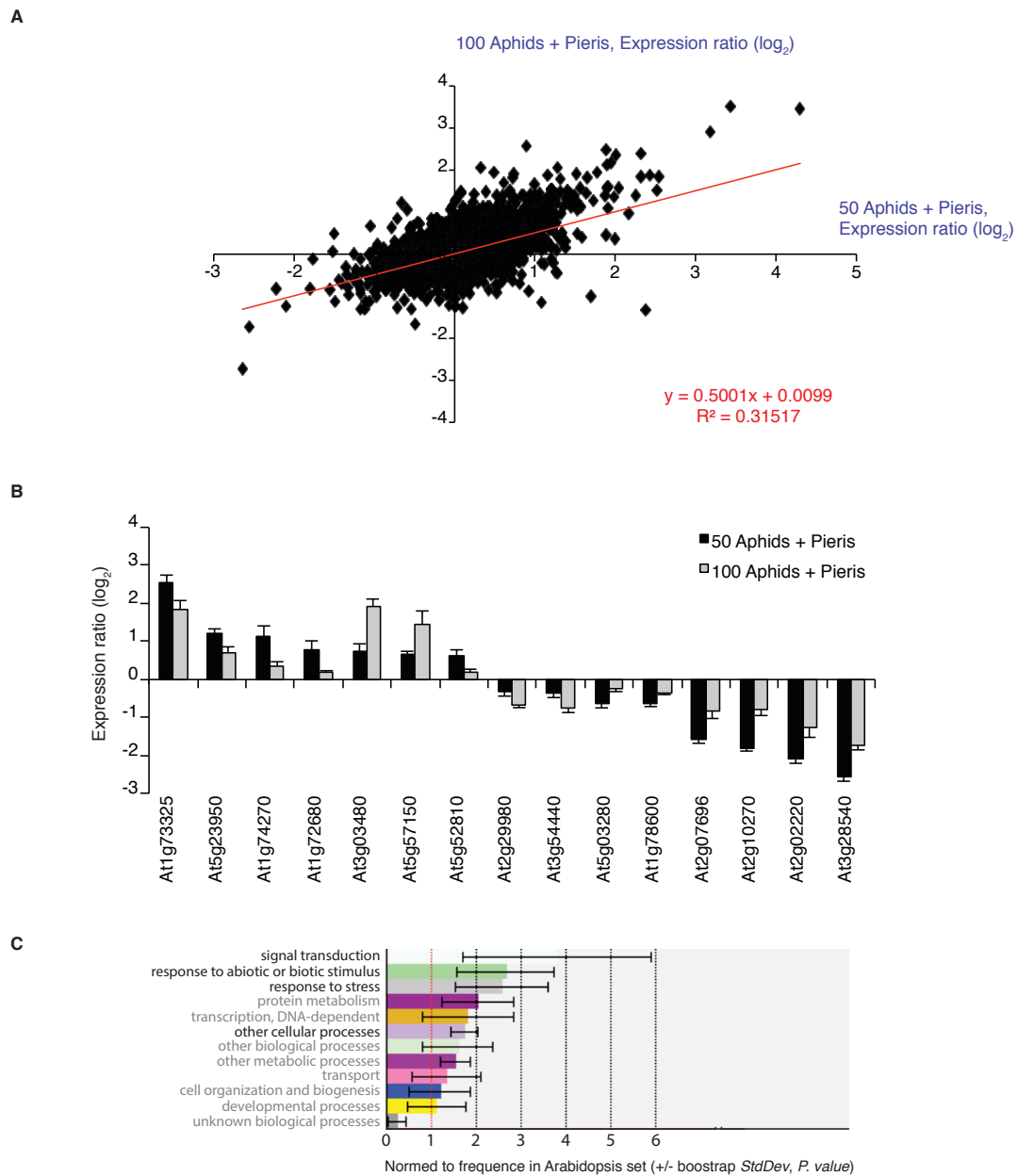
A total of 164 induced genes and 68 repressed genes were observed. Interestingly, the number of differentially regulated genes was higher during this dual stress than the one observed after

*Xcc* treatment alone, which comprised only 11 up-regulated and 21 down-regulated genes. GO class repartition of up-regulated genes showed that JA and GS marker genes were the most represented in comparison to other phytohormone pathways, with 10 % of all induced genes including 13 genes specific to the JA pathway and 3 to GS biosynthesis. A large part of up-regulated genes were allocated to biotic and abiotic stress groups with 13 % and 18 % respectively. 35 % of down-regulated genes were grouped in the “energy” GO class (Figure 14).

### **c. Transcriptome analysis of plants treated with *B. brassicae* aphids and *P. brassicae* larvae**

*B. nigra* transcriptome after 50 or 100 aphid pretreatment during 2 days followed by *P. brassicae* feeding during 24 hours was analyzed. In the case of 50 aphids / *P. brassicae* dual stress, there were 241 up-regulated genes and 96 down-regulated genes. For 100 aphids / *P. brassicae* dual stress, we totalized 232 induced genes and 72 repressed genes. Expression ratios between the 2 aphids densities were plotted against each other (Figure 15 A). The correlation coefficient is low (0.32) but comparison between the transcriptome of 50 or 100 aphids / *P. brassicae* dual stress revealed that only 15 genes were significantly differentially expressed between the two densities (Figure 15 B). Analysis by Classification SuperViewer showed that most of the 15 differentially expressed genes between aphids density were important for signal transduction. A few of them were also involved in response to stress (Figure 15 C). Moreover, comparison of gene expression for highly induced genes showed a similar induction between the two aphid densities. For example *LOX3* (At1g17420), expression ratio ( $\log_2$ ) was 3.42 and 3.51 with 50 and 100 aphids, respectively, and *LOX2* (At3g45140) 2.00 and 2.37 (Annex 1). Thus there was no striking difference in the transcriptomic signature of insect-attacked plants, whether the primary treatment was done with 50 or 100 aphids.

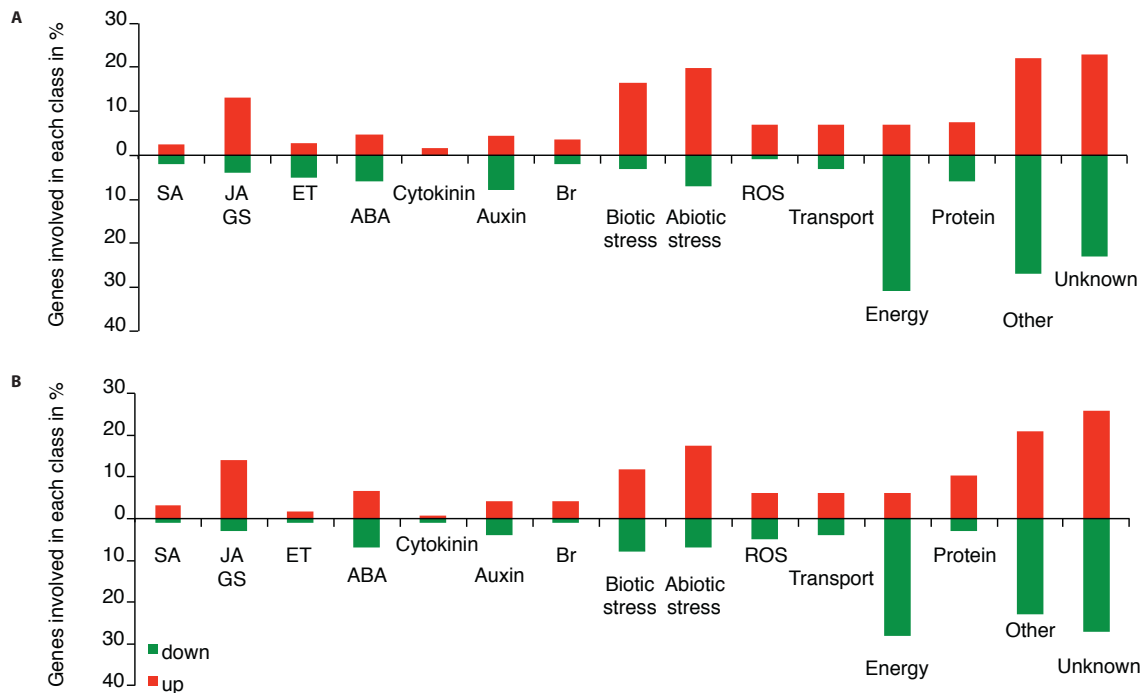
Furthermore, after pretreatment with 50 and 100 aphids density, insect-induced genes were predominantly grouped in JA pathway and GS biosynthesis classes, with respectively 13 % and 14 % of the total up-regulated genes. Surprisingly, contrary to aphid single stress, the dual stress showed a weak induction of SA and ET signaling pathways, with around 3 % of all induced genes for each class. Furthermore, the energy GO class regrouped most of the repressed genes, with respectively 31 % and 28 % after 50 or 100 aphids pretreatment (Figure 16 A and B).



**Figure 15: Transcriptome analysis of plants treated by 50 or 100 *B. brassicae*, during two days, followed by *P. brassicae* larvae during 24 hours.**

- A- Comparison of gene expression ratio mean between aphids density in the dual stress *B. brassicae* / *P. brassicae*.
- B- Genes with expression ratios that are significantly different (Student's *t*-test, P-value < 0.05) between aphids density in dual stress.
- C- Classification by GO of the 15 genes significantly different between aphids density in dual stress, using classification SuperViewer of the Bio-Analytic Resource for Plant Biology. Biological process class with a P-value > 0.05 are represented in grey or in black with a P-value < 0.05.





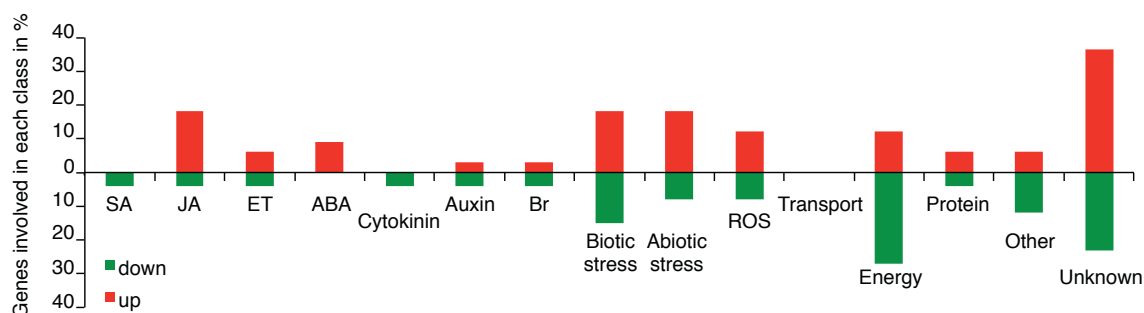
**Figure 16: Repartition of up- and down-regulated genes after the dual stress, aphids followed by *P. brassicae* larvae.**

- A- Transcriptome analysis of *B. nigra* after the dual stress 50 *B. brassicae* / *P. brassicae* comprised 241 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 96 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ .
- B- Transcriptome analysis of *B. nigra* after the dual stress 100 *B. brassicae* / *P. brassicae* comprised 232 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 72 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ .

Each gene was classified in a GO class based on information retrieved from the literature.

#### **d. Transcriptome analysis of plants treated with ozone and *P. brassicae***

Finally, the transcriptome of *B. nigra* pretreated by ozone during 5 days followed by *P. brassicae* larvae feeding during 24 hours was analyzed. We found 32 induced genes and 25 repressed genes. Gene classification by GO showed a clear involvement of the JA pathway (18 % of induced genes), ABA pathway (9 %) and ET pathway (6 %). Genes involved in the SA pathway were not up-regulated and few of them (4 %) were repressed. Moreover, 12 % of induced genes were classified on the ROS production GO class. Thus, repressed genes were mostly (27 %) involved on photosynthesis and electron transport and 23 % were genes of unknown function (Figure 17; Annex 2).

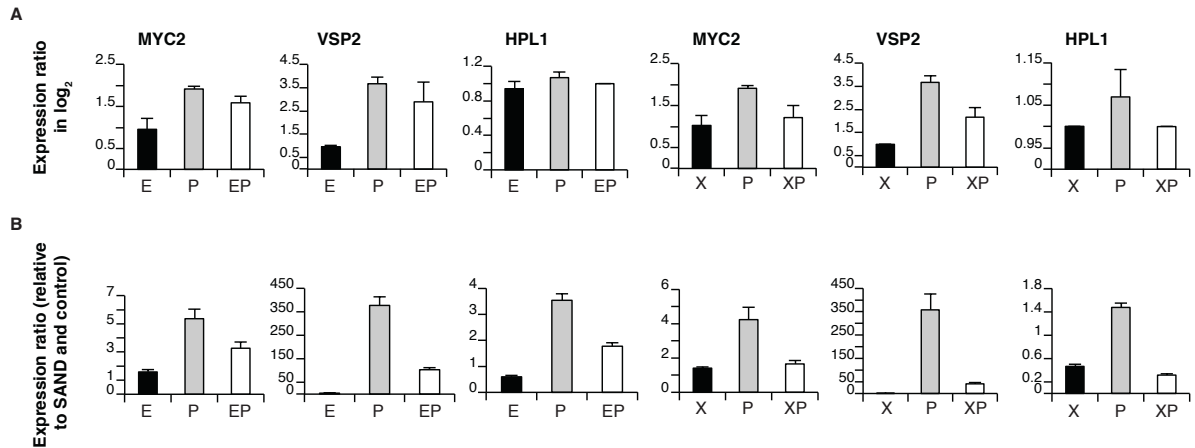


**Figure 17: Repartition of up- and down-regulated genes after the dual stress, ozone exposure followed by *P. brassicae* feeding.**

Transcriptome analysis of *B. nigra* after the dual stress ozone / *P. brassicae* comprised 32 up-regulated genes with an expression ratio in  $\log_2 > 0.585$  and a P-value  $< 0.05$  and 25 down-regulated genes with an expression ratio in  $\log_2 < -0.585$  and a P-value  $< 0.05$ . Each gene was classified in a GO class based on information retrieved from the literature.

#### e. Microarray validation

Quantitative RT-PCR (qPCR) analysis was used to confirm microarrays data for the JA marker genes *MYC2* (At1g32640), *VSP2* (At5g24770) and *HPL1* (At4g15440). Data from CATMAv4 microarrays (Figure 18 A) were compared to qPCR (Figure 18 B) data obtained with samples from plants treated by egg extract 3 days, *P. brassicae* 24 hours, and egg extract / *P. brassicae* herbivory. We also tested samples of *Xcc* treatment during 3 days, *P. brassicae* 24 hours and *Xcc* / *P. brassicae* herbivory. For all genes and treatments analyzed, expression ratios followed the same trend between microarrays and qPCR. We however observed larger amplitude of expression ratios with qPCR. This could be due to the fact that we designed specific primers for *B. nigra* genes for qPCR experiments, whereas transcriptomic analyses were realized using Arabidopsis CATMAv4 microarrays. It is also known that the dynamic range of qPCR is larger than that of microarrays. Thus, we conclude that results from qPCR experiments confirmed microarray data.



**Figure 18: Validation of microarray data.**

Expression level of *MYC2* (At1g32640), *VSP2* (At5g24770), and *HPL1* (At4g15440), was compared between results obtained from CATMAv4 microarrays (A) and qPCR (B) after different experiments. Egg extract during 3 days (E), *P. brassicae* feeding during 24 hours (P), dual stress egg extract / *P. brassicae* (EP). *Xcc* treatment during 3 days (X), *P. brassicae* feeding during 24 hours (P), dual stress *Xcc* / *P. brassicae* feeding during 24 hours (XP).

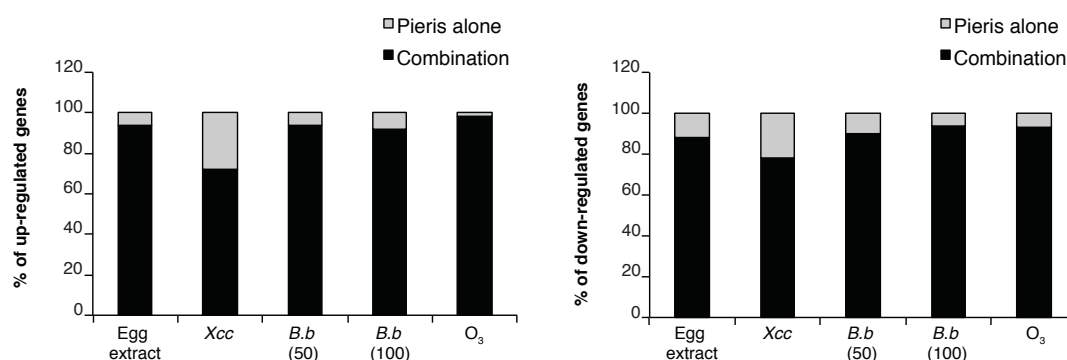
A- Means (log<sub>2</sub>), ± standard error, of at least five replicates are shown.

B- Means (± SE) of three technical replicates are shown. Expression levels were normalized with respect to the housekeeping gene *SAND* (At2g28390) and expressed relative to unchallenged control samples. These experiments were repeated, independently, at least twice with similar results.

#### 4- Effect of a pretreatment on the transcriptional response to a second stress

##### a. Gene repartition between single and dual stresses

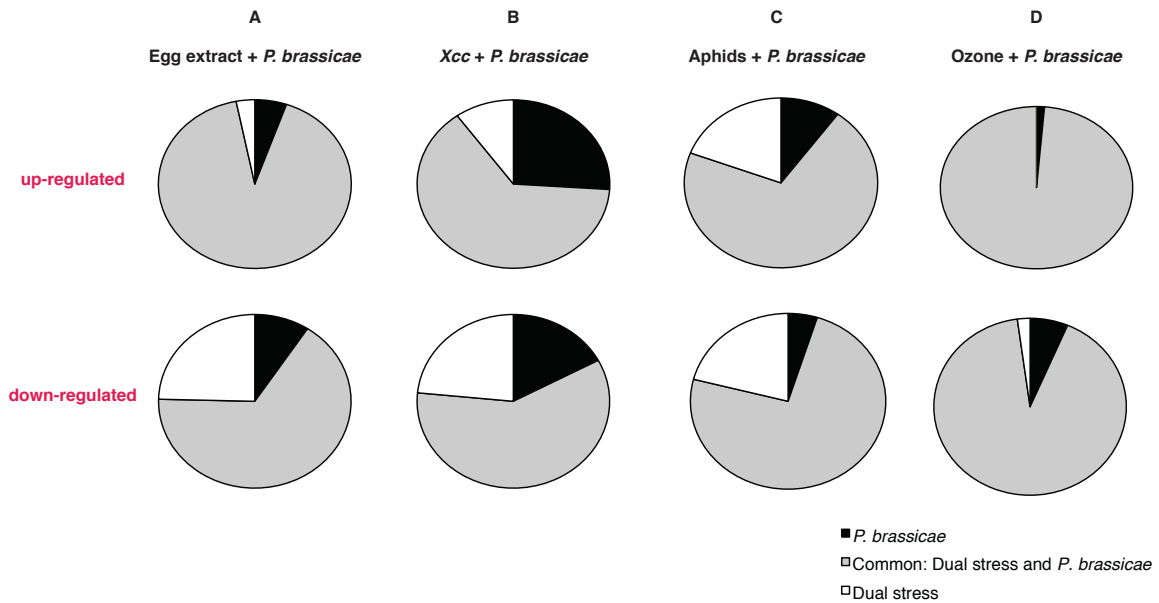
The effect of each pretreatment on the insect-induced transcriptome was further studied. We selected all genes that were significantly regulated in *P. brassicae* feeding and checked whether their expression ratio was significantly different in dual stress conditions (Student's *t*-test; P-value < 0.05). More than 90 % of the insect-regulated genes were also significantly up- or down-regulated in a dual stress with egg extract or aphids. *Xcc* pretreatment had a stronger effect, since only 72 % and 78 % respectively of insect-regulated genes were up- and down-regulated in the dual stress (Figure 19). Strikingly, ozone pretreatment had almost no impact in insect-regulated genes, because 98 % and 93 % of them were still induced or repressed in a dual stress (Figure 19).



**Figure 19: Percentage of common induced and repressed genes between single and dual stress.**

Proportion of *P. brassicae*-regulated genes that are also regulated during a dual stress (black bars). Each pretreatment is indicated under each column: Egg extract during 3 days, *X. campestris* pv. *campestris* during 3 days (*Xcc*), *B. brassicae* at density of 50 or 100 aphids during 2 days (*B.b*) and 70 ppb ozone ( $O_3$ ) during 5 days, all followed by 24 hours of *P. brassicae* feeding. Genes only regulated by herbivory are set to 100 % (grey bars).

Having shown that pretreatments have a small impact on insect-regulated genes, we looked if a dual stress affected genes that were not regulated by each single stress. We selected genes that were significantly induced in a dual stress and whose expression ratios were significantly lower in either the pretreatment alone or in response to herbivory alone (Student's *t*-test, *P*-value < 0.05). For all combined stresses, the proportion of dual-stress specific genes ranged from 1 % to 25 % of the total of insect-induced genes (Figure 20). For example, in the case of egg extract / *P. brassicae* dual stress, only 3 % of the total amount of induced genes was specific to a combined stress. For repressed genes, 25 % were specific to egg extract and herbivore treatment. For ozone, no gene was induced specifically during the dual stress and only 2 % of repressed genes were specific to the dual stress. Thus depending on the pretreatment, we found that the response to *P. brassicae* feeding can generate a specific signature that is unique to the dual stress, although the number of affected genes is relatively limited.



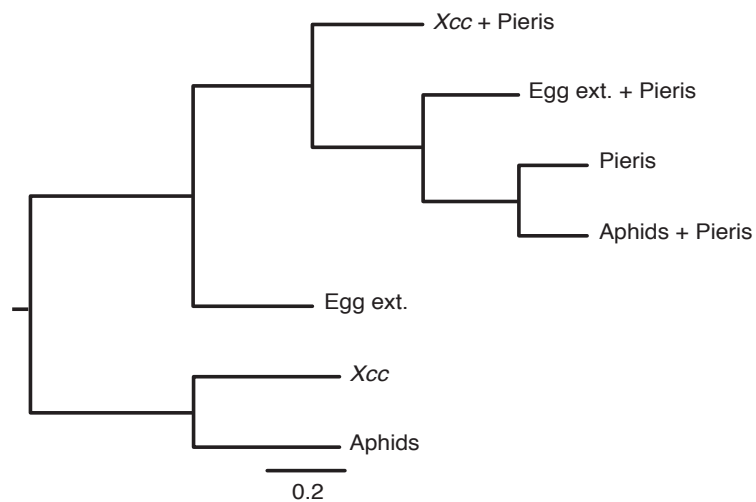
**Figure 20: Proportion of common and specific induced and repressed genes between single and dual stress.**

Proportion of genes specifically regulated by *P. brassicae* feeding (black) and dual stresses (white) or expressed in both conditions (grey).

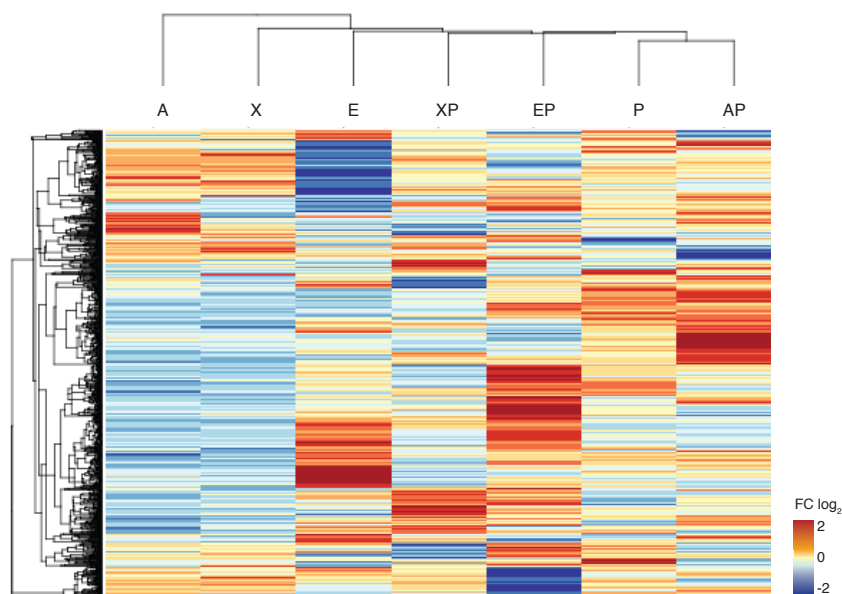
A) *P. brassicae* during and egg extract / *P. brassicae*. B) *P. brassicae* and *Xcc* / *P. brassicae*. C) *P. brassicae* and 100 *B. brassicae* / *P. brassicae*. D) *P. brassicae* and ozone / *P. brassicae*.

To further compare the expression profile in response to different pretreatments, a clustering analysis was performed including all induced and repressed genes in at least one of the experiment. Clustering of experiments was done by Pearson's correlation, with MultiExperiment Viewer software and represented by FigTree software (Figure 21 A) or by RStudio using d3heatmap library (Figure 21 B). In both representations, we clearly observed two distinct groups. One group was constituted by the single stresses (egg extract, *Xcc*, aphids) and another group contained both the single stress *P. brassicae* and dual stresses (egg extract / *P. brassicae*, *Xcc* / *P. brassicae* and aphids / *P. brassicae*) (Figure 21). These clustering nicely confirm that biotic pretreatments have a weak effect on the transcriptomic signature of *B. nigra* to *P. brassicae* feeding and that the secondary stress dominates the plant transcriptional activity.

A



B



**Figure 21: Clustering of expression profiles in response to single or dual stress.**

Correspondence analysis of expression profiles including all induced or repressed genes in at least one experiment ( $-0.585 > \log_2 \text{ ratio} > 0.585$ , P-value  $< 0.05$ ,  $n = 961$ ). Egg extract (Egg ext.; E), *Xcc* (*Xcc*; X), 100 *B. brassicae* (Aphids; A) or *P. brassicae* (Pieris; P) and the dual stress egg extract / *P. brassicae* (Egg ext. + Pieris; EP), *Xcc* / *P. brassicae* (*Xcc* + Pieris; XP) or 100 *B. brassicae* / *P. brassicae* (Aphids + Pieris; AP). Clustering and node length calculations were performed with MultiExperiment Viewer 4.8.1 using Pearson's correlation method and represented as tree in FigTree 1.4.2 (A) or as a heatmap in RStudio using d3heatmap library (B).

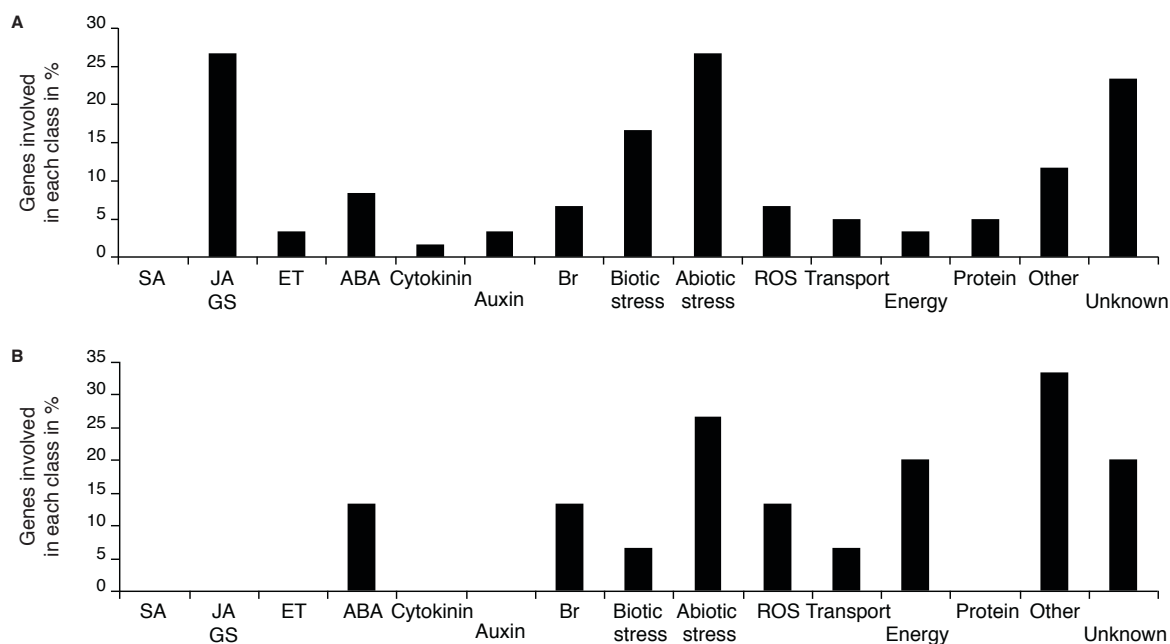
### **b. Effect of pretreatment on herbivory transcriptomic signature**

To analyze deeper the effect of pretreatment on *P. brassicae* transcriptomic signature, we tested how the top 50 genes that were induced (Figure S1) and repressed (Figure S2) by herbivory responded to each dual stress. Only a pretreatment with *Xcc* had a marked effect on insect-regulated genes. Almost half of the 50 induced genes were significantly less induced, indicating that bacterial infection impaired a strong response to *P. brassicae* (Figure S1 B). This effect was less pronounced for repressed genes as only 10 genes were affected by the pretreatment (Figure S2 B). For dual stresses with egg extract and *B. brassicae* followed by *P. brassicae* only 2 and 7 genes, respectively, were significantly less induced than with herbivory alone, and 8 and 4 were less repressed (Figure S1 A, C; Figure S2 A, C). Finally ozone pretreatment had no effect on the expression of the top 50 insect-regulated genes (Figure S1 D, Figure S2 D). We conclude that, except for *Xcc*, pretreatments have a really weak effect on the most differentially regulated genes that respond to herbivory.

### **c. Effect of *Xcc* pretreatment on plant response to *P. brassicae***

As previously shown, *Xcc* pretreatment had the strongest effect on insect-induced genes (Figures 19, 20, 21, S1 and S2). Comparison of genes up-regulated after single *P. brassicae* herbivory and *Xcc* / *P. brassicae* dual stress revealed 60 genes that were differentially expressed between the two experiments (Table S3). In comparison, only 15 genes were differentially expressed between the dual stress egg extract / *P. brassicae* and herbivory alone (data not shown). In most cases, genes were less induced in the dual stress than in the single herbivory treatment. For all 60 differentially expressed genes, only 2 had a higher expression ratio in dual stress than single stress, including a gene with unknown function and a member of the HSP70 family, *BIP1* (At5g28540). Furthermore, analysis of GO classes for these 60 genes revealed that most of them (ca. 30%) are involved in the JA signaling pathway and GS biosynthesis, whereas no one was classified in the SA signaling pathway (Figure 22 A). For example, the list contains *LOX3* (At1g17420), *LOX1* (At1g55020), *MYC2* (At1g32640), and *MPK3* (At3g45640), with an expression ratio after *P. brassicae* feeding always significantly higher than after the combined stress. Further, for the 16 genes belonging to the JA and GS class, only two (*LOX3*, and *BCAT4*, At3g19710) are still induced after *Xcc* pretreatment, with, respectively, an expression ratio of 2.31 and 1.47 after single herbivory and 0.97 and 0.60 after *Xcc* / *P. brassicae* dual stress (Table S3). In comparison, if we classify the 15 genes differentially expressed between herbivory and the dual stress egg extract / *P. brassicae* feeding, none of them belong to JA and GS class (Figure 22 B).

Thus, *Xcc* pretreatment seems to inhibit the induction of genes involved in the JA pathway and the GS biosynthesis.



**Figure 22: Repartition of insect-induced genes that are differentially expressed in dual stress.**

A- Repartition of 60 insect-induced genes differentially expressed in the dual stress *Xcc* / *P. brassicae* (Student's *t*-test, *P*-value < 0.05).

B- Repartition 15 insect-induced genes that are differentially expressed in the dual stress egg extract / *P. brassicae* (Student's *t*-test, *P*-value < 0.05)

Each gene was classified in a GO class based on information retrieved from the literature.

#### d. JA-induced response after *P. brassicae* or dual stresses

We found that the percent of genes belonging to GO classes is most of the time the same between *P. brassicae* and dual stresses. We compared expression ratios of genes involved in the major signaling pathway triggered after insect herbivory, the JA signaling pathway, with expression ratios between the *P. brassicae* single stress and dual stresses: egg extract / *P. brassicae*, *Xcc* / *P. brassicae* and *B. brassicae* (100) / *P. brassicae* (Table S5). Most of JA-induced genes in *P. brassicae* were still differentially regulated in dual stresses. Some of them had a *P*-value > 0.05 (Student's *t*-test) due to a high variability between samples but expression ratios were above 0.585 ( $\log_2$ ).

Only in *Xcc* / *P. brassicae*, as shown previously, some JA marker genes were not induced such as *LOX1* (At1g55020), *OPR3* (At2g06050) or *MYC2* (At1g32640) (Table S5). Moreover, we observed an induction of *PDF1.2* (At5g44420) and *ERF1* (At1g12920) in *Xcc* / *P. brassicae* (Table S5). *PDF1.2* was induced also in *Xcc* single stress while *ERF1* not (Annex 1). Further, *MYC2* (At1g32640) was not induced in both *Xcc* and *Xcc* / *P. brassicae*. As previously described by Lorenzo and coworkers (2003), activation of JA and ET signaling pathway is effective through the ERF branch and induces JA / ET-responsive genes such as *PDF1.2*. In contrast, JA-signaling pathway is activated through MYC2 branch. MYC2 is described to be a positive regulator of JA-marker genes and a negative regulator of JA / ET signaling pathway (Lorenzo *et*

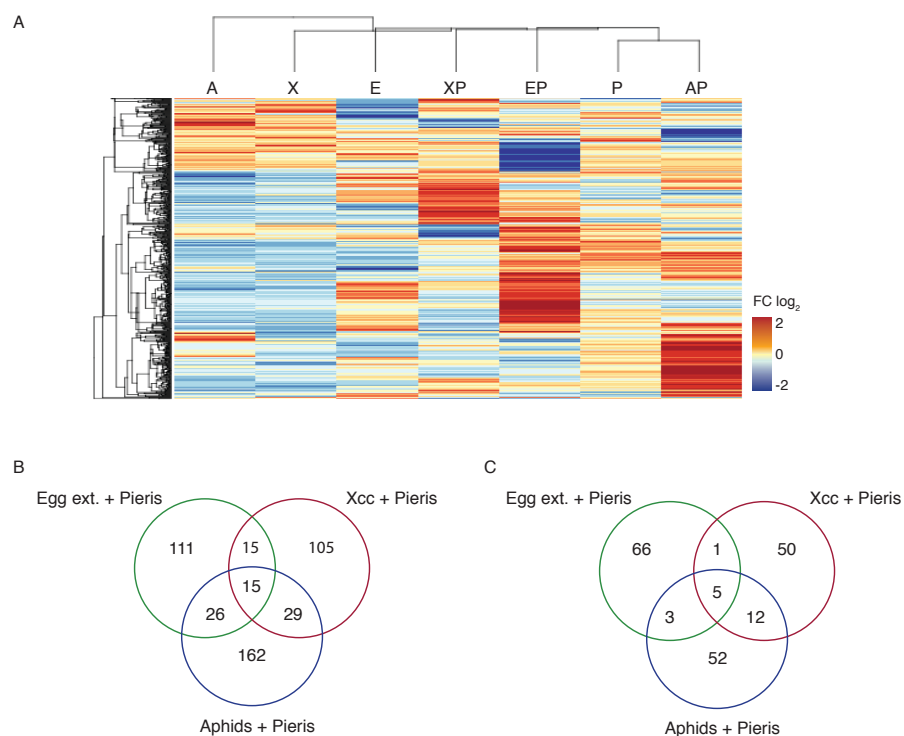


*al.*, 2003). This result confirmed that *Xcc* infection triggers plant response through the JA / ET signaling pathway (Ton *et al.*, 2002).

Thus, genes belonging to the JA signaling pathways were commonly expressed between *P. brassicae* and dual stresses egg extract / *P. brassicae* and *B. brassicae* / *P. brassicae*. Some differences observed in expression ratios of JA marker genes in *Xcc* / *P. brassicae* could be explained by the activation of the JA response in combination with ET through the ERF branch and the antagonistic effect of SA / JA crosstalk observed after bacterial infection (Figures 9 and 22).

#### e. Analysis of dual stresses responses in *Brassica nigra*

As shown above, we demonstrated that prior stress had a weak effect on *P. brassicae* herbivory response in *B. nigra*. To go further, we wondered if dual stresses regulate some genes specifically. We performed a clustering analysis including all induced or repressed genes (641 genes) in at least one of the dual stress experiment: egg extract / *P. brassicae*, *Xcc* / *P. brassicae*, *B. brassicae* / *P. brassicae*.

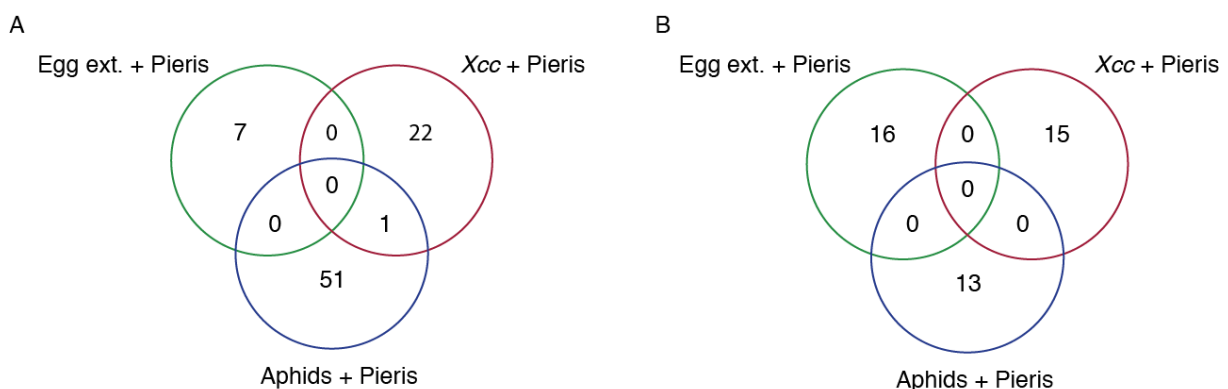


**Figure 23: Clustering of dual stress-specific genes.**

All differentially expressed genes (641 genes) in at least one of the dual stress were clustered in RStudio (d3heatmap library) using Pearson's correlation method (A) and the common induced- (B) or repressed (C) genes were compared between each combined-stresses.

We cannot distinguish a cluster of genes induced or repressed specifically by all dual stresses (Figure 23 A). This is confirmed by the comparison of induced and repressed genes after each combined stresses. Only 15 up- and 5 down-regulated genes were common to all dual stresses (Figure 23 B, C). Analysis of these 20 genes revealed that only 3 of them were not induced by *P. brassicae* single stress, a gene coding for a 60S ribosomal protein (At2g40205), for a myosin-like protein (At2g33240) and for a cytosol aminopeptidase family protein (At4g30910) and one was down-regulated after *P. brassicae* herbivory, *TUB9* (At4g20890). Unfortunately, these 4 genes were not all statistically different to *P. brassicae* single stress (Student's *t*-test, *P*-value < 0.05) (Table S4).

Furthermore, we also compared all the genes coming from the list (Figure 20) of genes specifically induced or repressed only after a dual stress and not by *P. brassicae* or the prior stress alone. Surprisingly, no genes were common to all or a subset of dual stresses, except one gene, a transcription elongation factor-related gene (At5g11430), that was common to *Xcc* / *P. brassicae* and *B. brassicae* / *P. brassicae* (Figure 24).

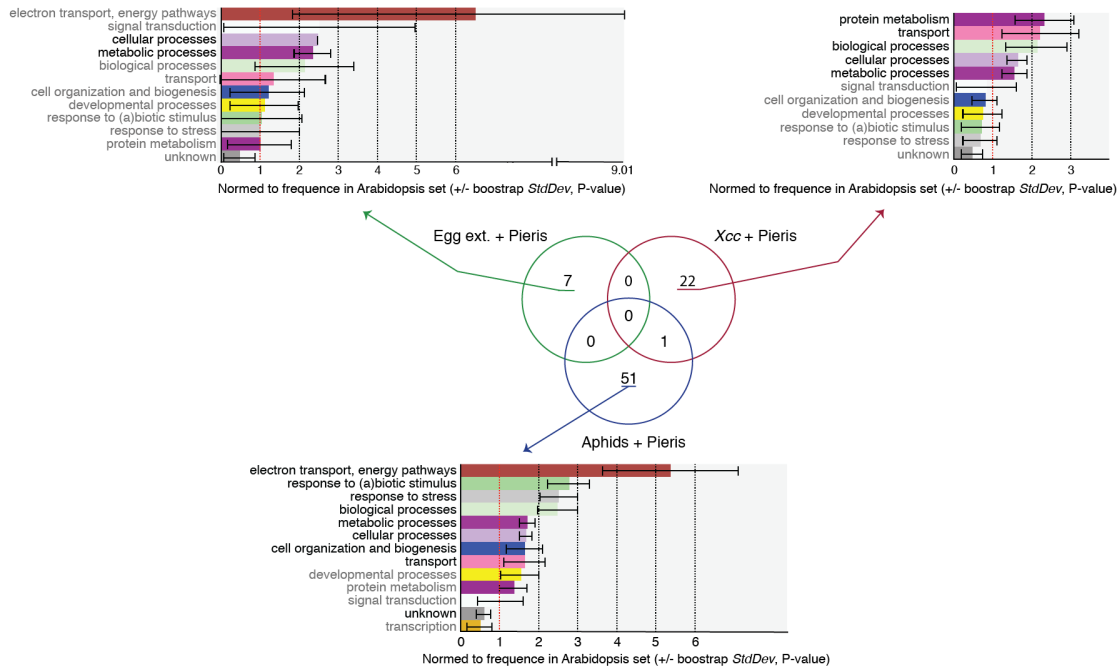


**Figure 24: Genes only induced or repressed after a dual stress.**

The number of up- (A) and down-regulated (B) genes differentially regulated only after a dual stresses (egg extract / *P. brassicae* (Pieris), *Xcc* / *P. brassicae* or *B. brassicae* (Aphids) / *P. brassicae*) and not after herbivory or the prior stress alone.

Furthermore, we analyzed the GO class of all genes specifically induced after each dual stress and not by *P. brassicae* or a single stress (genes list used for figures 20 and 24). *B. brassicae* / *P. brassicae* was the dual stress with the higher number of specifically induced genes (52 genes). These genes belonged to important classes involved in plant defense such as response to stress or response to (a)biotic stimulus (Figure 25). This difference in comparison to egg extract / *P. brassicae* or *Xcc* / *P. brassicae* dual stresses could be explained by the fact that aphids induced very few genes (Figure 10). Moreover, *B. brassicae* induced only genes involved in JA-signaling pathway or GS biosynthesis (Figure 10) but no other phytohormone pathways were regulated by aphids feeding in comparison to the dual stress *B. brassicae* / *P. brassicae*

where SA, JA, ET, ABA, Br and Auxin were triggered (Figure 16). Furthermore, we showed that most of the induced or repressed genes in *P. brassicae* or in *B. brassicae* / *P. brassicae* were common (Figure 20).



**Figure 25: Classification of dual stress-specific genes.**

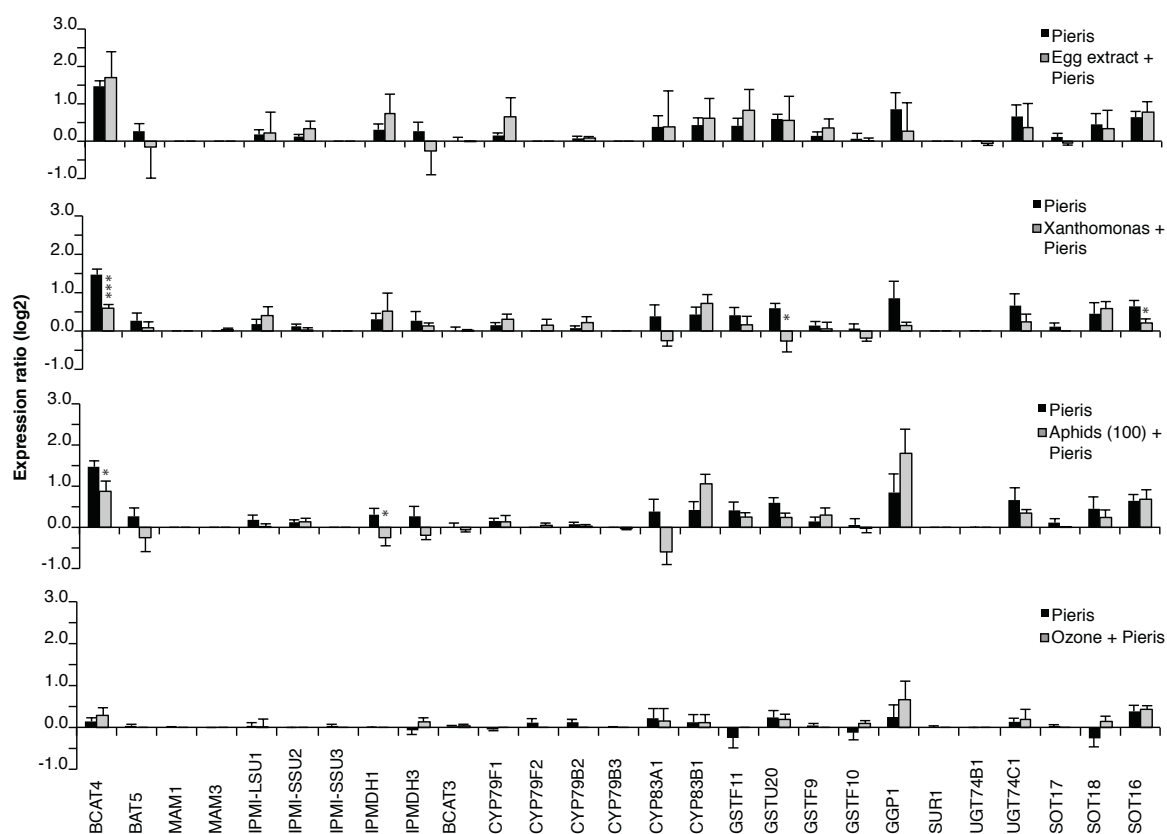
Classification of genes specific to each dual stress significantly different between egg extract / *P. brassicae*, *Xcc* / *P. brassicae* and *B. brassicae* (Aphids) / *P. brassicae*, using classification SuperViewer of the Bio-Analytic Resource for Plant Biology. Biological process class with a P-value > 0.05 are represented in grey or in black with a P-value < 0.05.

Moreover, egg extract / *P. brassicae* and *Xcc* / *P. brassicae* had both few specifically induced genes (7 and 22 respectively) and all these genes did not belong to GO classes important for plant responses to stress (Figure 25).

Thus, these analyses revealed that consecutive stresses do not generate an important and specific transcript signature in *B. nigra*. Moreover, the dual stress-specific genes are different depending on the type of dual stress. As discussed previously, most of the regulated genes in combined stresses were genes that were up- and down-regulated by the second stress, *P. brassicae* herbivory (Figures S2 and S3).

### f. Effect of *P. brassicae* feeding and combined stresses on glucosinolate biosynthesis gene expression

Expression of all genes important for glucosinolates biosynthesis in *Arabidopsis* (Sønderby *et al.*, 2010; Schweizer *et al.*, 2013) was compared between *P. brassicae* single stress and the different combined stresses to evaluate the potential importance of GS biosynthesis in *B. nigra*.



**Figure 26: Expression of glucosinolate biosynthesis genes after *P. brassicae* feeding compared to dual stress.**

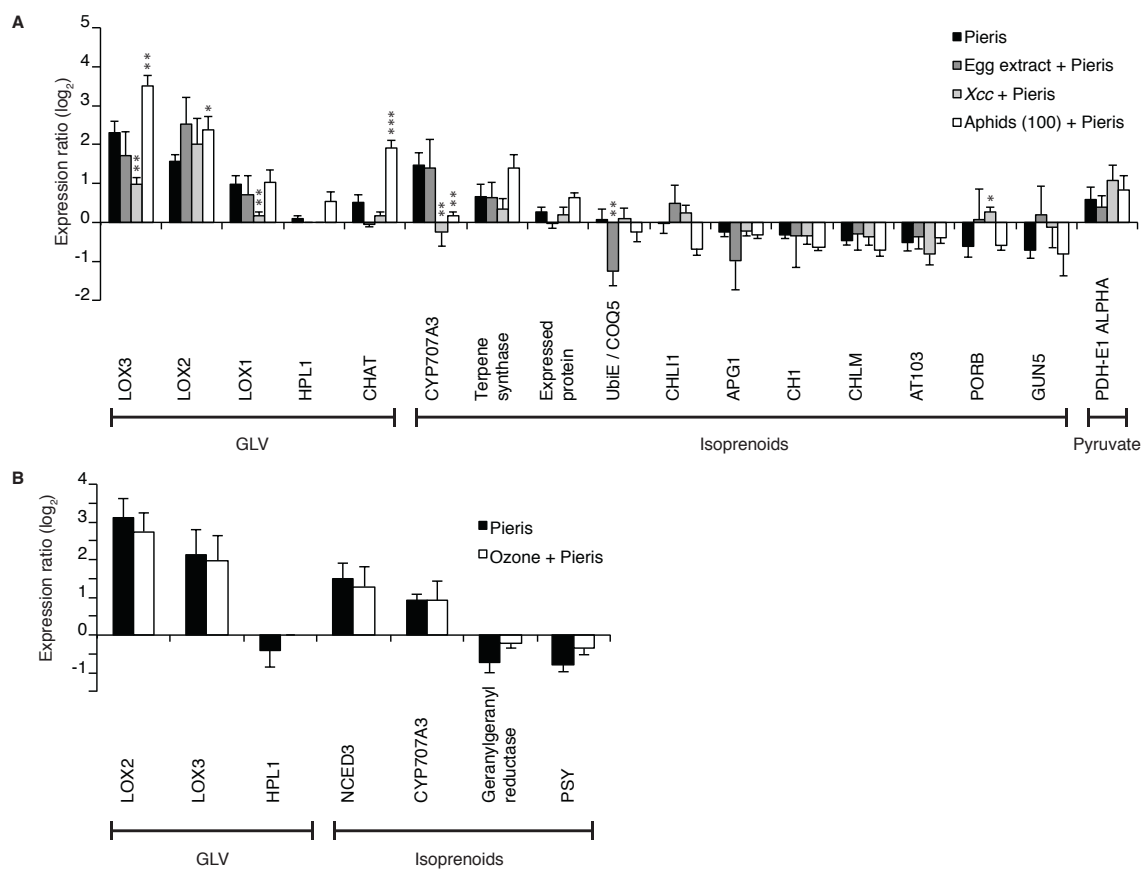
Expression ratio ( $\log_2$ ) of glucosinolate biosynthesis genes after 24 hours of *P. brassicae* feeding are compared to the expression after pretreatment with *P. brassicae* egg extract, *Xcc*, 100 aphids or ozone followed by 24h of herbivory in 5 week-old *B. nigra* plants. Values are the mean ( $\pm$  SE) of at least five biological replicates. Statistical differences between herbivory and dual stress are indicated (Student's *t*-test, \* P-value < 0.05, \*\*\* P-value < 0.001).

First of all, out of ca. 27 GS genes, only 3 genes were induced after *P. brassicae* feeding, including *BCAT4* (At3g19710; expression ratio of 1.47), *GSTU20* (At1g78370; expression ratio of 0.59) and *SOT16* (At1g74100; expression ratio of 0.65) (Figure 26). For both egg extract and ozone pretreatments, no difference was observed in expression ratios between a single herbivory stress and each combined stresses. In *Xcc* / *P. brassicae* dual stress, these 3 genes had a significantly smaller induction compared to *P. brassicae* single stress. Finally, in 100 aphids / *P.*

*brassicae* dual stress only 2 genes were statistically different to *P. brassicae* single stress, *BCAT4* and *IPMDH1* (At1g31180) (Figure 26). In conclusion, contrary to *Arabidopsis*, herbivory does not seem to strongly impact GS biosynthesis genes in *B. nigra*.

### g. Effect of *P. brassicae* feeding and combined stresses on BVOC gene expression

Genes important for the BVOC biosynthesis were analyzed for each stress. Expression of all genes differentially regulated after at least one treatment was compared to other stresses (Figure 27).



**Figure 27: Expression of genes important for BVOC biosynthesis after *P. brassicae* feeding or combined stresses.**

Expression ratio ( $\log_2$ ) of all genes important for the BVOC biosynthesis that were induced in at least one stress are included: *P. brassicae* (The Netherlands and Switzerland samples), egg extract / *P. brassicae*, *Xcc* / *P. brassicae*, 100 *B. brassicae* / *P. brassicae* (A) and *P. brassicae* (Finland samples) and ozone / *P. brassicae* (B).

Values are the mean ( $\pm$  SE) of at least five biological replicates. Statistical differences between herbivory and dual stress are indicated (Student's *t*-test, \* P-value < 0.05; \*\* P-value < 0.01; \*\*\* P-value < 0.05).

Very few genes (less than 5 %) of all BVOC biosynthesis genes were up- or down-regulated after single herbivory or after dual stresses (Figure 25 A). *LOX1* (At1g55020), *LOX2* (At3g45140), and

*LOX3* (At1g17420), were all induced after each treatment, except *LOX1* after *Xcc* / *P. brassicae* dual stresses. However, *HPL1* (At4g15440) which is important to convert products from *LOXs* activity into GLV (General Introduction, Figure 8) was never induced. We may conclude that *LOXs* genes induced in our experiments were rather up-regulated for the synthesis of JA and not for GLV biosynthesis. Moreover, we observed the same effect after single stresses: egg extract treatment, *Xcc* infection or *B. brassicae* (100 aphids) feeding. Less than 2 % of all BVOC biosynthesis genes were differentially regulated in at least one single stress. Interestingly, all belonging to the isoprenoids family, and all were down-regulated only after *P. brassicae* egg extract (data not shown).

In the case of experiments done in Finland, herbivory or ozone / *P. brassicae* dual stress, less than 2 % of genes known to be involved in the biosynthesis or emission of BVOCs were differentially regulated (Figure 27 B). Comparison of gene expression between herbivory and the dual demonstrated that none of them were differentially expressed between the two types of treatment. Moreover, *LOX2* and *LOX3* were induced by *P. brassicae* single stress and by ozone / *P. brassicae* but *HPL1* was not.

Finally, we can conclude that BVOC biosynthesis seems not regulated at the transcriptional level in response to herbivory in *B. nigra*.

## Discussion

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The effect of two or more (a)biotic stresses can be more detrimental to plants than an individual stress. The ability of plants to recognize and respond to combined and specific stresses appears thus to be important, especially if pathogens or herbivores trigger different plant defense pathways. In addition, the effect of one or more stresses, at the same time, is known to change BVOC biosynthesis and emission. One stress response can influence the other or sometimes their effects can be additive or opposite (Holopainen and Gershenzon, 2010). Nevertheless, few studies have been conducted on plant transcriptomes in the case of multiple stresses, and gene expression studies often focus on the plant model *Arabidopsis*.

Here, the black mustard *B. nigra* was used to investigate how plants respond to dual stresses and what was their impact on gene expression involved in BVOC biosynthesis. Surprisingly, transcriptomic responses of *B. nigra* to different pretreatments followed by *P. brassicae* larvae showed a weak impact of the first stress on subsequent herbivory (Figures 19 and 20). A recent study by Davila Olivas and co-workers (2016) reported the same effect. In *Arabidopsis*, a whole-genome RNAseq analysis revealed that drought stress or *B. cinerea* pretreatments followed by *P. rapae* feeding yielded only few specific differentially regulated genes in comparison to single herbivory. A time-course analysis clearly showed that pre-exposure shifts the timing of caterpillar-induced responses. Plants responded faster against *P. rapae* if they were pretreated by drought or *B. cinerea* (Davila Olivas *et al.*, 2016; Coolen *et al.*, 2016). On the contrary, other studies on multiple stresses showed that single and dual stress resulted in different gene expression profiles. For example, Rasmussen and co-workers (2013) showed that combined stresses trigger a very different response than individual stress, and that *Arabidopsis* response cannot be predicted by the simple addition of two stresses (Rasmussen *et al.*, 2013). Also in *Arabidopsis*, the combination of drought and heat stresses differentially regulated 770 genes that were specific to this dual stress, in comparison to drought or heat alone (Rizhsky *et al.*, 2004). Moreover, a recent study in *Solanum dulcamara* demonstrated that drought combined with herbivory had an additive effect on plant responses, including gene expression changes (Nguyen *et al.*, 2016). These results confirm the complex nature of responses to multiple stresses and the importance of studying plant stress factors in combination, like it really happens in nature. Atkinson and coworkers (2013) postulated that during multiple attacks plants respond preferentially to the more damaging stress. In *Arabidopsis* challenged by drought and / or nematodes, they observed that 96 % of differentially regulated genes were common to the dual stress and the water stress, and that only 2 % overlapped with nematode feeding stress alone (Atkinson *et al.*, 2013). We can hypothesize that *B. nigra* prioritized a response to larval feeding

rather than to ozone, aphids, eggs or bacteria for the sake of their own fitness, as *P. brassicae* larvae are known to inflict large damage in Brassicacea plants and mainly to be florivorous when reaching the second and subsequent instars (Smallegange *et al.*, 2007). Plants may have to respond efficiently and quickly against *P. brassicae* caterpillars to avoid impacting their fitness. It would be interesting to perform reciprocal experiments to see if a pretreatment with *P. brassicae* feeding is overruled by a second biotic or abiotic stress or if plants prioritize the response to herbivory over other stresses. A recent analysis in *B. nigra* validated our hypothesis that this plant seems to prioritize plant defense response against *P. brassicae* rather than against *Xcc*, *P. brassicae* eggs or *B. brassicae* in case of combined stresses. The authors showed that the presence of prior stress had no impact on indirect defense and attraction of *Cotesia glomerata*. The volatile blend emitted by the plant during a dual stress, eggs / *P. brassicae*, *Xcc* / *P. brassicae* or *B. brassicae* / *P. brassicae*, was the same than after *P. brassicae* herbivory. However, BVOCs were different between combined and single stresses (Ponzio *et al.*, 2014).

We however observed that *Xcc* pretreatment had a significant effect on *P. brassicae* transcriptome, underlying a potential antagonistic effect of SA on JA gene expression. Indeed, 30 % of genes differentially regulated by the dual stress belonged to the JA and GS GO classes (Figure 22 A). Furthermore, these genes were either not induced anymore or significantly less induced after *Xcc* pretreatment (Table S3). Since *Xcc* single treatment up-regulated genes belonging to the SA pathway (Figure 9), we hypothesize that a phenomenon of SA / JA crosstalk occurred in the *Xcc* / *P. brassicae* dual stress. The same effect was however not observed with egg extract pretreatment, as no JA and GS marker genes were less expressed in this dual stress (Figure S1). Indeed, we could not detect a reduced insect-triggered induction after egg pretreatment involving the SA pathway although SA-responsive genes, including *PR1*, were clearly up-regulated by egg treatment indicating that the SA pathway was activated by eggs, as in *Arabidopsis* (Little *et al.*, 2007; Bruessow *et al.*, 2010). This apparent absence of SA / JA crosstalk in response to egg pretreatment in *B. nigra* may represent a specific response of this plant or may be explained by a reduced response to insect eggs. More experiments will be necessary to distinguish between these two hypotheses. On the other hand, we had no apparent antagonistic effect of larvae-induced JA on SA gene expression, like in *Arabidopsis* (Thaler *et al.*, 2012). We postulate that JA and SA activation and their mutualistic antagonistic effects can depend on the strength of the treatment that triggers each one of these pathways. For example, a previous study in *Arabidopsis* reported a synergistic effect on JA- and SA-induced genes (*PDF1-2*, and *PR1* respectively) if plants were treated with low concentrations of each hormone. However, at high concentrations of SA and JA, the effects were antagonistic (Mur *et al.*, 2006).

Furthermore, we did not observe a dramatic change in genes important for GS biosynthesis (Sønderby *et al.*, 2010; Schweizer *et al.*, 2013) even after *P. brassicae* feeding alone (Figure 26).



As was shown for Arabidopsis, we expected an increase in the expression GS pathway genes in response to insect feeding (Mewis *et al.*, 2006; Schweizer *et al.*, 2013). In our case, very few genes were induced after *P. brassicae* feeding during 24 hours. Moreover, since aphids are known to increase GS levels in Arabidopsis (Mewis *et al.*, 2006), we could have observed a priming effect of aphid treatment in the dual stress aphid / *P. brassicae*. However, only 2 genes were expressed differently with *P. brassicae* single stress and the combined stresses, *BCAT4* and *IPMDH1*. Analysis of leaf GS levels after each stress could be interesting to compare with our results on GS gene expression. Noteworthy, Mewis and coworkers (2006) analyzed GS gene expression in response to insects after one week of feeding by aphids and after three days of feeding by caterpillars (Mewis *et al.*, 2006). Also, Schweizer and coworkers (2013) measured GS genes expression after 48 hours of *S. littoralis* feeding. We might repeat experiments with at least two days of larval feeding to see if we obtain the same increase of GS pathway genes (Schweizer *et al.*, 2013). Alternatively, GS accumulation may not be regulated at the transcriptional level in *B. nigra*. Regulation of enzymatic activity by herbivory or elevated constitutive levels may represent other strategies to control the defense metabolites.

Finally, we observed a weak expression of BVOC biosynthesis or emission genes in all treatments (Figure 27). However, it is well established that plants produce volatiles after herbivory (Kessler and Baldwin, 2001; Dicke and Baldwin, 2010) or egg deposition, to attract egg and larval parasitoids (Fatouros *et al.*, 2012). Also, studies have shown that *B. nigra* plants emit numerous compounds in their volatile blend (Gols *et al.*, 2008; Ponzio *et al.*, 2014). A previous experiment, conducted in *B. nigra*, showed that BVOCs blend composition varied according to the treatment: *Xcc* infection, *P. brassicae* eggs oviposition, *P. brassicae* herbivory or *B. brassicae* feeding. Whereas, BVOCs emitted after combined stresses: eggs / *P. brassicae*, *Xcc* / *P. brassicae* or *B. brassicae* / *P. brassicae* cannot be separated from those released after *P. brassicae* herbivory (Ponzio *et al.*, 2014). Strikingly, no experiments were done on gene expression analysis for genes involved in BVOC biosynthesis. Here we show that BVOC biosynthesis is not regulated at the transcriptional level, we do not have important genes differentially regulated after both single and dual stresses. Activation of enzymes by protein phosphorylation, control of metabolic fluxes and availability of precursors may explain our results. Alternatively, homology of BVOC and GS biosynthesis genes between Arabidopsis and *B. nigra* might be low and our microarray might not have detected *B. nigra* transcripts. A further analysis of the *B. nigra* genome by RNAseq, when available, might help in addressing this issue.

In conclusion, this study reveals that a pretreatment has a weak effect on the transcriptomic response to *P. brassicae* in *B. nigra* and that the second stress dominates the expression profile. Moreover, we show that GS and BVOC gene expression is not affected by any of treatments applied, suggesting that these biosynthetic pathways are regulated post-transcriptionally.

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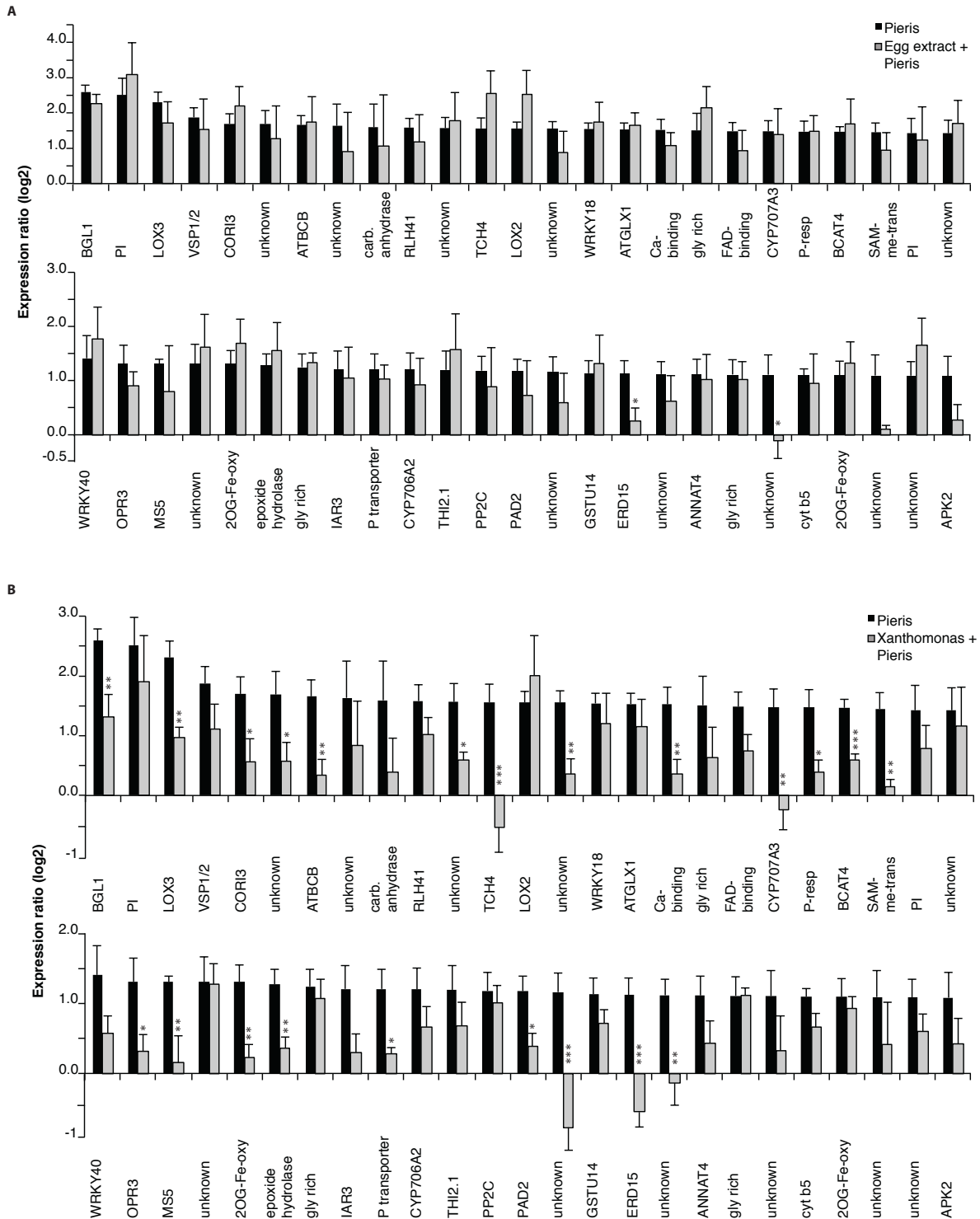
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## CHAPTER I

Wittstock, U., Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology*, 5(4), 300–307.

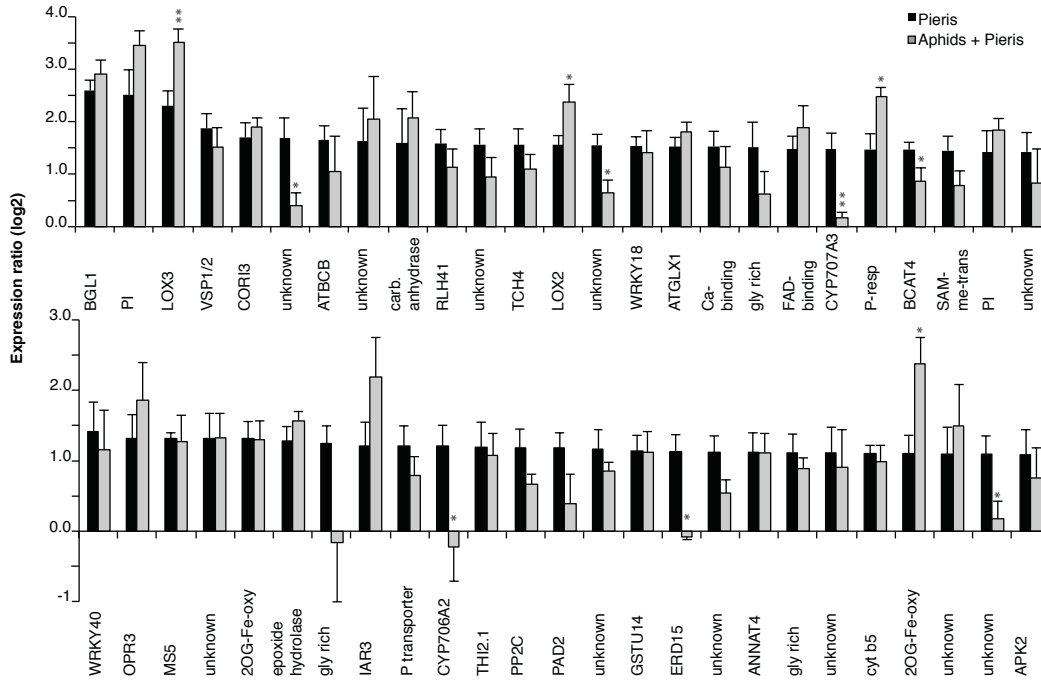
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# Supplementary data

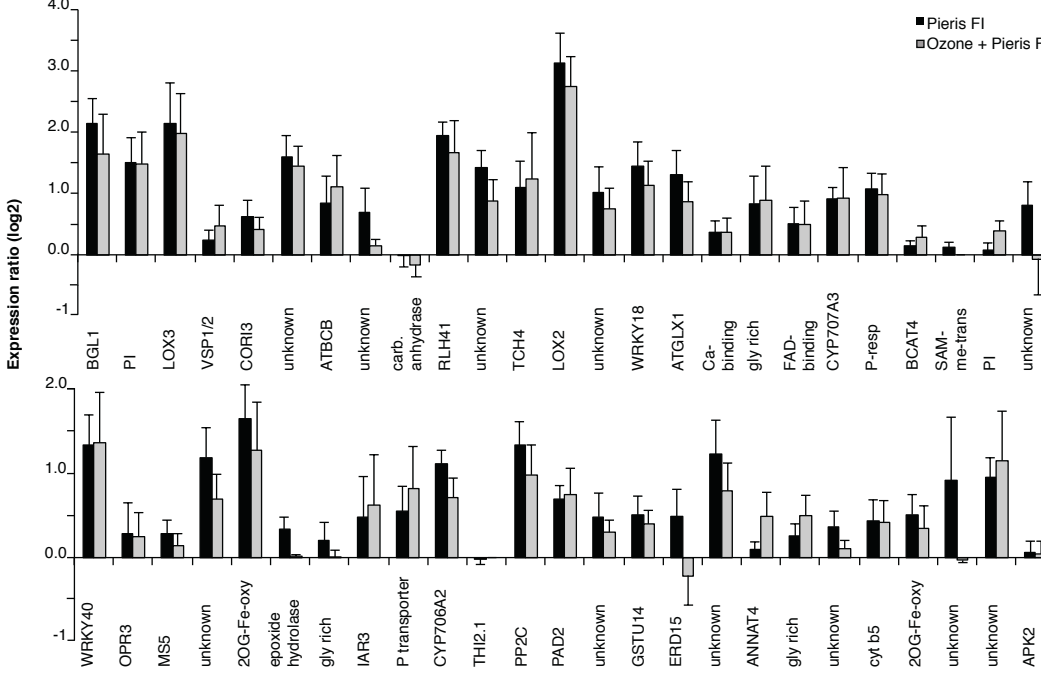


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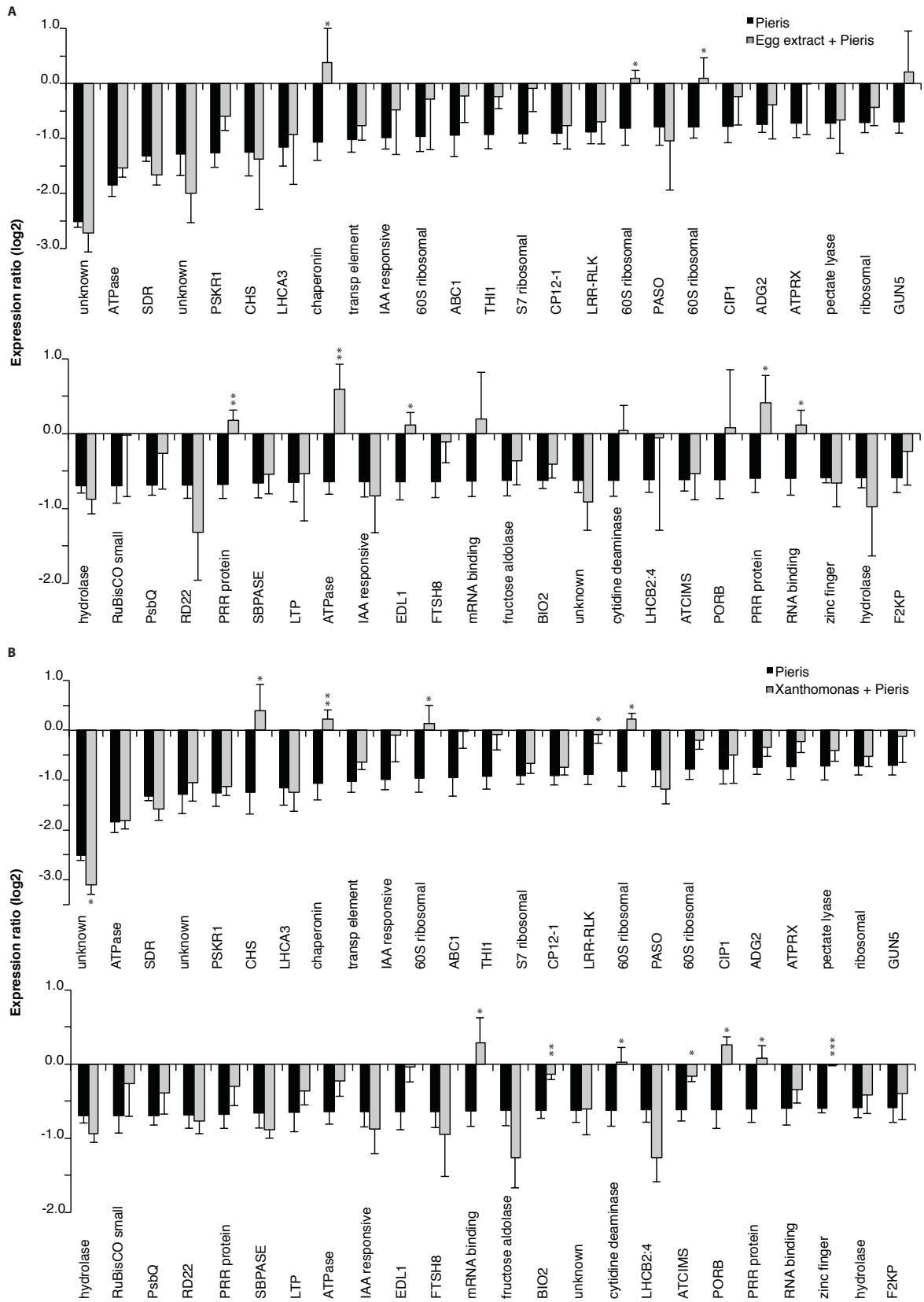


**Figure S1: Top 50 induced genes after *P. brassicae* feeding compared to dual stress.**

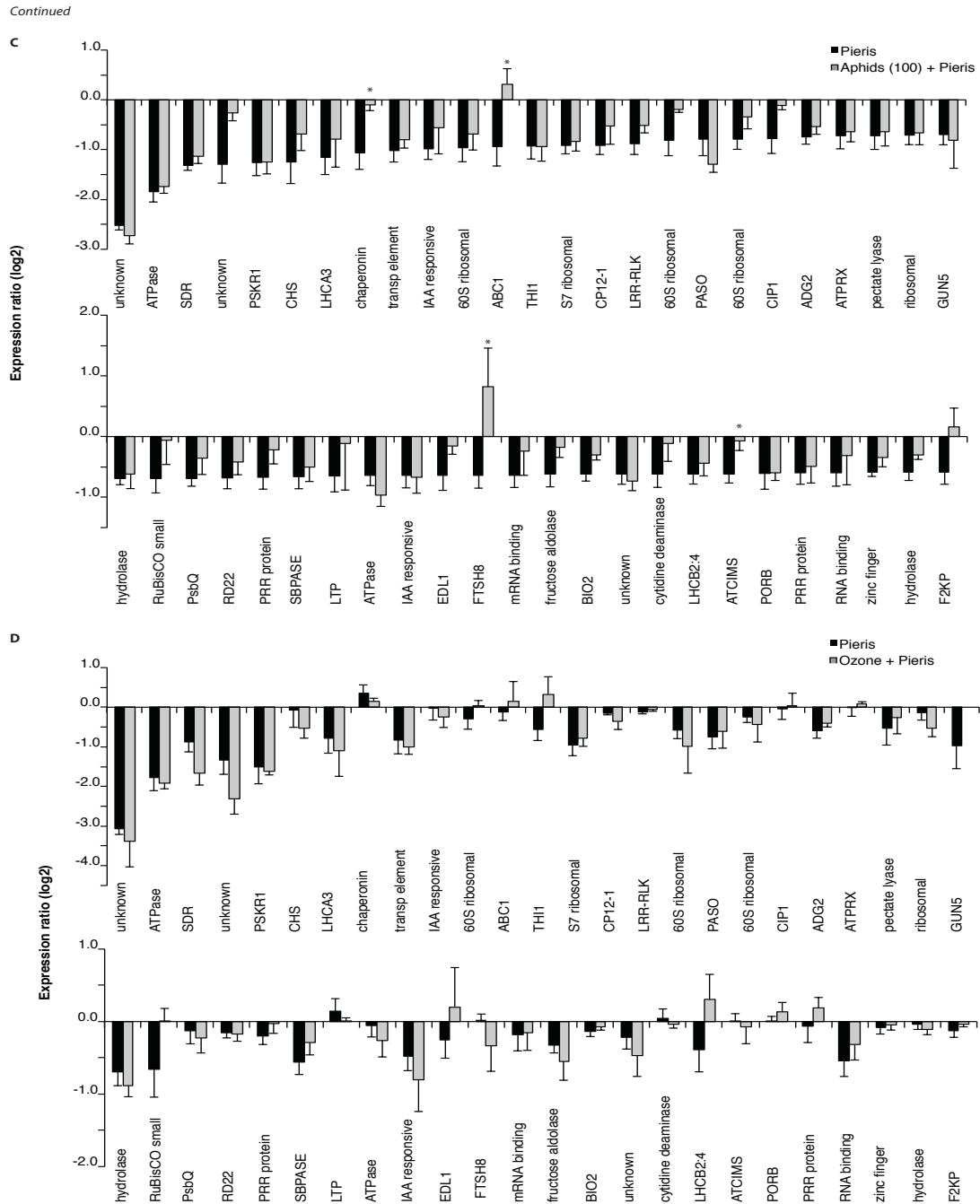
Expression ratio ( $\log_2$ ) of the 50 most induced genes after 24h *P. brassicae* feeding are compared to the expression ratio after the dual stress in 5 week-old *B. nigra* plants.

- A- Comparison between *P. brassicae* and egg extract / *P. brassicae*.
- B- Comparison between *P. brassicae* and *Xcc* / *P. brassicae*.
- C- Comparison between *P. brassicae* and *B. brassicae* (100 aphids) / *P. brassicae*.
- D- Comparison between *P. brassicae* and ozone / *P. brassicae*.

Values are the mean ( $\pm$  SE) of at least five biological replicates. Statistical differences between herbivory and dual stress are indicated (Student's *t*-test, \* P-value < 0.05; \*\* P-value < 0.01; \*\*\* P-value < 0.001).







**Figure S2: Top 50 repressed genes after *P. brassicae* feeding compared to dual stress.**

Expression of the 50 most repressed genes after 24 hours *P. brassicae* feeding are compared to the expression of dual stress in 5 week-old *B. nigra* plants.

- A- Comparison between *P. brassicae* and egg extract / *P. brassicae*.
- B- Comparison between *P. brassicae* and *Xcc* / *P. brassicae*.
- C- Comparison between *P. brassicae* and *B. brassicae* (100 aphids) / *P. brassicae*.
- D- Comparison between *P. brassicae* and ozone / *P. brassicae*.

Values are the mean ( $\pm$  SE) of at least five biological replicates. Statistical differences between herbivory and dual stress are indicated (Student's *t*-test, \* P-value < 0.05; \*\* P-value < 0.01; \*\*\* P-value < 0.001).

**Table S3: Genes differentially expressed between *P. brassicae* and dual stress *Xcc* followed by *P. brassicae*.**

AGI code	Gene name <sup>a</sup>	Pieris <sup>b</sup>	P - value	<i>Xcc</i> + Pieris <sup>b</sup>	P - value	P - value Student's <i>t</i> -test
At1g52400	BGL1	2.594	0.000	1.317	0.016	0.007
At1g17420	LOX3	2.310	0.000	0.969	0.003	0.003
At4g23600	CORI3	1.697	0.001	0.561	0.205	0.032
At5g20230	ATBCB	1.660	0.000	0.341	0.259	0.006
At3g57450	Unknown	1.568	0.001	0.592	0.006	0.021
At5g57560	TCH4	1.562	0.001	-0.539	0.260	0.001
At4g27280	Ca-binding	1.527	0.001	0.355	0.214	0.013
At5g45340	CYP707A3	1.477	0.002	-0.236	0.526	0.003
At5g64260	P-responsive protein	1.473	0.002	0.395	0.109	0.018
At3g19710	BCAT4	1.468	0.000	0.597	0.002	0.001
At1g66690	SAM	1.451	0.001	0.151	0.251	0.002
At2g06050	OPR3	1.318	0.006	0.321	0.236	0.045
At5g48850	MS5	1.315	0.000	0.155	0.706	0.006
At2g38240	2OG-Fe-oxy	1.314	0.001	0.231	0.255	0.006
At4g15960	Epoxide hydrolase	1.278	0.001	0.359	0.079	0.007
At1g12500	P transporter	1.212	0.004	0.283	0.024	0.019
At4g23100	PAD2	1.182	0.001	0.391	0.085	0.019
At5g43880	Unknown	1.167	0.004	-0.779	0.067	0.001
At2g41430	ERD15	1.128	0.002	-0.548	0.049	0.000
At3g57930	Unknown	1.122	0.002	-0.133	0.690	0.006
At1g08930	ERD6	1.068	0.011	-0.364	0.329	0.009
At5g62570	CBP60A	1.063	0.002	-0.184	0.637	0.009
At3g04717	Unknown	1.056	0.036	2.621	0.008	0.048
At2g28520	VHA-A1	1.049	0.007	0.083	0.171	0.012
At5g67300	ATMYB44	1.045	0.000	-0.032	0.922	0.005
At2g15900	PHOX	1.039	0.019	-0.301	0.233	0.011
At1g73630	Ca-binding	0.997	0.004	-0.064	0.516	0.003
At1g55020	LOX1	0.983	0.004	0.160	0.235	0.014
At1g19550	Glutathione S-transferase	0.964	0.000	0.221	0.333	0.004
At3g26980	MUB4	0.962	0.008	-0.121	0.050	0.004
At5g36910	THI2.2	0.957	0.008	0.016	0.363	0.009
At1g32640	ATMYC2	0.936	0.000	0.280	0.365	0.024
At5g01710	Methyltransferases	0.933	0.012	-0.339	0.085	0.003
At1g67310	Calmodulin-binding	0.890	0.006	-0.516	0.255	0.007
At3g49720	CGR2	0.874	0.001	-0.230	0.268	0.001
At4g19200	Unknown	0.858	0.011	0.016	0.960	0.050
At3g28450	BIR2	0.853	0.001	0.277	0.149	0.023
At5g47240	ATNUDT8	0.850	0.001	0.031	0.363	0.001
At3g51660	MIF superfamily	0.849	0.001	0.248	0.257	0.033
At4g37270	HMA1	0.813	0.002	-0.400	0.404	0.014
At3g45640	ATMPK3	0.802	0.034	-0.078	0.512	0.034
At2g32150	HAD	0.800	0.006	-0.707	0.046	0.001
At3g25780	AOC3	0.759	0.035	0.007	0.363	0.047
At5g28540	BIP1	0.751	0.006	1.709	0.003	0.017
At1g74620	RING/U box	0.744	0.049	-0.113	0.491	0.047
At1g02660	Lipase	0.743	0.016	-0.456	0.051	0.002
At4g25030	Unknown	0.729	0.000	0.057	0.797	0.012
At1g29690	CAD1	0.725	0.002	-0.288	0.054	0.000
At5g12140	ATCYS1	0.723	0.000	0.207	0.292	0.019
At1g21000	PLATZ	0.698	0.007	0.008	0.970	0.027
At5g09960	Unknown	0.664	0.000	-0.433	0.026	0.000
At5g52320	CYP96A4	0.662	0.017	0.041	0.363	0.029
At1g74100	ATSOT16	0.646	0.004	0.213	0.080	0.049
At1g07890	APX1	0.642	0.001	-0.003	0.992	0.038
At1g01140	CIPK9	0.632	0.013	-0.318	0.111	0.004
At1g27770	ACA1	0.630	0.010	-0.349	0.033	0.001
At3g47960	ATNPF2.10	0.620	0.001	0.129	0.307	0.009
At5g63370	CDKG1	0.617	0.014	-0.196	0.180	0.006
At1g78370	ATGSTU20	0.591	0.002	-0.263	0.422	0.014
At2g01540	CAR10	0.587	0.004	0.124	0.157	0.021

<sup>a</sup> List of the 60 induced genes after herbivory differentially expressed in the combined stresses *Xcc* and *P. brassicae*.

<sup>b</sup> Mean expression ratios calculated from at least five biologically independent experiments.

Table S4: Genes commonly differentially regulated between dual stresses.

AGI code	Name	Pieris			Egg extract + Pieris			Xcc + Pieris			B. brassicae + Pieris				
		Mean	SE	P-value	Mean	SE	P-value vs P	Mean	SE	P-value vs P	Mean	SE	P-value vs P		
A12g40205	<b>RPL41C</b>	0.700	0.301	0.053	1.639	0.169	0.001	1.066	0.155	0.001	0.348	0.784	0.121	0.003	0.837
A15g42635	Glycine-rich protein	1.114	0.268	0.004	1.019	0.335	0.038	1.119	0.109	0.000	0.990	0.889	0.150	0.004	0.548
A15g05600	Oxidoreductase	1.101	0.258	0.004	1.323	0.393	0.028	0.928	0.175	0.003	0.617	2.381	0.372	0.003	<b>0.014</b>
A11g07600	Metallothionein	1.081	0.280	0.006	1.472	0.417	0.024	0.810	0.293	0.039	0.523	1.366	0.354	0.018	0.540
A13g04717	CBP20 precursor	1.056	0.409	0.036	3.874	0.555	0.002	2.621	0.162	0.008	<b>0.048</b>	1.134	0.309	0.021	0.895
A11g17420	LOX3	2.310	0.280	0.000	1.721	0.601	0.046	0.969	0.178	0.003	<b>0.003</b>	3.514	0.249	0.000	<b>0.013</b>
A11g24909	Anthraniolate synthase	1.065	0.264	0.005	1.563	0.409	0.019	0.936	0.365	0.050	0.774	1.468	0.350	0.014	0.373
A13g45140	LOX2	1.559	0.182	0.000	2.528	0.681	0.021	2.004	0.671	0.031	0.480	2.372	0.336	0.002	<b>0.040</b>
<b>A12g33240</b>	<b>XID</b>	0.497	0.164	0.019	1.253	0.389	0.032	0.775	0.177	0.007	0.276	0.642	0.181	0.024	0.576
<b>A14g30910</b>	<b>Cytosol aminopeptidase</b>	0.217	0.168	0.237	1.106	0.382	0.044	0.838	0.302	0.039	0.079	0.633	0.210	0.040	0.152
A15g28540	BIP1	0.751	0.191	0.006	1.548	0.410	0.020	1.709	0.308	0.003	<b>0.017</b>	1.925	0.247	0.001	<b>0.003</b>
A11g52400	BGL1	2.594	0.197	0.000	2.260	0.272	0.001	1.317	0.371	0.016	<b>0.007</b>	2.910	0.268	0.000	0.353
A14g23880	Unknown	1.008	0.266	0.007	1.078	0.357	0.039	0.684	0.152	0.006	0.875	1.087	0.239	0.010	0.841
A12g04220	Unknown	0.727	0.201	0.009	1.302	0.421	0.037	0.717	0.221	0.023	0.709	1.033	0.113	0.001	0.559
A11g07600	MT1A	0.844	0.234	0.009	1.305	0.169	0.002	0.187	0.221	0.008	0.221	-0.805	0.162	0.008	0.514
A12g10270	Transposable element gene	-1.021	0.229	0.003	-0.762	0.272	0.049	-0.638	0.151	0.008	0.916	-0.741	0.135	0.000	0.731
A13g28540	ATPase family protein	-1.843	0.210	0.000	-1.540	0.162	0.001	-1.813	0.173	0.000	0.104	-0.716	0.188	0.019	0.201
<b>A14g20890</b>	<b>TUB9</b>	-0.461	0.093	0.002	-1.204	0.380	0.034	-0.732	0.128	0.002	<b>0.014</b>	-2.732	0.162	0.000	0.252
A13g53490	Unknown	-2.515	0.101	0.000	-2.724	0.342	0.001	-3.100	0.194	0.000	<b>0.014</b>	-2.732	0.162	0.000	0.252
A14g20760	SDR family protein	-1.316	0.100	0.000	-1.660	0.191	0.001	-1.582	0.224	0.001	0.258	-1.128	0.148	0.002	0.296

AGI code	Name	Egg extract			Xcc			B. brassicae						
		Mean	SE	P-value	Mean	SE	P-value vs XP	Mean	SE	P-value vs AP				
A12g40205	<b>RPL41C</b>	1.382	0.332	0.014	0.509	0.238	0.245	0.385	0.064	0.153	0.091	0.414	0.002	0.002
A15g42635	Glycine-rich protein	0.445	0.238	0.135	0.200	0.206	0.222	0.406	<b>0.004</b>	0.083	0.326	0.195	<b>0.001</b>	0.001
A15g05600	Oxidoreductase	0.094	0.144	0.551	<b>0.019</b>	0.000	0.000	1.000	<b>0.001</b>	0.797	0.368	0.096	0.297	0.007
A11g07600	Metallothionein	0.231	0.531	0.686	0.104	-0.032	0.613	0.961	0.221	0.019	0.199	0.374	<b>0.007</b>	<b>0.008</b>
A13g04717	CBP20 precursor	3.376	0.755	0.011	0.609	1.233	0.656	0.134	0.160	1.744	0.441	0.017	<b>0.008</b>	0.514
A11g17420	LOX3	0.775	0.741	0.355	0.351	0.279	0.198	0.232	<b>0.029</b>	1.059	0.485	0.094	0.514	0.011
A11g24909	Anthraniolate synthase	0.437	0.654	0.541	0.182	0.328	0.238	0.240	0.216	1.003	0.243	0.015	0.201	0.011
A13g45140	LOX2	-1.307	0.310	0.001	<b>0.001</b>	1.050	0.719	0.218	0.358	0.302	0.164	0.140	0.201	0.011
<b>A12g33240</b>	<b>XID</b>	1.649	0.172	0.001	0.379	-0.058	0.058	0.374	<b>0.003</b>	0.285	0.172	0.173	0.237	0.002
<b>A14g30910</b>	<b>Cytosol aminopeptidase</b>	0.636	0.348	0.141	0.391	0.478	0.246	0.124	0.393	0.674	0.347	0.124	<b>0.019</b>	<b>0.000</b>
A15g28540	BIP1	0.426	0.414	0.361	<b>0.000</b>	0.012	0.012	0.374	<b>0.001</b>	0.000	0.000	1.000	<b>0.000</b>	<b>0.002</b>
A14g23880	Unknown	0.808	0.341	0.077	0.599	0.128	0.128	0.374	<b>0.022</b>	0.000	0.000	1.000	<b>0.002</b>	0.115
A11g52400	BGL1	-0.535	0.303	0.153	0.090	0.000	0.000	1.000	<b>0.011</b>	0.000	0.000	1.000	<b>0.000</b>	0.362
A12g04220	Unknown	1.037	0.203	0.007	0.586	0.266	0.038	0.063	<b>0.038</b>	0.247	0.167	0.214	0.115	0.115
A11g07600	MT1A	0.804	0.250	0.032	0.136	0.963	0.378	0.063	<b>0.038</b>	0.764	0.255	0.040	0.763	0.763
A12g10270	Transposable element gene	-1.143	0.299	0.019	0.374	-1.401	0.261	0.006	<b>0.027</b>	-0.927	0.353	0.059	0.709	0.709
A13g28540	ATPase family protein	-2.110	0.190	0.000	0.052	-2.290	0.153	0.000	0.073	-1.584	0.385	0.015	0.220	0.220
<b>A14g20890</b>	<b>TUB9</b>	-0.818	0.188	0.012	0.389	-0.921	0.251	0.021	0.495	-0.360	0.190	0.132	<b>0.024</b>	<b>0.024</b>
A13g53490	Unknown	-2.632	0.114	0.000	0.809	-3.305	0.219	0.000	0.500	-1.480	0.421	0.025	<b>0.024</b>	<b>0.024</b>
A14g20760	SDR family protein	-1.299	0.164	0.001	0.190	-1.045	0.379	0.051	0.235	-0.258	0.112	0.082	<b>0.002</b>	<b>0.002</b>

List of the 20 differentially regulated genes common after combined stresses and their respective expression ratio in single stresses. Mean expression ratios, in log<sub>2</sub>, calculated from at least five biologically independent experiments. Values with a Student's t-test P-value < 0.05 are represented in bold.

**Table S5: Expression ratios of genes belonging to the JA signaling pathway in *P. brassicae* herbivory or combined stresses.**

AGI code	Gene name <sup>a</sup>	P <sup>b</sup>	P-value	EP <sup>b</sup>	P-value	t-test	XP <sup>b</sup>	P-value	t-test	AP <sup>b</sup>	P-value	t-test
At1g55020	LOX1	<b>0.98</b>	0.004	0.72	0.208	0.581	0.16	0.235	0.014	<b>1.03</b>	0.034	0.914
At3g45140	LOX2	<b>1.56</b>	0.000	<b>2.53</b>	0.021	0.119	<b>2.00</b>	0.031	0.480	<b>2.37</b>	0.002	0.040
At1g17420	LOX3	<b>2.31</b>	0.000	<b>1.72</b>	0.046	0.337	<b>0.97</b>	0.003	0.003	<b>3.51</b>	0.000	0.013
At1g67560	LOX6	0.18	0.391	0.03	0.594	0.570	0.00	1.000	0.448	0.16	0.203	0.930
At5g42650	AOS	0.21	0.039	0.79	0.066	0.052	0.00	1.000	0.051	0.33	0.081	0.463
At3g25760	AOC1	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At3g25770	AOC2	-0.01	0.351	0.02	0.374	0.158	0.00	1.000	0.408	0.00	1.000	0.453
At3g25780	AOC3	<b>0.76</b>	0.035	0.47	0.140	0.507	0.01	0.363	0.047	0.51	0.260	0.617
At1g13280	AOC4	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At1g76680	OPR1	0.11	0.375	-0.21	0.547	0.290	0.11	0.486	0.968	0.07	0.568	0.840
At1g76690	OPR2	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At2g06050	OPR3	<b>1.32</b>	0.006	<b>0.90</b>	0.024	0.407	0.32	0.236	0.045	<b>1.86</b>	0.024	0.381
At1g19640	JMT	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At2g39940	COI1	0.04	0.849	0.41	0.389	0.375	-0.16	0.193	0.413	-0.41	0.009	0.102
At2g46370	JAR1	0.09	0.176	-0.11	0.507	0.179	-0.08	0.332	0.097	<b>0.66</b>	0.028	0.006
At5g03730	CTR1	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At1g66340	ERT1	-0.11	0.351	-0.16	0.420	0.802	0.08	0.363	0.213	0.01	0.941	0.466
At2g40940	ERS1	-0.09	0.118	0.01	0.972	0.602	0.00	1.000	0.152	0.17	0.221	0.039
At5g03280	EIN2	-0.48	0.003	-0.73	0.142	0.468	-0.11	0.231	0.025	-0.25	0.053	0.155
At3g20770	EIN3	0.37	0.158	-0.18	0.764	0.317	-0.06	0.636	0.165	0.20	0.382	0.623
At1g12920	ERF1-2	0.85	0.088	0.73	0.417	0.884	<b>1.05</b>	0.010	0.731	<b>1.08</b>	0.017	0.708
At1g72260	THI2.1	<b>1.20</b>	0.011	1.58	0.074	0.583	0.68	0.100	0.327	<b>1.08</b>	0.026	0.822
At5g44420	PDF1.2	0.24	0.351	1.20	0.076	0.079	<b>1.48</b>	0.007	0.010	0.25	0.530	0.983
At5g24770	VSP2	<b>1.88</b>	0.000	1.53	0.150	0.657	<b>1.12</b>	0.043	0.139	<b>1.52</b>	0.014	0.448
At1g32640	MYC2	<b>0.94</b>	0.000	<b>0.66</b>	0.011	0.090	0.28	0.365	0.024	<b>1.14</b>	0.004	0.277
At5g46760	MYC3	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At4g17880	MYC4	0.00	1.000	-0.03	0.374	0.220	0.00	1.000	1.000	-0.10	0.374	0.220
At1g17380	JAZ5	0.01	0.351	0.03	0.374	0.534	0.00	1.000	0.408	0.00	1.000	0.453
At1g74950	JAZ2	0.10	0.110	0.10	0.601	0.979	0.12	0.363	0.869	0.11	0.192	0.918
At3g17860	JAZ3	0.17	0.138	0.54	0.077	0.109	0.00	1.000	0.177	0.15	0.374	0.908
At1g48500	JAZ4	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.00	1.000	1.000
At1g72450	JAZ6	0.00	1.000	0.00	0.374	0.220	0.00	1.000	1.000	0.00	1.000	1.000
At2g34600	JAZ7	0.02	0.351	0.00	1.000	0.453	0.00	1.000	0.408	0.00	1.000	0.453
At1g30135	JAZ8	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	-0.06	0.374	0.220
At1g70700	JAZ9	0.77	0.067	<b>1.17</b>	0.021	0.458	0.06	0.310	0.117	1.31	0.092	0.421
At5g13220	JAZ10	0.01	0.171	0.07	0.374	0.320	0.00	1.000	0.215	0.11	0.114	0.046
At3g43440	JAZ11	0.00	1.000	0.00	1.000	1.000	0.00	1.000	1.000	0.03	0.374	0.220
At5g20900	JAZ12	0.09	0.083	0.10	0.136	0.978	-0.04	0.147	0.037	0.09	0.582	0.985

<sup>a</sup> List of genes belonging to the JA signaling pathway.

<sup>b</sup> Mean expression ratios calculated from at least five biologically independent experiments. Expression ratios with a P-value < 0.05 were bolded.

Expression ratio between *P. brassicae* single stress (P) and the dual stress: egg extract / *P. brassicae* (EP), *Xcc* / *P. brassicae* (X), 100 *B. brassicae* / *P. brassicae* (AP) was compared using a Student's *t*-test (*t*-test).



## Chapter II:

# Effect of pretreatments on insect performance and plant defense in *Brassica nigra*

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**Abstract:** Plants face multiple attackers in nature or in fields and have developed different arms to decrease pathogen infection or insect feeding. Constitutive defense is the first barrier and is comprised of secondary metabolites toxic to a large range of bacteria, fungi or insects. In this category, the most important in Brassicaceae species are glucosinolates (GS). GS are known to be important in fighting herbivores but also pathogens. The second barrier consists of inducible defenses that are triggered specifically after perception and recognition of the invader through phytohormone signaling pathways. The jasmonic acid (JA) pathway is activated after herbivore feeding, both JA and ethylene (ET) pathways after a necrotrophic infection, and the salicylic acid (SA) pathway after a biotrophic invasion or oviposition by *P. brassicae* butterflies. Notably, in the case of dual stresses, some antagonistic or synergistic effects can occur between each phytohormone pathway. In this study, we focused on the effect of single and dual stresses on plant defense response and insect performance in *Brassica nigra*. Interestingly, *Pieris brassicae* and *Spodoptera littoralis* larvae were both affected by *P. brassicae* eggs or *Xanthomonas campestris* pv. *campestris* (*Xcc*) pretreatments and performed differently depending on the stress applied. Surprisingly, *Xcc* pretreatment had an opposite effect on insect performance when larvae were constrained to feed on infected tissue, compared to insect feeding freely on the whole-leaf. Moreover, we demonstrated that SA, JA and GS, as well as SA and JA marker genes, accumulated specifically at the site of treatment. SA / JA crosstalk occurred in *B. nigra* but the antagonistic effect of SA or JA depended on the pretreatment and on the insect applied. Thus, we show that plant defense response can vary in the same leaf and is highly specific to the history of attacks.



## Introduction

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Biotic and abiotic stresses have a negative impact on plant fitness. Without control measures, crop losses are evaluated at around 50 to 80 % of their potential yield (Bruce, 2010). Plants have no immunity specialized cells, and thus after each pathogen or insect attack every damaged plant cell reprograms its normal cellular activity to defense. Phytohormones are small signal molecules, important during all stages of plant life: for growth and reproduction but also for defense. Variation of hormone concentration after recognition of (a)biotic stress triggers an adaptive plant response (Spoel and Dong, 2008; Pieterse *et al.*, 2009). The main phytohormones implicated in plant defense are salicylic acid (SA), which plays a role against biotrophic attackers and after insect oviposition, jasmonic acid (JA), which is involved against herbivory and, both JA and ethylene (ET), which are used to fight necrotrophs (Pieterse *et al.*, 2009; Bruessow *et al.*, 2011). Through crosstalk between phytohormone pathways, plants have a flexible and finely regulated network to respond to multiple stresses. Crosstalk can have synergistic or antagonistic effects (Glazebrook *et al.*, 2003; Spoel and Dong, 2008; Pieterse *et al.*, 2009; Thaler *et al.*, 2012). For example, the SA pathway is triggered by *Pieris brassicae* egg deposition onto *Arabidopsis* leaves and inhibits the expression of JA-responsive genes, providing an advantage for some insect herbivores (Bruessow *et al.*, 2011). Moreover, pathogens and insects are known to manipulate plant defense signaling network to their own benefit (Spoel and Dong, 2008; Pieterse *et al.*, 2009; Denancé *et al.*, 2013). On the other hand, activation of plant defense by an (a)biotic stress can have an impact on the fitness of another pathogen or insect (Al-Naemi and Hatcher, 2013; Lazebnik *et al.*, 2014; Cheruiyot *et al.*, 2015). A recent study in *Nicotiana attenuata* showed that a bacterial quorum-sensing compound increased *Manduca sexta* performance when caterpillars fed on infected plants, potentially through inhibition of the JA signaling pathway (Heidel *et al.*, 2009). On the contrary, Hilfiker and coworkers (2014) demonstrated that *P. brassicae* larvae performed less well on *Arabidopsis* leaves pretreated with *Pseudomonas syringae* bacteria (Hilfiker *et al.*, 2014). Moreover, *Pieris rapae* increased plant resistance against bacteria such as *Xanthomonas campestris* and *P. syringae* (De Vos *et al.*, 2006). Effects of a first stress on plant defense to a second attacker seem thus highly complex and difficult to predict. Analysis of phytohormones concentration and marker genes of SA, JA, or ET signaling pathways may be useful to predict the impact of multiple stresses on plant defense and on each member of the phyllosphere.

Analysis of secondary metabolites provides another way to understand plant defense against successive stresses. An important defense compound family that is only present in the order of Brassicales is glucosinolates (GS). GS are nitrogen- and sulfur-containing products that become toxic when tissue is damaged. During cell disruption, GS and their enzymes, myrosinases, are mixed and toxic breakdown compounds are then formed (Wittstock and Halkier,



2002; Sønderby *et al.*, 2010; Hopkins *et al.*, 2009). Each Brassicaceae plant species contains a few selected compounds, whereas the GS family is composed of more than 120 different members. GS products can differ between species, ecotypes and individuals depending on the environment, development stages or also on the type of tissue (Redovnikovi *et al.*, 2008). GS were discovered to be important against herbivores (Wittstock and Gershenzon, 2002; Smallegange *et al.*, 2007; Hopkins *et al.*, 2009; Schweizer *et al.*, 2013a) and pathogens (Bednarek *et al.*, 2009; Clay *et al.*, 2009; Buxdorf *et al.*, 2013; Sotelo *et al.*, 2014). Interestingly, herbivores are not equal when facing GS. For more than 25 insect species in the Coleoptera, Lepidoptera and Diptera family GS act as potent oviposition and feeding stimulants, whereas they are repellent in others (Hopkins *et al.*, 2009). Specialists of the Brassicales have developed methods to digest or detoxify GS. Moreover, some insects avoid production of breakdown products thanks to their own feeding mode or sequester intact GS (Winde and Wittstock, 2011). In general, specialists are supposed to be less affected by host plant defenses than generalists (Ali and Agrawal, 2012). Although efforts have been made to understand the role of GS after a single stress, very little is known on the effect of multiple stresses on GS and their impact on plant defense.

In this chapter, we focus on the impact of a first stress, *P. brassicae* egg extract or infection by the bacterial *Xanthomonas campestris* pv. *campestris* (*Xcc*), on insect performance and the biosynthesis of hormones or defense metabolites. We use *Pieris brassicae* L., the large white butterfly, which is specialized on Brassicaceae. Interactions between this insect and its host plants have been explored in detail for many years and have become a model in the plant-insect field (Feltwell, 1982). For comparison, we also use the generalist *Spodoptera littoralis*, the Egyptian cotton leafworm. *Brassica nigra*, the black mustard, belongs to the Brassicaceae family. *B. nigra* is used as a plant model for single stress research at an ecological level, because it is closer to cultivated species than *Arabidopsis* (Broekgaarden *et al.*, 2011).

## Materials and Methods

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### 1- Biological material

Seeds of *Brassica nigra* were collected from a wild population near Wageningen University in The Netherlands. Plants were grown in growth chambers in L : D 16 h : 8 h 22 - 25 °C, 60 % relative humidity, under white fluorescent light ( $170 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Seeds were always stratified for 3 days at 4 °C after sowing.

Bacterial strains of *Xanthomonas campestris* pv. *campestris* were obtained from the Plant-Microbe Interactions group of Utrecht University (Netherlands) in -80 °C glycerol stocks containing King B liquid medium: 20 g / l peptone bacterio, 1.5 g / l dipotassium hydrogen phosphate, 1.5 g / l magnesium sulphate heptahydrate, 12 g / l agar, at a final pH of 7.2. Antibiotic used was rifampycine at 25  $\mu\text{g}$  / ml. Bacteria were transferred into 10 ml of liquid culture in King B with antibiotic and grown in a shaker at 28 °C, 200 rpm, during 48 h. For infection by infiltration, *Xcc* culture was centrifuged at 7000 rpm during 2 min. The supernatant was discarded and the pellet washed in 10 mM  $\text{MgCl}_2$  before centrifugation at 7000 rpm during 2 min. The supernatant was discarded and the pellet diluted in 10 mM  $\text{MgCl}_2$  to an OD 600 of 0.07 to obtain a concentration of  $10^7$  cfu / ml in the leaf.

*Pieris brassicae* colony was reared in 1 m<sup>3</sup> cages in the greenhouse. Larvae were fed with *Brassica oleracea* and butterflies with sugar/water. Eggs were collected from the plants and crushed with a pestle in Eppendorf tubes. After centrifugation (15 000 g, 3 min), the supernatant (egg extract) was stored at -20 °C.

*Spodoptera littoralis* (Egyptian cotton worm) eggs were obtained from Syngenta and stored at 10 °C until further use. Eggs were placed in a beaker covered with plastic film in an incubator at 28 °C for 48 h to allow hatching.

### 2- Plant treatments

Plants were five weeks old at the time of the treatment. For *P. brassicae* or *S. littoralis* feeding, 30 first-instar larvae were placed evenly, just after hatching, on the three highest fully developed leaves during 24 h.

For egg extract treatment, we added 12 egg extract droplets (2  $\mu\text{l}$ ) per leaf on the three highest leaves of each plant. Egg extract droplets were kept for three days.

For infection with *Xcc*, the three highest leaves were treated by three infiltrations per leaf of  $10^7$  cfu / ml using a 1 ml needleless syringe. Each infiltration zone represented a circle of 1.5 cm<sup>2</sup>. Samples were harvested three days after infection.

For egg extract treatment followed by *P. brassicae* or *S. littoralis* larval feeding, plants were exposed to 12 x 2  $\mu$ l of *P. brassicae* egg extract on the three highest leaves of each plant during three days. Thirty larvae freshly hatched were then placed on treated leaves for 24 h.

For *Xcc* followed by *P. brassicae* or *S. littoralis* larvae dual stress, plants were infected on the three highest leaves by three spots of  $10^7$  cfu/ml of bacteria during three days. Thirty larvae freshly hatched were then placed on treated leaves for 24 h.

As controls for the dual stress egg extract followed by insect, plants grown in the same conditions without any stress were used. As controls for the dual stress *Xcc* followed by insect, the same number of MgCl<sub>2</sub> infiltrations was done on the three highest leaves of each plant.

Each treatment was done on three different plants for each biological replicate. All experiments were repeated a minimum of three times independently at intervals of several weeks. Treated and control leaves were frozen in liquid nitrogen for RNA extraction.

### 3- Quantitative PCR

Total RNA was extracted from plant tissue using the ReliaPrep RNA tissue protocol (Promega). Five hundred nanograms of total RNA were transcribed to cDNA using M-MLV reverse transcriptase (Invitrogen) and oligo dT primers according to commercial instructions. cDNA synthesis was done in triplicates. RT-qPCR analysis was performed in a final volume of 25  $\mu$ l according to the Brilliant III Fast SYBR Green instruction manual (Agilent). Specific primers (Table 1) were designed on conserved sequences identified by multiple alignments of genes from different species of Brassica family. Sequences were found using the Brassica database ([www.brassicadb.org](http://www.brassicadb.org)). Each primer has a T<sub>m</sub> (melting temperature) of 60 °C and gives an amplicons length between 100 and 250 bp (base pairs) in the conserved part of the cDNA strand. The efficiency of each primer pair was calculated using a PCR product from a cDNA as a template. Five dilutions (0.1 pg /  $\mu$ l, 0.01 pg /  $\mu$ l, 1fg /  $\mu$ l, 0.1 fg /  $\mu$ l, 0.01 fg /  $\mu$ l) of the PCR product were done and a qPCR was performed. For each primer pair, a standard curve was designed from qPCR data in order to calculate the slope of the curve, and the efficiency was calculated with this formula:  $E = 10^{(-1/\text{slope})}$ . For normalization, SAND gene was used as housekeeping gene.

**Table 1: Primers used for gene expression analysis by qPCR.**

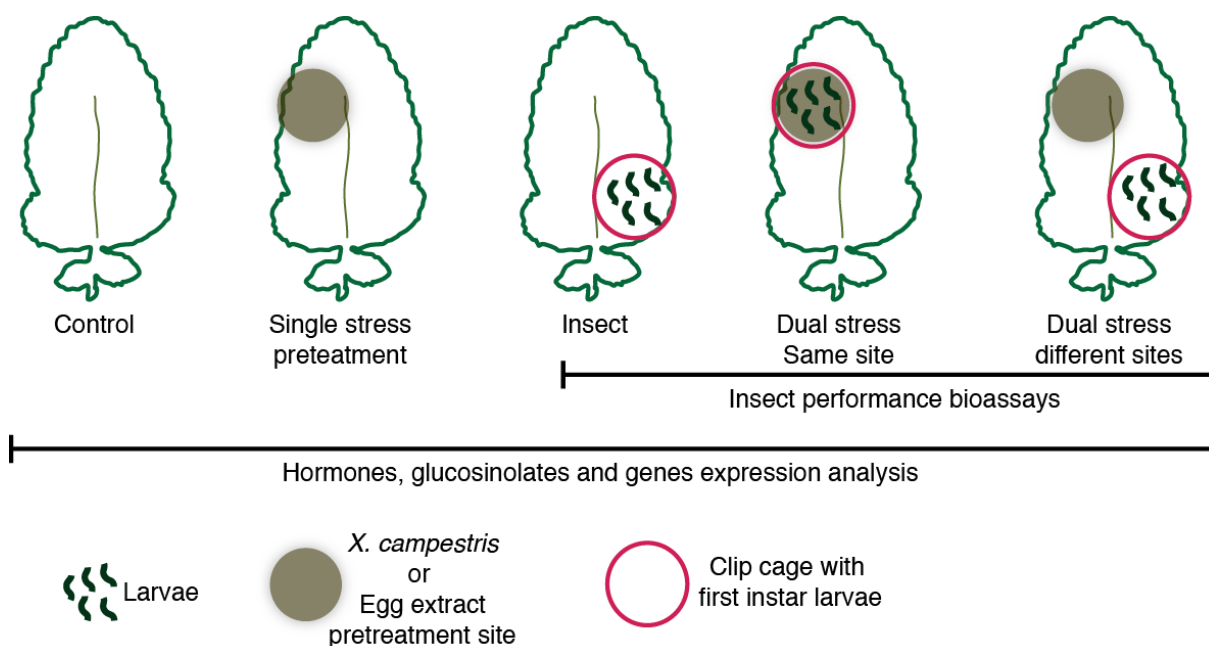
Involved in	Description	Gene Name	AGI	Efficacy	Primer seq 5'-3'	Amplicon size
JA	Jasmonate insensitive 1	MYC2	At1g32640	1.69	FW: GTGGAATCGAGCAAGAGGAA RV: ATCGTTAACCCCGACATACT	102 bp
JA	Vegetative storage protein	VSP2	At5g24770	1.85	FW: GGGAACTAGCCGAACCTTT RV: CGAAGTCCTTTGGCATAGAAA	200 bp
SA	Senescence-associated gene	SAG13	At2g29350	1.87	FW: AAACATGGAACCGTTTCC RV: TAGATGGATCCAACATTAATATG	241 bp
SA	Pathogenesis related gene 2	PR2	At3g57260	1.82	FW: GTGATAGATTCTTGGTAAGCA RV: ACCACGATTCCAACGATCC	230 bp
Housekeeping gene	SAND family protein	SAND	At2g28390	1.90	FW: TGCTTGGAGGGACAGATGC RV: AACCTTGTGCTGCACATTAG	247 bp

#### 4- Insect bioassays

Insect performance bioassays were done in two different ways. The first one was realized on entire leaves and the second with the help of a clip cage system. All were performed on 5-week-old *B. nigra* plants, in plastic tents of 60 x 60 x 60 cm (Bugdorm company) in a growth chamber (20 °C, 65 % relative humidity, 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 10 / 14 h photoperiod).

For insect bioassays performed on entire leaves after *Xcc* or *P. brassicae* eggs extract pretreatment during three days, ten neonate caterpillars were deposited on each of the three pretreated leaves for a total of 30 larvae per plant. As controls, plants were inoculated with  $\text{MgCl}_2$  for *Xcc* experiments and were not treated for egg extract experiments. Larval weight was measured after 7 days of feeding for *P. brassicae* and after 11 days for *S. littoralis*.

The second type of performance bioassay was accomplished with clip cages of 36.5 x 25.4 x 9.5 mm (BioQuip Products, Rancho Dominguez, USA). Plants were pretreated as described above with *Xcc* or *P. brassicae* egg extract during three days. Five neonate of *P. brassicae* or *S. littoralis* caterpillars were placed in a clip cage, on each of the three treated leaves for a total of 15 larvae per plant. Clip cages were placed at different sites in order to analyze plant response diffusion in the leaf, as described in Figure 1.



**Figure 1: Experimental design for insect performance bioassays, hormones and glucosinolates analyses using clip cages.**

Five first-instar larvae were placed in clip cages to force them to feed on pretreated site or on an adjacent site. For hormones, glucosinolates and gene expression analysis, pretreatment was performed during three days before larvae addition for 24 h. For insect performance bioassays, insects were added after 3 days of pretreatment. Larval weight was measured after 4 days for *P. brassicae* and after 7 days for *S. littoralis*. As control, plants were infiltrated with  $MgCl_2$  for *Xcc* experiments and untreated for egg extract experiments.

Control plants were inoculated with  $MgCl_2$  for *Xcc* experiments and were not treated for egg extract experiments. Larval weight was measured after 4 days for *P. brassicae* and after 7 days for *S. littoralis* (Figure 1).

### 5- Hormones, glucosinolates and gene expression analyses

For GS, hormones and RNA extraction, 5-week-old *B. nigra* plants were pretreated with *Xcc* or *P. brassicae* egg extract during three days before deposition of first-instar larvae during 24 h. Caterpillars were added freely on each of the three entire treated leaves or in a clip cage in the same way than described earlier for insects bioassays (Figure 1). Control plants were infiltrated with  $MgCl_2$  for *Xcc* analysis or untreated for *P. brassicae* egg extract experiments. After 24 h of feeding, entire leaves or 2.4 cm of leaf discs were harvested and frozen in liquid nitrogen (Figure 2). Three biologically independent replicates were analyzed for each treatment to quantify hormones, GS, and expression of marker genes. Extraction method, UHPLC-QTOFMS measurements and analysis were conducted as described earlier for hormones (Glauser *et al.*, 2014) and GS (Glauser *et al.*, 2012).

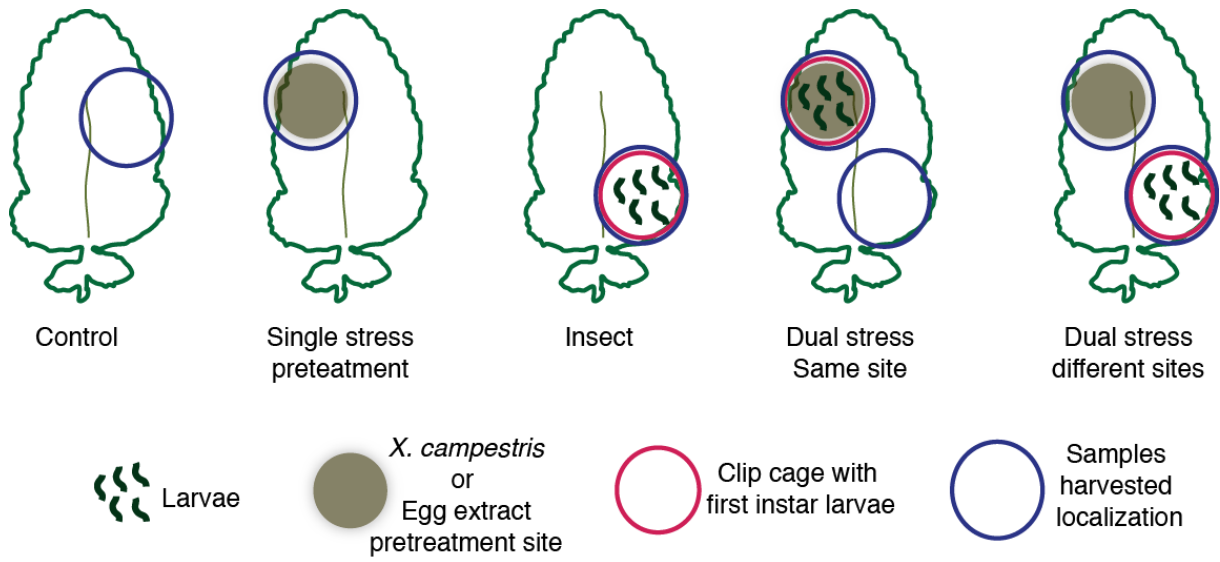


Figure 2: Localization of samples harvested using a clip cage experimental design.

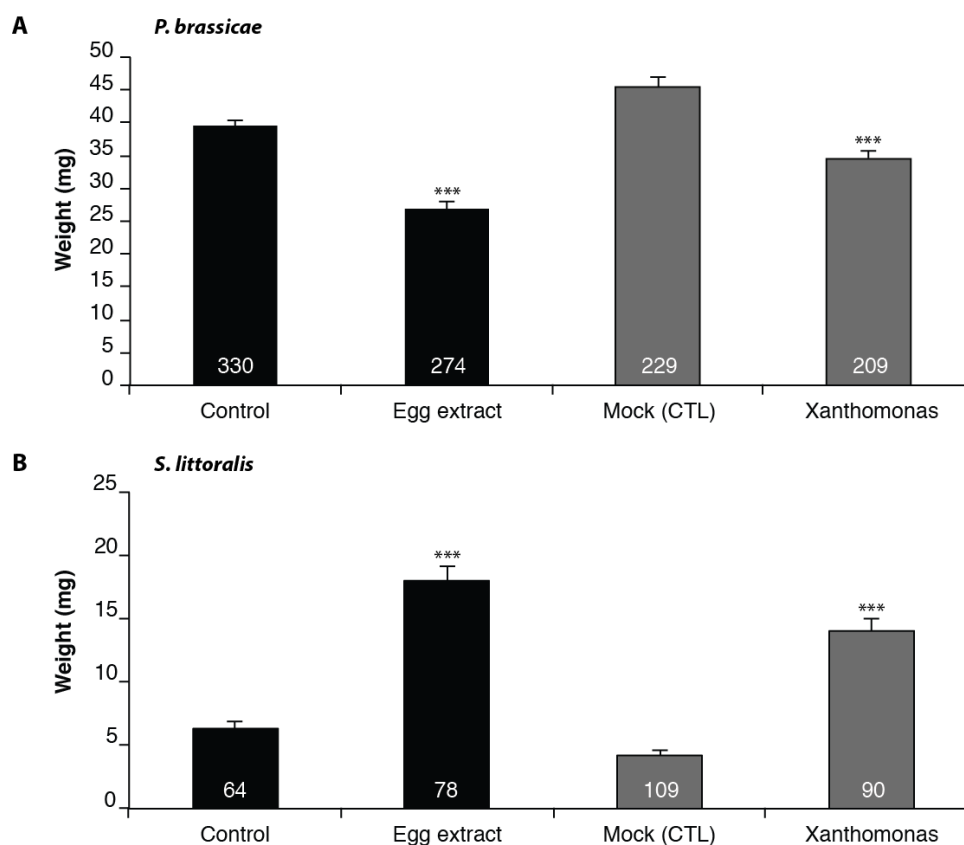
## Results

Transcriptomic analyses of different combined stresses in *B. nigra* revealed that pretreatments have a weak effect on insect induced-genes (Chapter I). In this chapter, we analyzed the effect of pretreatment on insect performance and on the concentration of phytohormones and GS after single or dual stresses.

### 1- Effect of pretreatment on plant defenses

#### a. Effect of egg extract or *Xcc* pretreatment on larval performance

Larval performance was measured using the specialist *P. brassicae* and the generalist *S. littoralis*.



**Figure 3: Impact of pretreatments on generalist and specialist larval performance.**

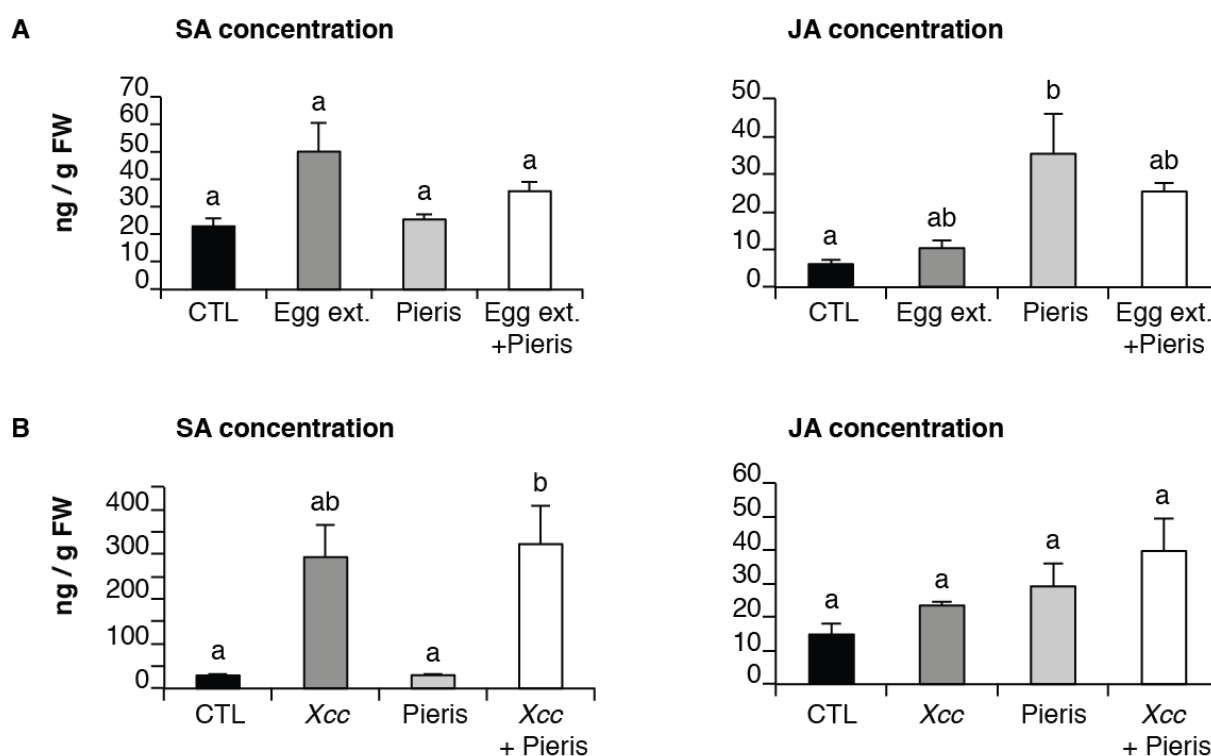
Performance of 1<sup>st</sup> instar larvae of the specialist *P. brassicae* (A) and the generalist *S. littoralis* (B) feeding on 5 week-old *B. nigra* plants pretreated with *P. brassicae* egg extract or *Xcc* (*Xanthomonas*) during 3 days.

Weight was measured 7 days (A) or 11 days (B) after larvae deposition. Values ( $\pm$  SE) are the mean of four (A) and three (B) independent experiments. The number of larvae is indicated in each column. Significant differences between control and pretreatment are indicated (Student's *t*-test, \*\*\* P-value < 0.001).

In case of *P. brassicae* egg extract or *Xcc* pretreatment, the performance of *P. brassicae* was significantly decreased in comparison to larvae feeding on control plants (Figure 3A). On the contrary, *S. littoralis* performance was significantly enhanced in *P. brassicae* egg extract- or *Xcc*-pretreated plants in comparison to untreated plants (Figure 3B).

### b. Effect of dual stresses on phytohormone levels

SA and JA were quantified in the entire leaf after *P. brassicae* egg extract and *Xcc* pretreatments followed by *P. brassicae* feeding. In the case of plants challenged by *P. brassicae* egg extract, *P. brassicae* larvae or dual stress, SA content did not differ significantly between each treatment. However, egg extract pretreatment had a tendency to increase SA levels. JA levels were significantly higher after single larval feeding but did not change in the other treatments (Figure 4 A).



**Figure 4: Hormones concentration after different treatments in whole-leaf of *B. nigra*.**

A- SA and JA concentration in control plants, after *P. brassicae* egg extract treatment during 3 days, *P. brassicae* feeding during 24 hours and dual stress, egg extract / *P. brassicae*.

B- SA and JA concentration in control plants, after *Xcc* treatment during 3 days, *P. brassicae* feeding during 24 hours and dual stress, *Xcc* / *P. brassicae*.

Values are the mean ( $\pm$  SE) of three biological replicates for each experiment. Statistical differences between treatments are indicated by letters (ANOVA followed by Tukey's honest significant difference test, (A - SA)  $F_{(3,7)} = 3.869$ , P-value = 0.0639; (A - JA)  $F_{(3,8)} = 5.877$ , P-value = 0.0202; (B - SA)  $F_{(3,7)} = 6.807$ , P-value = 0.0175; (B - JA)  $F_{(3,8)} = 2.72$ , P-value = 0.115).



The same analysis was made for *Xcc*, *P. brassicae* herbivory or the combined stress. A clear increase of SA content was observed after *Xcc* infiltration, with or without *P. brassicae* herbivory. For JA, there was no statistical difference between treatments (Figure 4 B). Large variation between samples from the same treatment was at the origin of the lack of statistical difference showed by the ANOVA test.

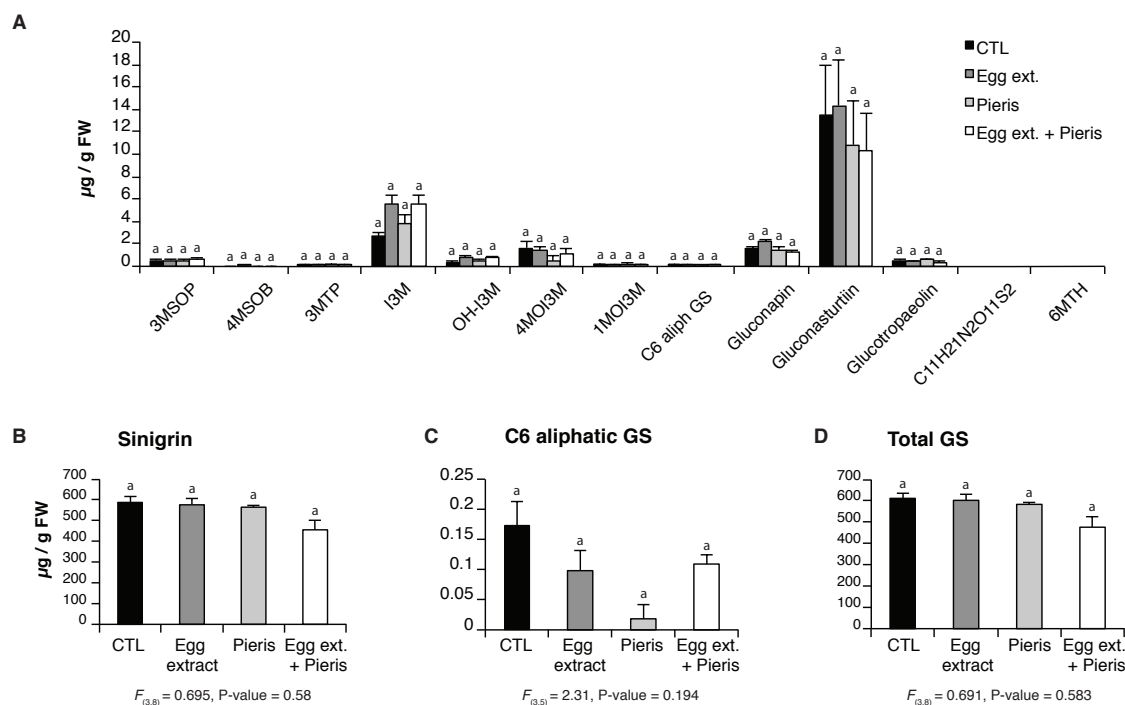
ABA concentration was also measured after different treatments. *P. brassicae* larvae, *P. brassicae* egg extract, *Xcc* or dual stresses application had no impact in the total amount of ABA which was around 90 ng / g FW (data not show).

Thus, combination of stresses had not a large effect on SA or JA concentration in whole *B. nigra* leaves and cannot explain the variation of herbivore performance.

### **c. Effect of dual stresses on glucosinolate levels**

Glucosinolates were quantified after *P. brassicae* egg extract or *Xcc* pretreatment, *P. brassicae* feeding and dual stresses on entire leaves.

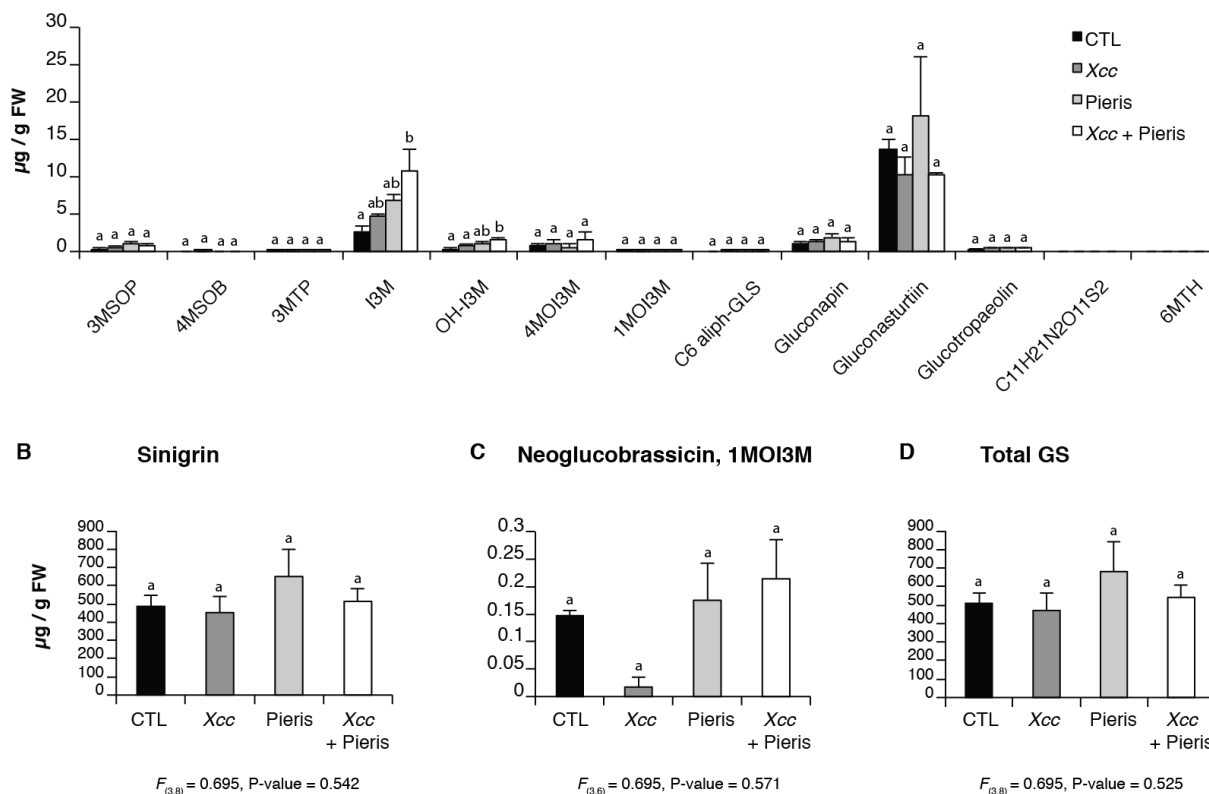
In all experiments, sinigrin represented more than 94 % of total GS amount in *B. nigra* (Figures 5 B and 6 B). In the case of experiments with *P. brassicae* egg extract, *P. brassicae* larvae or both treatments, only 2 compounds showed statistical differences between samples. The glucobrassicin I3M increased between control plant and egg extract treatment (2.7  $\mu\text{g}$  / g FW versus 5.6  $\mu\text{g}$  / g FW respectively; Student's *t*-test, P-value = 0.037; Figure 5 A) and the C6 aliphatic compound that decreased between control and *P. brassicae* treatment, with 0.17  $\mu\text{g}$  / g FW and 0.1  $\mu\text{g}$  / g FW respectively (Student's *t*-test, P-value = 0.023; Figure 5 C). However, no statistical difference was observed using ANOVA followed by Tukey's honest significant difference test (Figure 5). Moreover, no statistical difference was showed in the amount of the most abundant sinigrin or in the total amount of GS (Figure 5 B and 5 D).



**Figure 5: Quantification of glucosinolates after treatment with *P. brassicae* eggs, larvae or both stresses.**

Levels of glucosinolates were quantified in 5 week-old *B. nigra* treated with *P. brassicae* egg extract during 3 days, *P. brassicae* larvae during 24 hours and by the dual stress *P. brassicae* egg extract / *P. brassicae* feeding. Unchallenged plants were used as controls. All glucosinolates analyzed (A) except the most abundant, sinigrin (B); enlargement on C6 aliphatic GS (C) and total amount of GS (D) are represented. Values are the mean ( $\pm$  SE) of three biological replicates. Statistical differences between treatments are indicated by letters (ANOVA followed by Tukey's honest significant difference test, 3MSOP:  $F_{(3,8)} = 0.206$ , P-value = 0.889; 4MSOB:  $F_{(3,8)} = 1$ , P-value = 0.441; 3MTP:  $F_{(3,7)} = 1.162$ , P-value = 0.39; I3M:  $F_{(3,8)} = 3.754$ , P-value = 0.0598; OH-I3M:  $F_{(3,8)} = 2.741$ , P-value = 0.113; 4MOI3M:  $F_{(3,8)} = 1.784$ , P-value = 0.228; 1MOI3M:  $F_{(3,8)} = 0.7$ , P-value = 0.578; Gluconapin:  $F_{(3,8)} = 1.965$ , P-value = 0.198; Gluconasturtiin:  $F_{(3,8)} = 0.33$ , P-value = 0.804; Glucotropaeolin  $F_{(3,8)} = 1.589$ , P-value = 0.267; C11H21N2O11S2 and 6MTH, values = 0).

In experiments using *Xcc*, *P. brassicae* herbivory or dual stress, only 2 compounds showed a differential accumulation between treatments. Glucobrassicin (I3M) and hydroxyglucobrassicin (OH-I3M) were induced by dual stress in comparison to other treatments (Figure 6 A). Moreover, OH-I3M increased also between *Xcc* single treatment and the dual stress (Figure 6 A). On the contrary, neoglucobrassicin was reduced after *Xcc* treatment in comparison to control plants (Student's *t*-test, P-value = 0.003; Figure 6 C). Like for the previous analysis, we observed no statistical difference between samples for sinigrin or total GS content (Figure 6 B and 6 D).



**Figure 6: Quantification of glucosinolates after treatment with *Xcc*, *P. brassicae* larvae or both stresses.**

Levels of glucosinolates were quantified in 5 week-old *B. nigra* treated with *Xcc* during 3 days, *P. brassicae* larvae feeding during 24 hours or the dual stress *Xcc* / *P. brassicae*. Plants infiltrated with  $\text{MgCl}_2$  were used as controls. All glucosinolates analyzed (A) except the most abundant, sinigrin (B); enlargement on 1MOI3M GS (C) and total amount of GS (D) are represented. Values are the mean ( $\pm$  SE) of three biological replicates. Statistical differences between treatments are indicated by letters (ANOVA followed by Tukey's honest significant difference test, 3MSOP:  $F_{(3,8)} = 2.27$ , P-value = 0.157; 4MSOB:  $F_{(3,8)} = 1$ , P-value = 0.441; 3MTP:  $F_{(3,4)} = 0.161$ , P-value = 0.917; I3M:  $F_{(3,8)} = 5.345$ , P-value = 0.0259; OH-I3M:  $F_{(3,8)} = 8.432$ , P-value = 0.00737; 4MOI3M:  $F_{(3,5)} = 1.089$ , P-value = 0.434; C6 aliph-GLS:  $F_{(3,8)} = 0.575$ , P-value = 0.647; Gluconapin:  $F_{(3,8)} = 1.255$ , P-value = 0.353; Gluconasturtiin:  $F_{(3,8)} = 0.833$ , P-value = 0.513; Glucotropaeolin  $F_{(3,8)} = 0.537$ , P-value = 0.67; C11H21N2O11S2 and 6MTH, values = 0).

These results show that treatments have no major impact on GS concentration in whole *B. nigra* leaves and confirm our previous observation of a weak change in expression of GS biosynthesis genes in response to single or dual stresses (Chapter I, Figure 26). GS levels, like signaling hormones, cannot thus explain the variation of insect performance between untreated and pretreated plants.

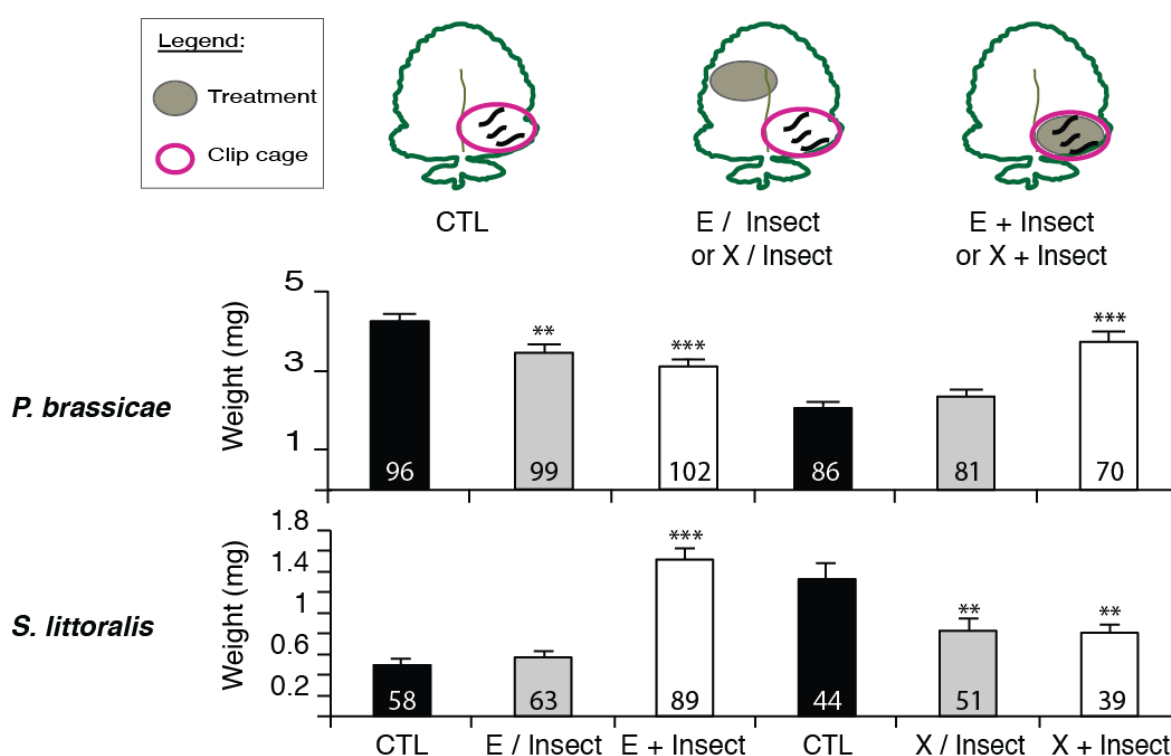
## 2- Impact of pretreatment on localized herbivory responses

Since the difference of larval performance between untreated and pretreated plants could not be easily explained by transcriptomic data, phytohormones concentration or by variation of GS amounts in whole *B. nigra* leaves, we then decided to study the effect of pretreatment localization

on feeding responses. The rationale was that locally-induced changes might be responsible for the observed effects.

### a. Herbivore performance depending on pretreatment site

Using a clip cage system, the impact of pretreatment localization on insect performance was analyzed. First-instar *P. brassicae* or *S. littoralis* larvae were constrained to feed at the site of pretreatment or on an untreated part of the leaf. After 4 days of feeding for the specialist and 7 days for the generalist caterpillar weight was measured (Figure 7).



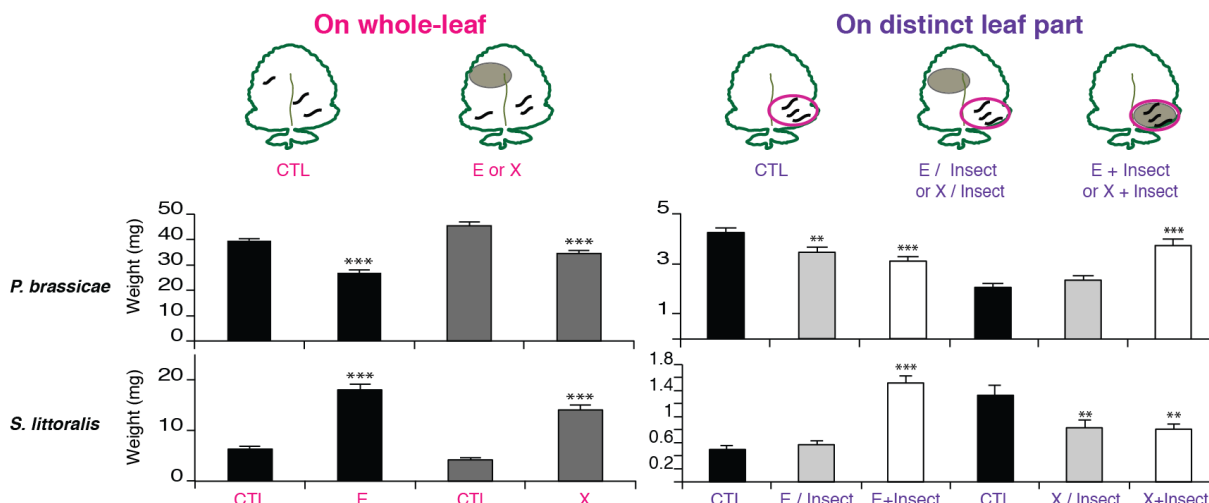
**Figure 7: Impact of pretreatment on insect performance using clip cages.**

Performance of 1<sup>st</sup> instar *P. brassicae* and *S. littoralis* after 4 and 7 days, respectively, in 5 week-old *B. nigra* plants pretreated during 3 days by *Xcc* (X) or by *P. brassicae* egg extract (E). Clip cages were placed on different sites on treated leaves. On mock treated ( $MgCl_2$ ) leaf, clip cages were placed at the infiltration site and for control leaf they were placed randomly. On *Xcc* or *P. brassicae* egg extract treated leaves, clip cages were placed on untreated part for half of the plants and labeled X / Insect or E / Insect. For the other half, clip cages were placed on the treated part and labeled X + Insect or E + Insect. Values ( $\pm$  SE) are the mean of at least three independent experiments. The number of larvae is indicated in each column. Significant differences between control and treatment are indicated (Student's *t*-test, \*\*, P-value < 0.01; \*\*\* P-value < 0.001).

In the case of *P. brassicae* egg extract pretreatment, specialist *P. brassicae* larvae showed a lower performance on treated plants, whether they fed on the site of pretreatment or not (Figure 7). Inversely, *S. littoralis* larvae gained more weight when they fed on the site of egg extract

pretreatment. However, their weight was not significantly different when they fed on control plants or on a site adjacent to the treatment (Figure 7).

For *Xcc* pretreatment, *P. brassicae* larvae were significantly heavier when they fed on the treated site but not when they fed on an adjacent site. On the opposite, *S. littoralis*, were significantly smaller on treated plants, irrespective of the feeding location (Figure 7).



**Figure 8: Impact of pretreatment on insect performance.**

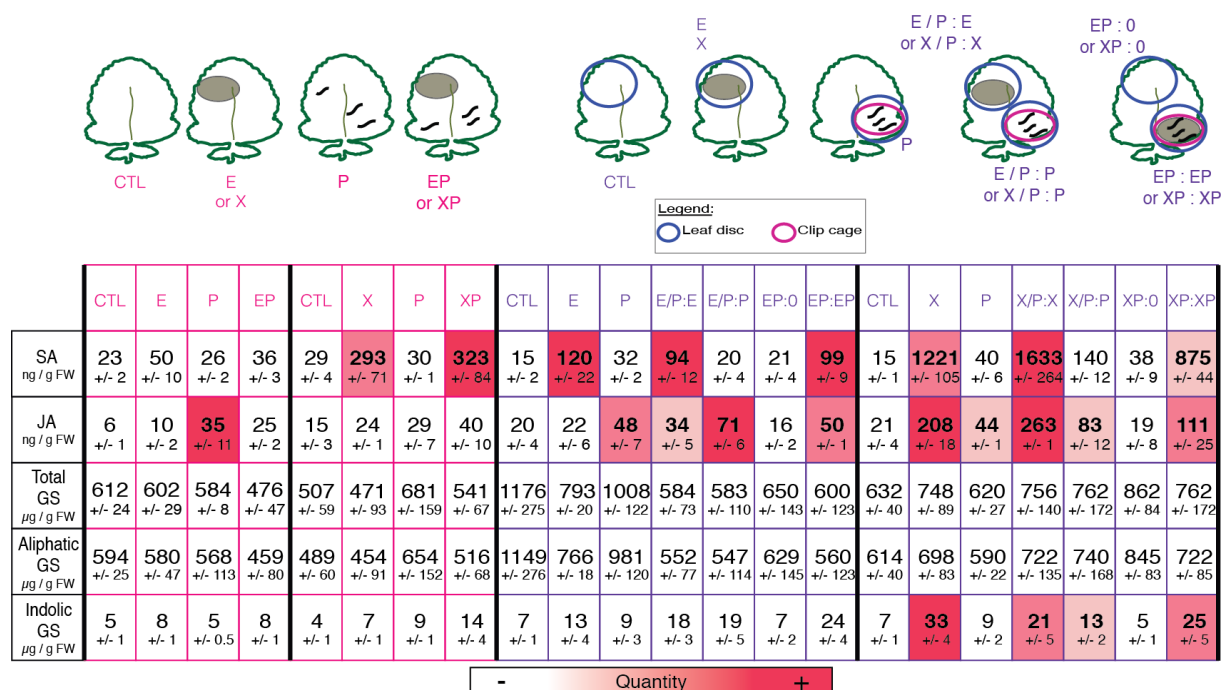
Larval performance of the specialist *P. brassicae* and the generalist *S. littoralis* were measured after *P. brassicae* egg extract (E) or *Xcc* (X) pretreatment during 3 days. The analysis was performed with neonates added to entire pretreated leaf or in specific parts of pretreated leaves (pink circles): adjacent to the site of pretreatments (E / Insect or X / Insect) or at the same site as pretreatments (E + Insect or X + Insect). Means ( $\pm$  SE) of three biological replicates are shown. Significant differences between control and treated plants are indicated (Student's *t*-test, \* P-value < 0.05, \*\* P-value < 0.01, \*\*\* P-value < 0.001).

In conclusion, performance of caterpillar depends on the type of pretreatment and on its localization. After *P. brassicae* egg extract treatment, herbivore performance was generally the same if bioassays were conducted with caterpillars moving freely on whole leaves or constrained to feed into egg-treated zone. In contrast, after *Xcc* pretreatment, herbivore performance gave opposite results if larvae fed freely on treated leaves or at the infiltration area (Figure 8).

#### **b. Impact of localized attack on hormone and glucosinolate accumulation**

Using clip cages, we analyzed SA, JA and GS concentrations at the site of single or dual stresses, or in adjacent sites. Samples from area treated by *P. brassicae* egg extract during 3 days (E), *Xcc* during 3 days (X) and *P. brassicae* feeding during 24 hours (P) were analyzed to assess the local impact of a single stress. To test the effect of pretreatment on herbivory, half of

pretreatments were done at sites adjacent to the feeding site and each stressed zones were harvested. For the other half, pretreatments and feeding were done at the same site (see bioassay design in the top of the Figure 9).



**Figure 9: Impact of localized attack on hormone and glucosinolate concentration.**

Hormones and glucosinolates (GS) were measured in 5 week-old plants in a whole leaf (left) or in leaf discs (right). For whole leaf: control (CTL), treated by *P. brassicae* egg extract (E), infiltrated by *Xcc* (X), *P. brassicae* feeding (P) or dual stresses (EP, XP). For local measurements, the same experiments were done using clip cages (pink circles). After a single stress, leaf discs (blue circles) were harvested from: control plant (CTL), *P. brassicae* egg extract (E) or *Xcc* (X) or *P. brassicae* feeding sites (P). After dual stresses applied at different sites, leaf discs were harvested from: *Xcc* or *P. brassicae* egg extract pretreatment zone (X / P : X or E / P : E) and adjacent from *P. brassicae* treatment (X / P : P or E / P : P). After dual stress applied at the same site, leaf discs were collected from untreated parts (XP : 0 or EP : 0) or from treatment areas (XP : XP or EP : EP). Means ( $\pm$  SE) of three biological replicates are shown. Color gradient indicates significant differences between samples, with the most significant values in dark red. ANOVA tests were done between samples coming from the same bioassay, separated by bold lines in the table (ANOVA followed by Tukey's honest significant difference test).

SA analysis showed a clear increase wherever *P. brassicae* egg extract was applied. All egg extract treated samples were not statistically different from each other (Figure 9). On the opposite, samples where *P. brassicae* egg extract was not directly applied had the same level of SA than control plants, indicating that eggs induce a local SA accumulation.

The highest level of SA was observed after *Xcc* infiltration. The concentration increased from 15 ng / g FW in control plants to 1221 ng / g FW in X. Interestingly, in case of dual stress the amount was statistically higher in X / P : X samples, reaching 1633 ng / g FW but decreased in XP : XP samples with 875 ng / g FW, in comparison to *Xcc* single stress.

As expected, JA showed a local increase after single herbivory (P) and also in dual stresses, when JA was measured at the herbivore-feeding site. The highest level was observed in samples coming from E / P : P (71 ng / g FW). A marginal JA increase was observed adjacent to the feeding site, in the egg-treated site. Surprisingly, a strong local JA accumulation was measured after *Xcc* infiltration. This was not observed in the whole leaf (Figure 9). Interestingly, X and X / P : X samples had a higher amount of JA than samples harvested after herbivory (P) and dual stress X / P : P. Furthermore, the JA concentration had an intermediate level in sample coming from dual stress XP : XP.

Finally, we showed that all area from leaves subjected to a dual stress, EP : 0 and XP : 0, had all the same amount of SA and JA than control tissues, indicating that there was no distal effect of a dual stress on hormone levels (Figure 9).

For glucosinolates, we observed a local variation of GS content only after *Xcc* treatment, with an increase of the indolic glucosinolates concentration, mainly on the site of infection. Herbivory did not induced indolic GS and inhibited slightly the increase induced by *Xcc* pretreatment (Figure 9). However, indolic GS corresponded on average to only 2.2 % of aliphatic GS levels, which were not affected by any treatment.

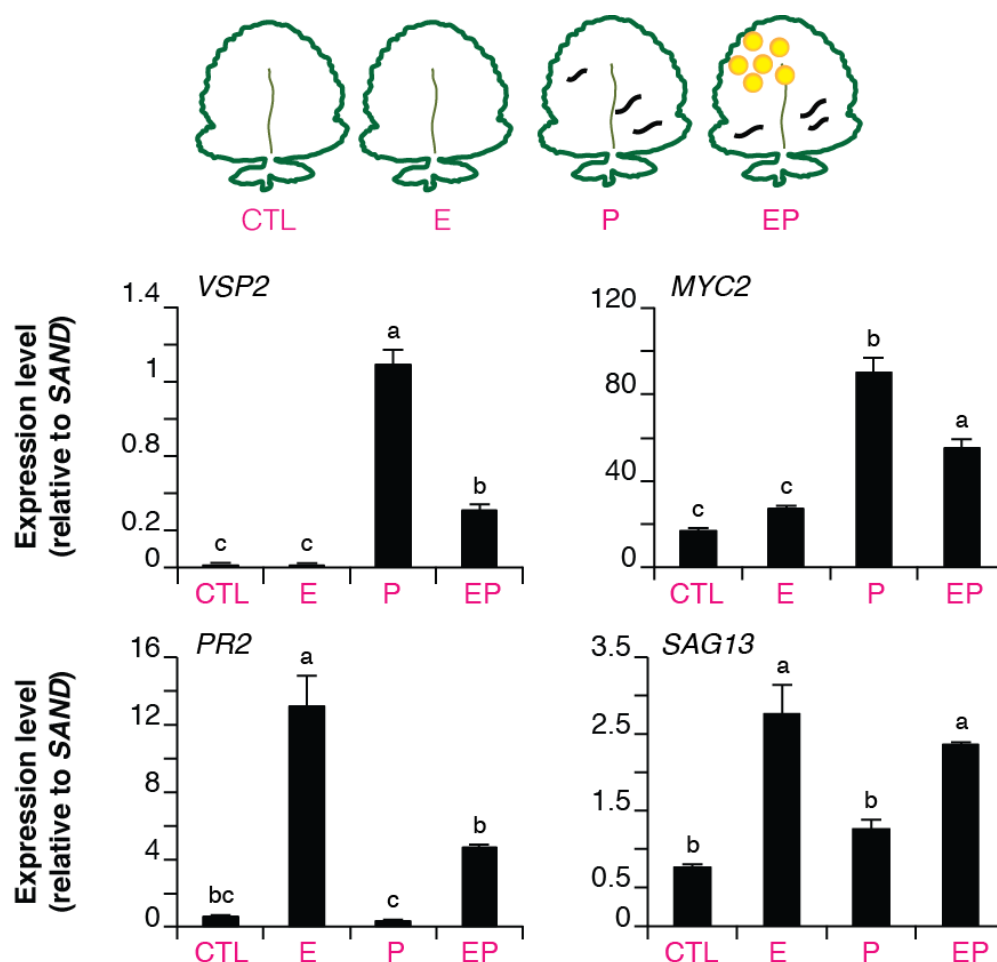
In conclusion, we showed that eggs of *P. brassicae* increase SA levels in *B. nigra*, like in *Arabidopsis* (Bruessow *et al.*, 2010) whereas *Xcc* triggered SA and JA accumulation (Ton *et al.*, 2002). We also found that the increase of phytohormones is preferentially confined to the treatment area. This explains why we did not observed the same SA and JA increase in whole-leaf analyses (Figure 4). In addition, except for minute changes of indolic-GS, GS levels are not regulated by single or dual stresses, even at the site of attack.

### c. SA / JA crosstalk in *B. nigra*

Transcriptomic analysis showed a weak effect of pretreatments on transcriptional responses to *P. brassicae* feeding (Chapter I). Only in case of *Xcc* pretreatment, few insect-induced genes belonging to the JA signaling pathway and GS biosynthesis were not induced anymore after *Xcc* / *P. brassicae* dual stress (Chapter I: Figures S1, S2, S3 and 22). However, we observed an impact of *Xcc* or *P. brassicae* egg extract on both generalist and specialist performance (Figure 8) and on SA and JA concentration (Figure 9). A further analysis of the impact of these different stresses and their localization on the leaf on SA / JA crosstalk were done on *B. nigra* marker genes. We performed whole-leaf and localized (using clip cages) experiments and analyzed the expression of SA (*PR2*, At3g57260, *SAG13*, At2g29350) and JA (*VSP2*, At5g24770, *MYC2*, At1g32640) marker genes by qPCR.

### b.1 Effect of egg extract and *P. brassicae* single or dual stress on SA / JA crosstalk

We analyzed the impact of *P. brassicae* egg extract during 3 days, *P. brassicae* during 24 hours and the dual stress egg extract / herbivory.



**Figure 10: Effect of *P. brassicae* egg extract, *P. brassicae* herbivory and the dual stress on SA / JA crosstalk in whole *B. nigra* leaf.**

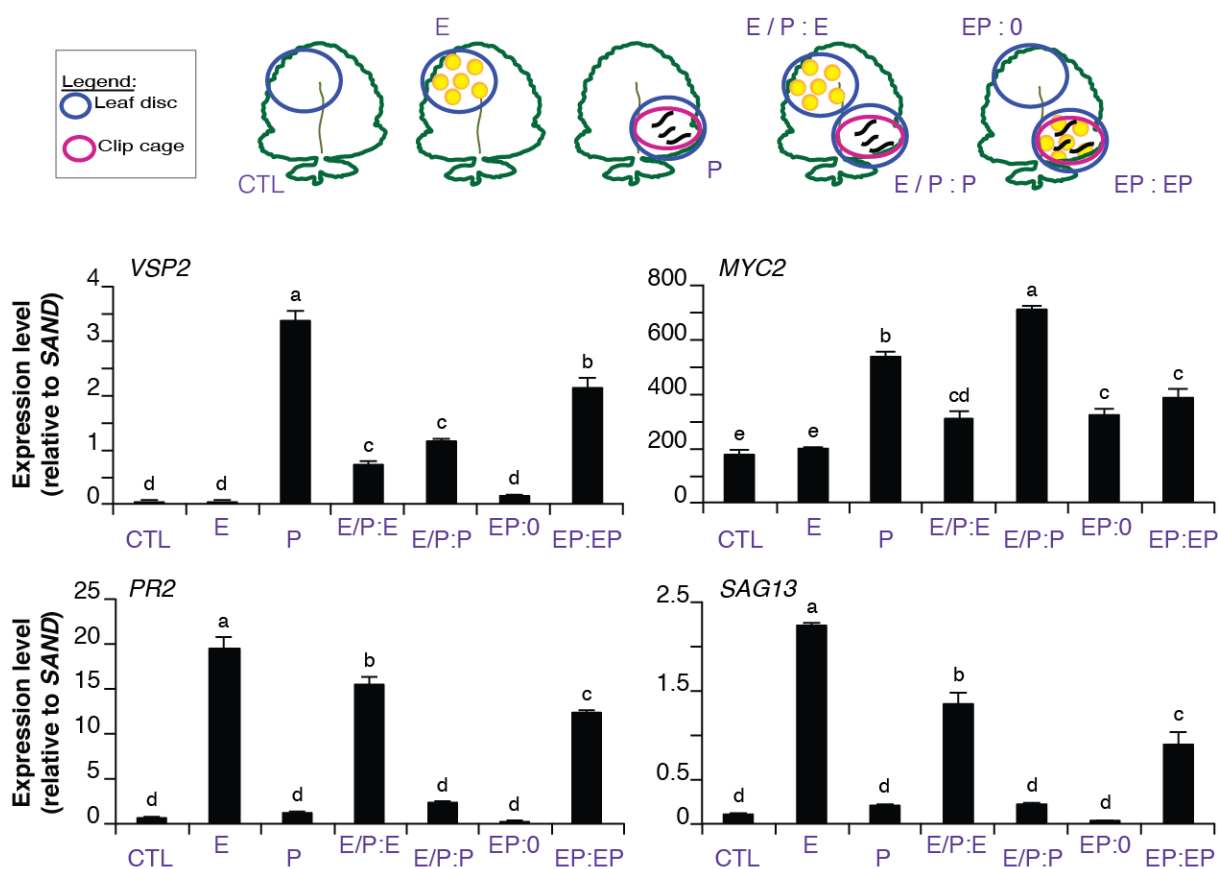
JA marker genes, *VSP2* and *MYC2*, and SA marker genes, *PR2* and *SAG13* were analyzed by qPCR in whole leaf. Samples were collected after 3 days of *P. brassicae* egg extract (E) treatment, 24 hours of *P. brassicae* feeding (P), the dual stress egg extract / herbivory (EP) treatment or in untreated leaves (CTL).

Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

On entire leaves, we clearly identified an increased expression ratio of both *VSP2* and *MYC2* after *P. brassicae* feeding (P), with respectively an induction of more than 375- and 5-fold in comparison to untreated plants. After dual stress (EP), both *VSP2* and *MYC2* were less



induced than after herbivory single stress (P) but expression levels did not reach the control state, with respectively an induction of more than 103- and 3-fold in comparison to untreated leaves. For SA marker genes, we observed a 21-fold increase of *PR2* expression and 4-fold increase of *SAG13*. After dual stress (EP), *PR2* was less induced than after egg extract single stress and this was not statistically different from control plants. In the case of *SAG13*, the expression level after dual stress (EP) was the same than after *P. brassicae* egg extract treatment (E). Thus, the dual stress egg extract / herbivory caused a reduced induction of both SA and JA marker genes (Figure 10).



**Figure 11 : Effect of *P. brassicae* egg extract, *P. brassicae* herbivory and the dual stress on SA / JA crosstalk in specific *B. nigra* leaf part.**

JA marker genes, *VSP2* and *MYC2*, and SA marker genes, *PR2* and *SAG13* were analyzed by qPCR in leaf discs. Leaf discs (blue circles) were harvested after 3 days of *P. brassicae* egg extract (E), *P. brassicae* feeding during 24 hours (P) or untreated leaves (CTL). In the case of dual stress, pretreatment and second stress were applied at the same place or in different sites. For leaves treated on different sites, samples were collected from 3 days of egg extract pretreatment (E / P : E) and from 24 hours of *P. brassicae* feeding site (E / P : P). For leaves treated at the same site, samples were harvested from untreated part (EP : 0) and from the dual stress site (EP : EP).

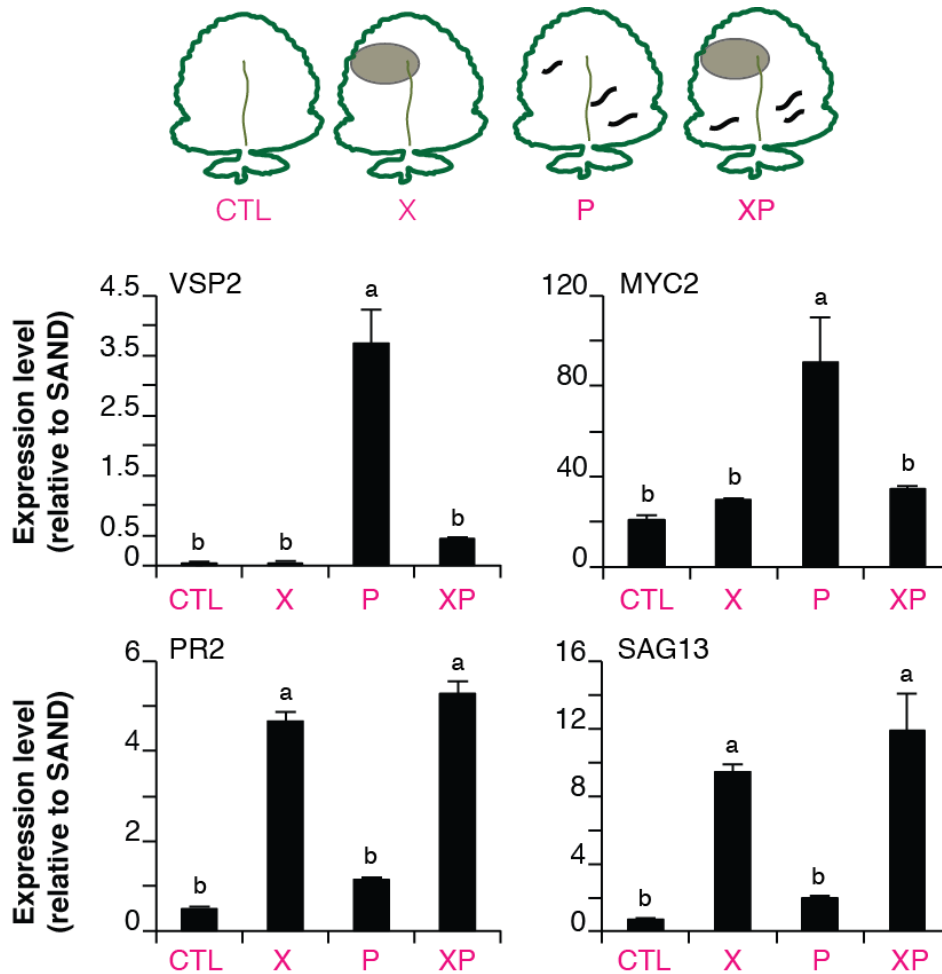
Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

Analysis of leaf disc samples showed that expression of JA and SA marker genes were higher than in whole-leaf experiments, except for *SAG13* (Figure 11). For JA marker genes, we noticed that SA / JA crosstalk operated at the site of dual stress for *VSP2* and *MYC2*, but that this effect was conserved only for *VSP2* when egg extract treatment was applied to a site adjacent to larvae. Egg-induced expression of *PR2* and *SAG13* was inhibited by in dual stress, both locally and when larval feeding was adjacent (Figure 11).

Thus, *B. nigra* responds to the dual stress egg extract / herbivory by a reduction of SA and JA marker genes, illustrating the known antagonistic effects of SA on the JA pathway and, reciprocally, of JA on the SA pathway. This is consistent with our observation of egg-induced SA accumulation and larvae-induced JA accumulation (Figure 9). Interestingly, these crosstalks seem to be effective in sites that are adjacent to the treatment.

### **b.2 Effect of *Xcc* and *P. brassicae* single or dual stress on SA / JA crosstalk**

The same experiment as above was done using *Xcc* during 3 days, *P. brassicae* feeding during 24 hours and the combined stresses *Xcc* / *P. brassicae* herbivory. On whole-leaf samples, *P. brassicae* feeding (P) significantly induced the expression of *VSP2* and *MYC2*, whereas this induction was clearly inhibited after *Xcc* pretreatment (Figure 12). *Xcc* treatment alone had no effect on the expression of these genes. In contrast, SA marker genes (*PR2* and *SAG13*) were significantly induced after *Xcc* treatment and this induction stayed the same in the dual stress (Figure 12). Thus, similar to egg extract pretreatment, *Xcc* triggered a SA / JA crosstalk in whole *B. nigra* leaves. However, larval-induced JA / SA crosstalk was not observed for *Xcc*-induced SA genes.

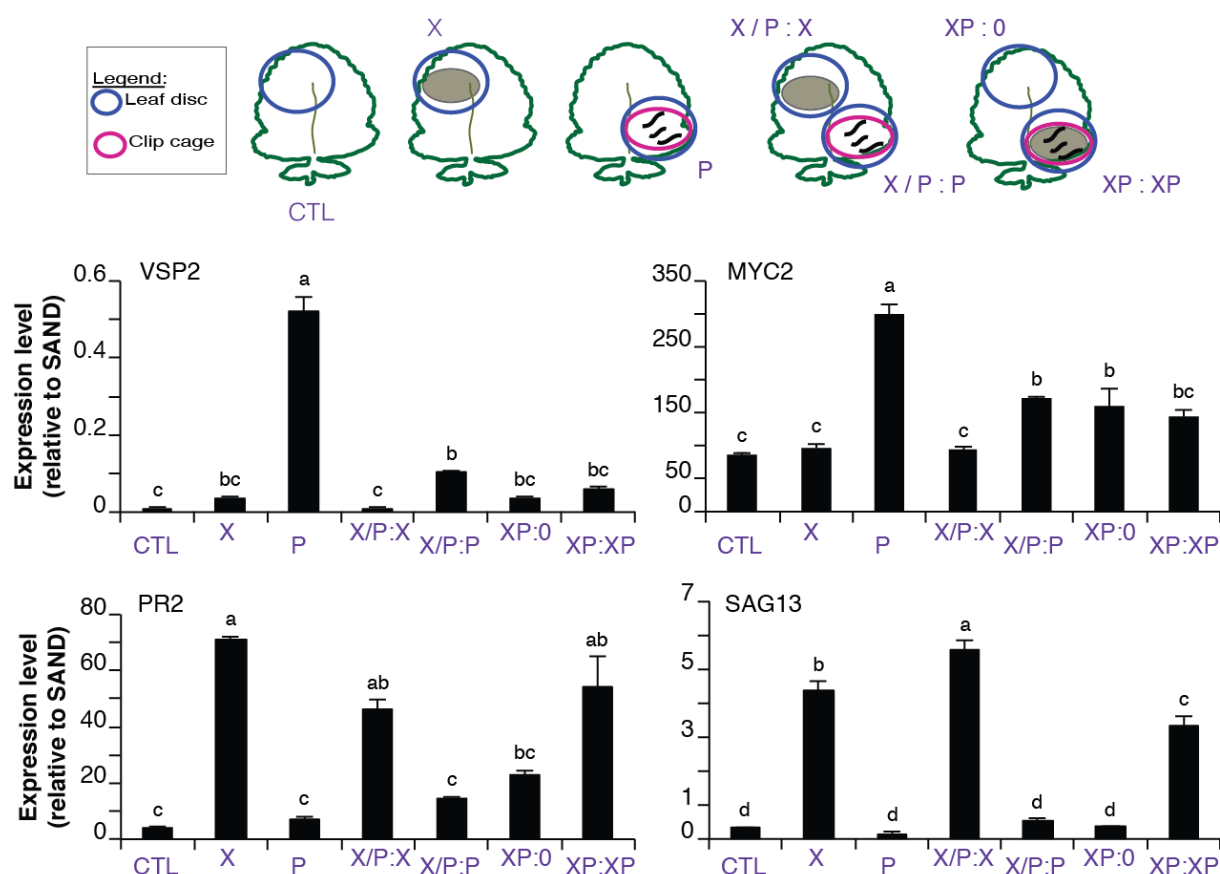


**Figure 12: Effect of *Xcc*, *P. brassicae* herbivory and the dual stress on SA / JA crosstalk in whole *B. nigra* leaf.**

JA marker genes, *VSP2* and *MYC2*, and SA marker genes, *PR2* and *SAG13* were analyzed by qPCR in whole leaves. Samples were collected after 3 days of *Xcc* (X), 24 hours of *P. brassicae* feeding (P), the dual stress *Xcc* / herbivory (XP) treatment or in untreated leaves (CTL).

Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

Further analysis with clip cages and local sampling gave similar results. *VSP2* and *MYC2* expression was induced by larval feeding and this induction was inhibited by *Xcc* pretreatment, both locally and distant to the feeding site (Figure 13). *PR2* and *SAG13* expression was induced by *Xcc* treatment, but was not inhibited by larval feeding, except for *SAG13*, whose expression was weakly inhibited at the site of dual treatment (Figure 13).



**Figure 13: Effect of *Xcc*, *P. brassicae* herbivory and the dual stress on SA / JA crosstalk in specific *B. nigra* leaf part.**

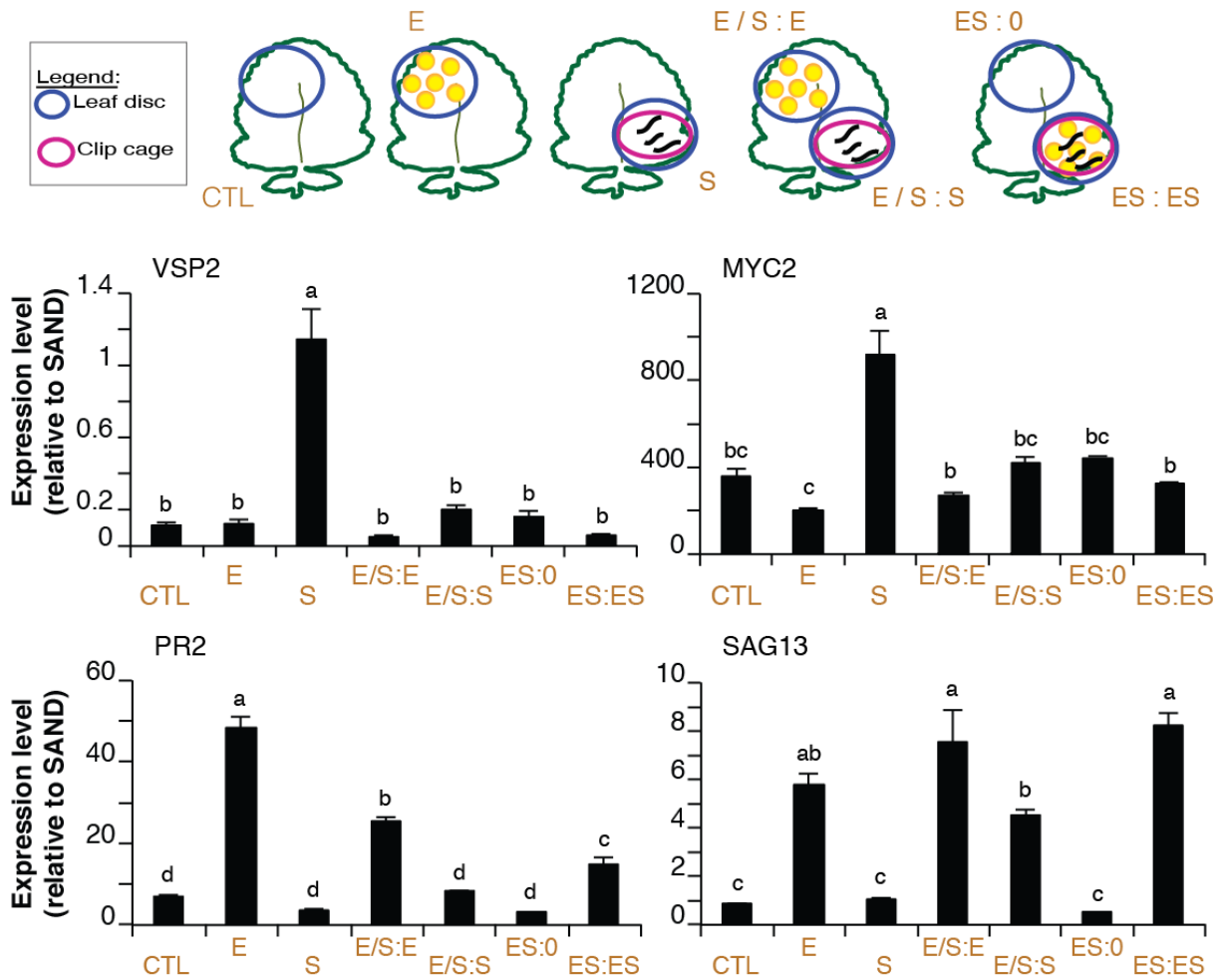
JA marker genes, *VSP2* and *MYC2*, and SA marker genes, *PR2* and *SAG13* were analyzed by qPCR in leaf discs. Leaf discs (blue circles) were harvested after 3 days of *Xcc* (X), *P. brassicae* feeding during 24 hours (P) or untreated laves (CTL). In the case of dual stress, pretreatment and second stress were applied at the same place or in different sites. For leaves treated at different sites, samples were extracted from 3 days of *Xcc* pretreatment (X / P : X) and from 24 hours of *P. brassicae* feeding site (X / P : P). For leaves treated at the same site, samples were harvested from untreated part (XP : 0) and from the dual stress site (XP : XP).

Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

In summary, *Xcc* pretreatment triggers a clear SA / JA crosstalk that inhibits insect-induced genes in *B. nigra*. Interestingly, the observation that herbivory does not inhibit *Xcc*-induced SA-dependent genes but inhibits egg extract-induced genes (Figures 10 and 11) may indicate that the efficacy by which JA blocks the SA pathway depends on the biological context in which the SA or the JA pathway is activated or the strength of their activation.

### b.3 Effect of *Xcc*, *P. brassicae* eggs extract or *S. littoralis* single or dual stresses on SA / JA crosstalk

Since *S. littoralis* performance assays after single or dual stress gave different results than with *P. brassicae*, we analyzed the expression of JA- and SA-marker genes with *Xcc* and *P. brassicae* egg extract pretreatment followed by *S. littoralis* herbivory.

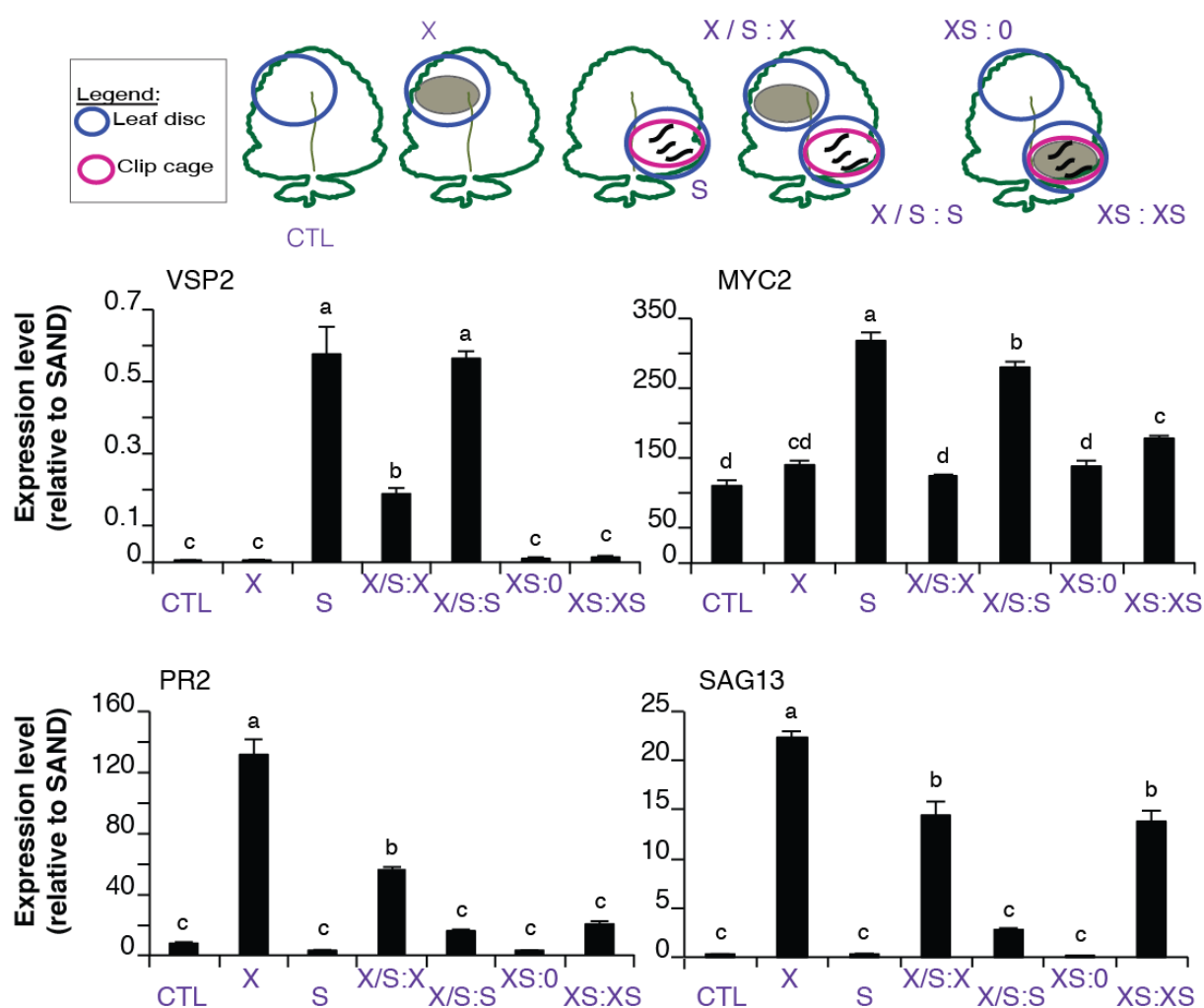


**Figure 14: Effect of single and dual stress, using *P. brassicae* egg extract as pretreatment and *S. littoralis* as second stress, on SA / JA crosstalk.**

JA marker genes, *VSP2* and *MYC2*, and SA marker genes, *PR2* and *SAG13* were analyzed by qPCR in leaf discs (blue circles). Samples were collected from plants treated by *P. brassicae* egg extract during 3 days (E), *S. littoralis* during 24 hours (S) or untreated leaves (CTL). In the case of dual stress, pretreatment and second stress were applied at the same site or in different sites. For leaves treated at different sites, samples were harvested from 3 days of *P. brassicae* eggs extract pretreatment (E / S : E) and from 24 hours of *S. littoralis* feeding site (E / S : S). For leaves treated at the same site, samples were collected from untreated part (ES : 0) and from the dual stress site (ES : ES).

Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

Like with *P. brassicae* larvae, *S. littoralis* larvae significantly induced the expression of *VSP2* and *MYC2* (Figures 14 and 15). SA / JA crosstalk was triggered by both egg-extract and *Xcc* pretreatments, leading to a reduced induction of *VSP2* and *MYC2* after *S. littoralis* feeding. In most cases, this crosstalk occurred when pretreatments were at the site or distant to the site of feeding (Figures 14 and 15). Egg-extract and *Xcc* pretreatments induced the expression of *PR2* and *SAG13*, like in previous experiments. This induction was inhibited by *S. littoralis* feeding in all dual stress conditions, except for *SAG13* in the case of egg-extract pretreatment (Figures 14 and 15).



**Figure 15: Effect of single and dual stress, using *Xcc* as pretreatment and *S. littoralis* as second stress, on SA / JA crosstalk.**

Samples were collected from plants treated by *Xcc* during 3 days (X), *S. littoralis* during 24 hours (S) or untreated laves (CTL). In the case of dual stress, pretreatment and second stress were applied at the same site or in different sites. For leaves treated at different sites, samples were harvested from 3 days of *Xcc* pretreatment (X / S : X) and from 24 hours *S. littoralis* feeding site (X / S : S). For leaves treated at the same site, samples were collected from untreated part (XS : 0) and from the dual stress site (XS : XS).

Means ( $\pm$  SE) of three replicates are shown. Different letters indicate significant differences at P-value < 0.05 (ANOVA followed by Tukey's honest significant difference test). These experiments were repeated at least twice with similar results.

## CHAPTER II

In conclusion, our experiments with dual stresses involving *S. littoralis* or *P. brassicae* reveal that both SA / JA and JA / SA crosstalks are generally activated to inhibit the expression of JA- and SA-dependent defense genes. However, the magnitude and strength of each crosstalk depends on the type of biotic pretreatment and on the herbivore. This complex transcriptional output may thus explain the contrasting effects of pretreatments on larval performance.

In this chapter, we observed that activation of plant defenses in *B. nigra* occurs close to the infection or feeding site and that analyses at the whole leaf level may mask some important changes due to a dilution effect. Moreover, SA / JA and JA / SA crosstalks occur in *B. nigra* but are finely modulated depending of the combination of biotic stresses. Finally, neither GS levels, hormones concentrations nor pathway crosstalk can easily explain the contrasting performance of a specialist and a generalist insect.

## Discussion

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Plant defense response in the case of dual stress is very difficult to predict. Analysis of insect performance, for the generalist *S. littoralis* or the generalist *P. brassicae*, has confirmed the complexity of plant responses to multiple attacks. We observed that insect performance after *P. brassicae* egg extract application was decreased for the specialist and increased for the generalist, regardless of herbivores feeding freely on the whole leaf or feeding locally in a clip cage (Figure 8). Surprisingly, insect performance in a plant already containing eggs of *P. brassicae* butterflies differs between plants species. In Arabidopsis, Bruessow and coworkers (2011) showed that *P. brassicae* eggs deposition has no effect on performance of the specialist *P. brassicae*, whereas *S. littoralis* performed better on pretreated plants. The weight gain of *S. littoralis* disappeared if plants pretreated with egg extract could not produce SA (Bruessow *et al.*, 2011). In Arabidopsis and *B. nigra*, other studies showed that caterpillars performed less in the presence of eggs regardless of whether experiments were conducted in laboratory or field settings (Geiselhardt *et al.*, 2013; Pashalidou *et al.*, 2013; Pashalidou *et al.*, 2015a). The same results were also found in other wild brassicaceous species, such as *Brassica oleracea*, *Moricandia moricandioides* and *Sinapsis arvensis* (Pashalidou *et al.*, 2015b). To the contrary, as we showed during our experiments with *S. littoralis* performance (Figure 8), generalists developed better on plants already treated with eggs of *P. brassicae* (Bruessow *et al.*, 2011). Interestingly, if plants were pretreated by generalist eggs such as *Mamestra brassicae*, performance of generalists and specialists were not affected (Pashalidou *et al.*, 2013). Our data and previous studies confirm that plant history really does have an impact on future herbivore fitness in *B. nigra* (Smallegenge *et al.*, 2007; Ali and Agrawal, 2012; Lucas-Barbosa *et al.*, 2012; Pashalidou *et al.*, 2013).

Furthermore, we showed that *P. brassicae* larvae feeding freely on *Xcc* pretreated leaves gained less weight in comparison to *P. brassicae* larvae deposited on an untreated leaf, whereas the generalist *S. littoralis* increased his performance (Figure 8). At the whole-leaf level, *Xcc* pretreatment impacts plant defense response against herbivores, depending of their feeding mode. An experiment done on *Capsicum annum* L. also showed an impact of *X. campestris* pv. *vesicatoria* tomato race 1, the incompatible strain, and *Xcv* pepper 1, the compatible strain, on *Spodoptera exigua* plant defense response. From 2 days post-infection with the incompatible strain *S. exigua* fed significantly more on infected plants than on healthy plants. This increased performance occurred only 4 days after bacterial inoculation with the compatible strain. This difference is explained by the fact that the compatible strain infects plant tissue more slowly, and



finally needs more time to trigger the SA pathway, which will inhibit plant defense to herbivory (Cardoza and Tumlinson, 2006).

**Table 2: Insect performance depending on the first stress applied.**

Plants	1 <sup>st</sup> stress	Insect	Effect	Publication
Arabidopsis	<b><i>Botrytis cinerea</i></b> Drought	<b><i>Pieris rapae</i></b> <i>Pieris rapae</i>	= CTL = CTL	Davila Olivas <i>et al.</i> , 2016
	<b><i>Pieris brassicae</i></b> eggs	<b><i>Pieris brassicae</i></b> <i>Spodoptera littoralis</i>	= CTL ↗	Bruessow <i>et al.</i> , 2011 Schmiesing <i>et al.</i> , 2016
	<b><i>Pseudomonas syringae</i></b> <b><i>P. brassicae</i></b> eggs + <b><i>P. syringae</i></b>	<b><i>Pieris brassicae</i></b> <b><i>Pieris brassicae</i></b>	↘ ↘	Hilfiker <i>et al.</i> , 2014
	<b><i>Pieris brassicae</i></b> eggs	<b><i>Pieris brassicae</i></b>	↘	Geiselhardt <i>et al.</i> , 2013
	<b><i>Pieris brassicae</i></b> <b><i>Brevicoryne brassicae</i></b>	<b><i>Pieris brassicae</i></b> <b><i>Pieris brassicae</i></b>	↘ ↘	Onkokesung <i>et al.</i> , 2016
<i>Brassica nigra</i>	<b><i>Pieris brassicae</i></b> eggs	<b><i>Pieris brassicae</i></b> <i>Mamestra brassicae</i>	↘ ↘	Pashalidou <i>et al.</i> , 2013
	<i>Mamestra brassicae</i> eggs	<b><i>Pieris brassicae</i></b> <i>Mamestra brassicae</i>	= CTL = CTL	
	<b><i>Pieris brassicae</i></b> eggs	<b><i>Pieris brassicae</i></b>	↘	Pashalidou <i>et al.</i> , 2014a
	Ozone	<b><i>Pieris brassicae</i></b>	↘	Khaling <i>et al.</i> , 2015
Brassicaceous	<b><i>Pieris rapae</i></b> <b><i>Phyllotreta crucifera</i></b>	<b><i>Pieris rapae</i></b>	↘	Traw and Dawson, 2002
	<b><i>Pieris brassicae</i></b> eggs	<b><i>Pieris brassicae</i></b>	↘	Pashalidou <i>et al.</i> , 2014b
<i>Nicotiana attenuata</i>	<b><i>Manduca sexta</i></b> eggs	<i>Spodoptera exigua</i>	↘	Bandoly <i>et al.</i> , 2015

Insect names of specialists are in bold oblique and generalists in italics. Insect performance was compared to untreated plants and can be not affected (= CTL), ↘ increased or ↗ decreased by pretreatment. Data were obtained from literature.

Finally, in Brassicaceae, pretreatment seems to really affect insect performance because in addition to *P. brassicae* eggs and *X. campestris*, other stresses such as ozone, *Pseudomonas syringae*, or prior herbivory by *Phyllotreta crucifera* or *Pieris rapae* were also shown to have a negative impact (Traw and Dawson, 2002; Hilfiker *et al.*, 2014; Khaling *et al.*, 2015; Table 2). Collectively, these data suggest that plant response to a first stress triggers either a specific protection from further herbivory or enhanced susceptibility (Reymond, 2013; Hilker and Fatouros, 2015). Analysis of physical changes, variation in nutrients or secondary metabolites could explain the difference in insect performance. For example, in *B. nigra* challenged by *P. rapae*, the number of trichomes increased, whereas the caterpillar growth rate decreased (Traw and Dawson, 2002). Also, a recent study in *Eucalyptus tereticornis* observed a correlation between foliar nitrogen content and immune responses of the insect *Paropsis atomaria* (Gherlenda *et al.*, 2016). Efforts have to be made in this direction to clearly understand the mechanisms beyond plant defense, not only to one stress but in general as well.

To our knowledge, this study is the first one that analyzed plant responses against herbivores after dual stresses, inside the same leaf, depending on the infection and feeding sites. Analysis of hormones and GS content was realized to provide a deeper overview of plant response in all the different combinations tested. Specific analysis of tissue from treated or untreated leaf discs was important to observe the increase of SA after *P. brassicae* egg extract treatment during 3 days. On a whole-leaf scale, SA concentration was slightly increased but not enough to be statistically different from the control leaf concentration, whereas in tissue coming specifically from the site of egg extract deposition, we clearly observed a rise that was not affected by *P. brassicae* herbivory second stress. In *Arabidopsis*, SA accumulated mainly underneath the eggs in response to oviposition or egg extract application and slightly in distal leaves (Bruessow *et al.*, 2011; Schmiesing *et al.*, 2016). Thus a localized response after oviposition seems to be the same between *Arabidopsis* and *B. nigra*. Furthermore, JA concentration was not statistically different between the dual stress egg extract / herbivory and single *P. brassicae* feeding. Egg extract application seems thus to have no impact on insect-induced JA accumulation.

The analysis of infected tissue gave clear evidence for an increase of both SA and JA after *Xcc* infiltration that was not observed on a whole-leaf scale. Thanks to this method, we can validate that *Xcc* triggers plant defense through both JA and SA pathways (Ton *et al.*, 2002). Interestingly, JA level obtained after *P. brassicae* feeding did not reach the same amount and was lower than after *Xcc* infiltration. Moreover, tissue harvested specifically at the site of the dual stress *Xcc* / *P. brassicae* had an intermediate level of JA in comparison to tissue coming from the *Xcc* infiltrated area or *P. brassicae* single stress. We expected that *Xcc* and *P. brassicae* together could have an additive effect on JA concentration. Moreover, in all of our experiments, we showed that plant defense response is really concentrated to the challenged area. Further analysis of SA / JA crosstalk revealed that the reciprocal antagonism between SA and JA depended on the pretreatment and insect applied. A study done by Vos and coworkers (2015) showed that hormonal crosstalk during multiple stresses seems to be important to allow plants to prioritize their defenses and to limit the fitness costs of induced defenses (Vos *et al.*, 2015). In addition, it is well established that synergistic or antagonistic effects of SA on JA, and inversely, is dependent on phytohormone concentration (De Vos *et al.*, 2006; Mur *et al.*, 2006; Koornneef *et al.*, 2008). Interestingly, *Xcc* increased both SA and JA hormones levels (Figure 9) while only the SA marker genes were induced after infection. After dual stress *Xcc* followed by insect feeding, JA marker genes, normally triggered after herbivory, were inhibited while SA marker genes were still induced (Figures 13, 15 and Chapter I). In contrast, after egg extract treatment, only the SA hormone and SA marker genes were induced. After combined stresses *P. brassicae* egg extract / *P. brassicae* feeding, the SA and JA pathways inhibited each other and marker genes were partially induced (Figures 10 and 11). Interestingly, plant response to *P. brassicae* egg extract

followed by *S. littoralis* feeding was not the same than with the specialist. Expression of *S. littoralis*-induced JA marker genes was fully inhibited whereas the effect was weaker with *P. brassicae* (Figures 10, 11 and 14). Analysis of SA and JA phytohormone concentration in dual stress using *S. littoralis* may show that *B. nigra* triggers the JA pathway with different strength in response to a specialist or a generalist.

Surprisingly, insect performance assays gave opposite conclusions using clip cages or not after *Xcc* pretreatment, while after egg extract the weight gain of insects was the same independently of the feeding assays (Figure 8). *P. brassicae* feeding specifically in an *Xcc* infiltrated area were bigger than larvae constrained to feed in a non-infected zone of an infiltrated leaf or in an untreated leaf. To the contrary, *S. littoralis* performed less well when they were constrained to feed in an *Xcc* infiltrated area or close to one, in comparison to larvae added in an untreated leaf. Secondary metabolites or plant nutrients produced close to the *Xcc* infection site seem to be detrimental for a generalist, whereas the specialist *P. brassicae* may in fact detoxify or benefit from them. These differences in insect performance depending on the feeding site can be explained by a bacterial density-dependent effect on plant defense. A study conducted on *Arabidopsis* showed that *Plutella xylostella* larval performance was dependent of *Brevicoryne brassicae* aphid density. At low aphid density, *P. xylostella* growth rate was increased and, inversely, at high density caterpillars performed less well. *B. brassicae* are known to involved both JA and SA pathways for plant defense response whereas *P. xylostella*, as chewing herbivores, induced JA pathway. Authors observed that SA and JA transcription factors *WRKY70* and *MYC2* were induced differently depending on aphid density. *WRKY70* was highly induced while *MYC2* was not under the dual stress caterpillars / low aphid density, whereas *WRKY70* was lower and *MYC2* highly induced after caterpillars / high aphid density. It was concluded that a SA / JA crosstalk occurred depending of the strength in the balance between SA and JA. At low aphid density / caterpillars, SA was triggered more than JA and antagonistic effect of SA on JA appeared. While at high aphid density / caterpillars, JA pathway was mainly induced compared to SA and *WRKY70* cannot abolish the JA-pathway (Kroes *et al.*, 2015). The SA / JA crosstalk apparition depending of phytohormones concentration, time scale or kinetics have been well described (De Vos *et al.*, 2006; Mur *et al.*, 2006, Koornneef *et al.*, 2008). For example, antagonist effect of SA on JA and inversely was observed in *Arabidopsis* only after a high concentrated and prolonged treatment of SA or JA, while at low concentration of both phytohormones a synergistic enhancement was measured (Mur *et al.*, 2006). We may hypothesize that an *Xcc* infiltrated zone, containing a higher density of bacteria than systemic tissue, leads to a higher increase of SA level and to a SA antagonistic effect on JA. This hypothesis is correlated with concentration of SA, which was less reduced (divided by 1.4 vs *Xcc*) than JA (divided by 2 vs *Xcc*) (Figure 9) and by the expression of SA- and JA-marker genes in XP : XP or XS : XS tissues. SA-marker genes

were still induced after the dual stress *Xcc* / insects in tissue challenged by both stresses, while JA-marker genes induction were abolished (Figures 13 and 15). This SA / JA crosstalk in favor of SA could be at the origin of the increase of *P. brassicae* performance in dual stress tissue, where *Xcc* and insects were applied together (XP : XP; Figure 8), as observed for the Brassicaceae specialist *P. xylostella* feeding simultaneously with low aphid density (Kroes *et al.*, 2015). This *P. brassicae* increase of performance by *Xcc* prior stress protection, through SA / JA antagonistic crosstalk, seems to decrease away from the infiltration zone. *P. brassicae* performance was not different in adjacent tissue than insect feeding in control plants (Figure 8) and SA-marker genes were not induced, while JA-marker genes were (Figures 13 and 15). We may hypothesize that *Xcc* bacterial concentration leads to this phenomenon. Unfortunately, this model cannot explain the performance of the generalist. If only the SA / JA antagonistic effect is at the origin of insect performance, we may imagine that generalists, more sensitive to herbivory plant defense than specialists, will have a larger weight gain if they are constrained to feed on *Xcc* infected tissue. Indeed, a previous study showed that *S. littoralis* performed better when SA / JA crosstalk occurs (Bruessow *et al.*, 2010; Schmiesing *et al.*, 2016).

Inversely, on a whole-leaf scale, the specialist performed less well on *Xcc*-pretreated and the generalist gained more weight (Figure 8). We could imagine that SA / JA crosstalk occurred also through the whole leaf. As previously demonstrated in *B. nigra*, *Xcc* infection inhibited JA - ET pathways (Chapter I). We may postulate that the decrease of JA-induced response against herbivory by the SA / JA crosstalk triggered indirect evolution of defense traits, in the entire leaf, that is beneficial to *S. littoralis* and detrimental to *P. brassicae*. Furthermore, this kind of event could also be linked to insect performance after egg extract application (Figure 8). No clear evidence of SA / JA crosstalk was observed in our previous transcriptomic analysis after *P. brassicae* egg extract pretreatment (Chapter I), while we demonstrated by qPCR that both SA and JA marker genes were decreased by the dual stress egg extract / *P. brassicae* and a SA / JA crosstalk occurred after egg extract / *S. littoralis* (Figures 10 and 14). Moreover, other important phytohormones pathway could be involved during plant defense after dual stress that impact insect fitness such as ET, BR, ABA, CK or Auxin (Robert-Seilaniantz *et al.*, 2011; Nguyen *et al.*, 2016). For example, chewing herbivores could manipulate plant defense and activate, through oral secretions, the ERF branch of the JA pathway to decrease herbivore resistance controlled by the MYC branch. In Arabidopsis, plants impaired in MYC branch were more attractive for *P. rapae* than wild-type plants (Verhage *et al.*, 2011), whereas *S. littoralis* gained more weight in *myc2myc3myc4* mutant (Fernandez-Calvo *et al.*, 2011). Moreover, Schweizer and coworkers (2013) demonstrated that some transcription factors activated defense genes expression against herbivory in JA-independent manner. Interestingly, Arabidopsis plants mutated for some of these transcription factors were more susceptible to *S. littoralis*. The authors concluded that additional defense against insect was triggered through this JA-independent pathway (Schweizer *et al.*,

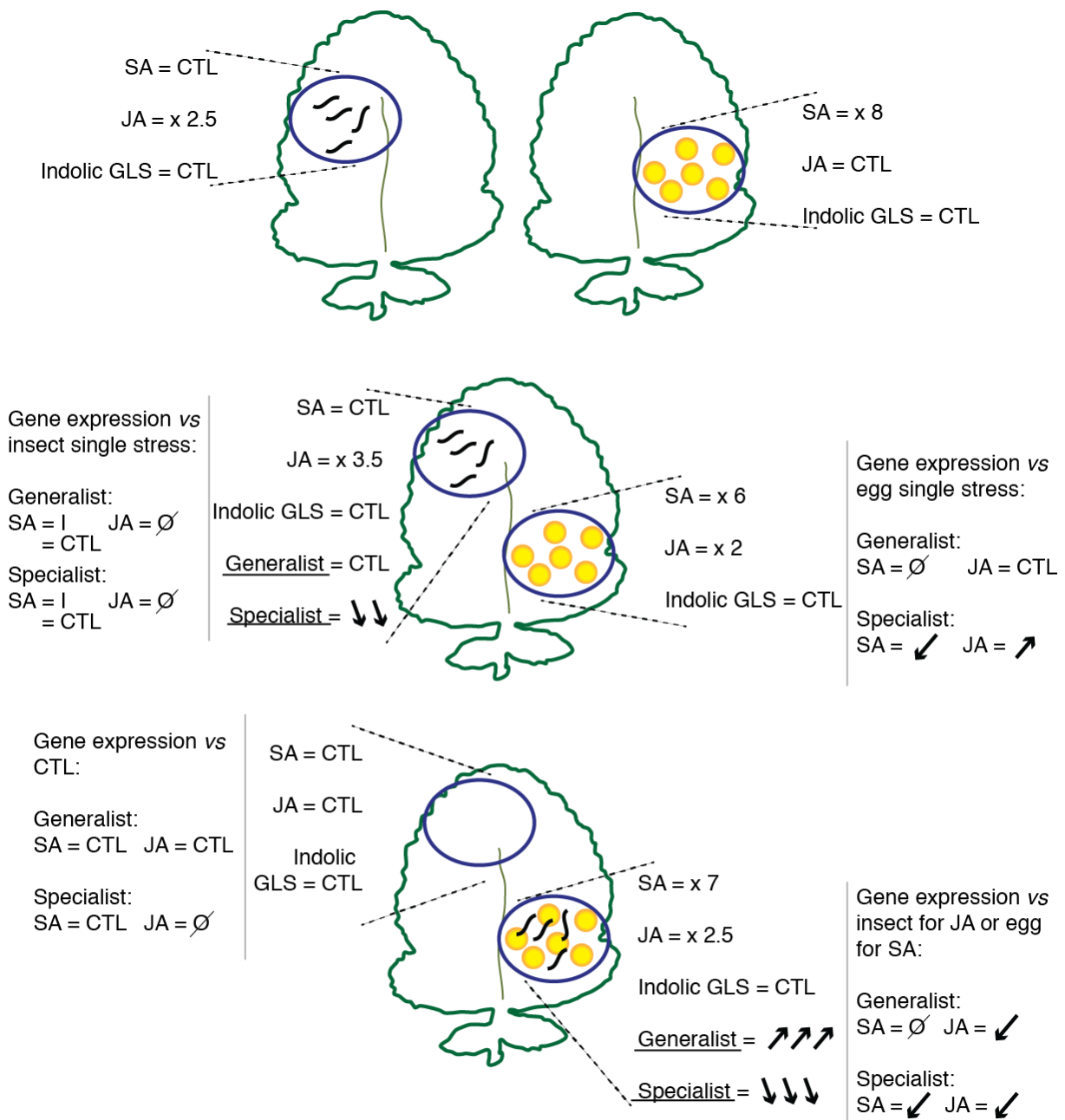
2013b). Further investigations have to be done in this direction to explain a direct or indirect link between phytohormone pathway crosstalks observed in *B. nigra* and insect performance depending of their feeding mode.

Likewise, GS content was analyzed using whole-leaf (Figure 4) or leaf discs and clip cages (Figure 9). First of all, we observed that the most abundant GS in *B. nigra* leaf was sinigrin, with more than 94 % of total GS amount. Our results validated what was previously shown by Gols and coworkers (2008) in *B. nigra*, where sinigrin represented more than 98 % of total GS content (Gols *et al.*, 2008a). Surprisingly, single GS molecules or total GS levels did not vary after *P. brassicae* feeding, neither in the whole leaf nor in feeding specific tissue. This is quite different from *Arabidopsis*, where it is well established that GS increase after herbivory (Mewis *et al.*, 2006; Schweizer *et al.*, 2013a). Also, studies in *B. nigra* showed that sinigrin increased in leaves after *P. rapae* and *P. brassicae* feeding in comparison to undamaged plants (Taw and Dawson, 2002; Smallegange *et al.*, 2007). This difference may be explained by the degree of plant domestication; Smallegange and coworkers (2007) used seeds coming from a Greek feral population and from two German advanced cultivars (Smallegange *et al.*, 2007) while in our experiments *B. nigra* seeds were collected from a wild population in Wageningen. It is known that GS levels can vary between domesticated, feral or wild cabbage populations of *Brassica oleracea*. The authors showed that in addition to containing higher amount of GS, only the wild cabbage showed increased GS levels after herbivory. This observation may explain that the performance of herbivores, whether generalists or specialists, was worse in the wild population in comparison to feral or cultivated cabbage (Gols *et al.*, 2008b). This is confirmed by the study of Soler and coworkers (2011) working with *Brassica oleracea* var. *gemmifera*. The authors did not observed any variation of GS in this Brussels sprout variety challenged by the phloem feeder *B. brassicae* or the leaf chewer *P. brassicae* (Soler *et al.*, 2011). But as described previously, *B. oleracea* var. *gemmifera* is the cultivar containing relatively higher levels of GS between Brussels sprout plants but considerably lower than a wild population (Gols *et al.*, 2008b). Analysis of GS amounts in *B. nigra* during its evolution could help to know if domestication had an impact on this secondary metabolites concentration and in plant defense strategies that depend on these molecules.

Secondly, only *Xcc* treatment increased the amount of indolic GS specifically in infected tissue, but not on a whole-leaf scale. This increase had however a very limited impact on the total amount of GS because of the high sinigrin concentration. Studies showed that both aliphatic and indolic GS have an inhibitory effect on *Xcc* growth and may play a role in the constitutive plant defense in *Brassicaceae* (Velasco *et al.*, 2013; Sotelo *et al.*, 2014). In addition, this rise in indolic GS by *Xcc* infection may underlie a defense mechanism to slow down infection progression under the tissue, through the biosynthesis of the phytoalexin camalexin (Frerigmann *et al.*, 2015).

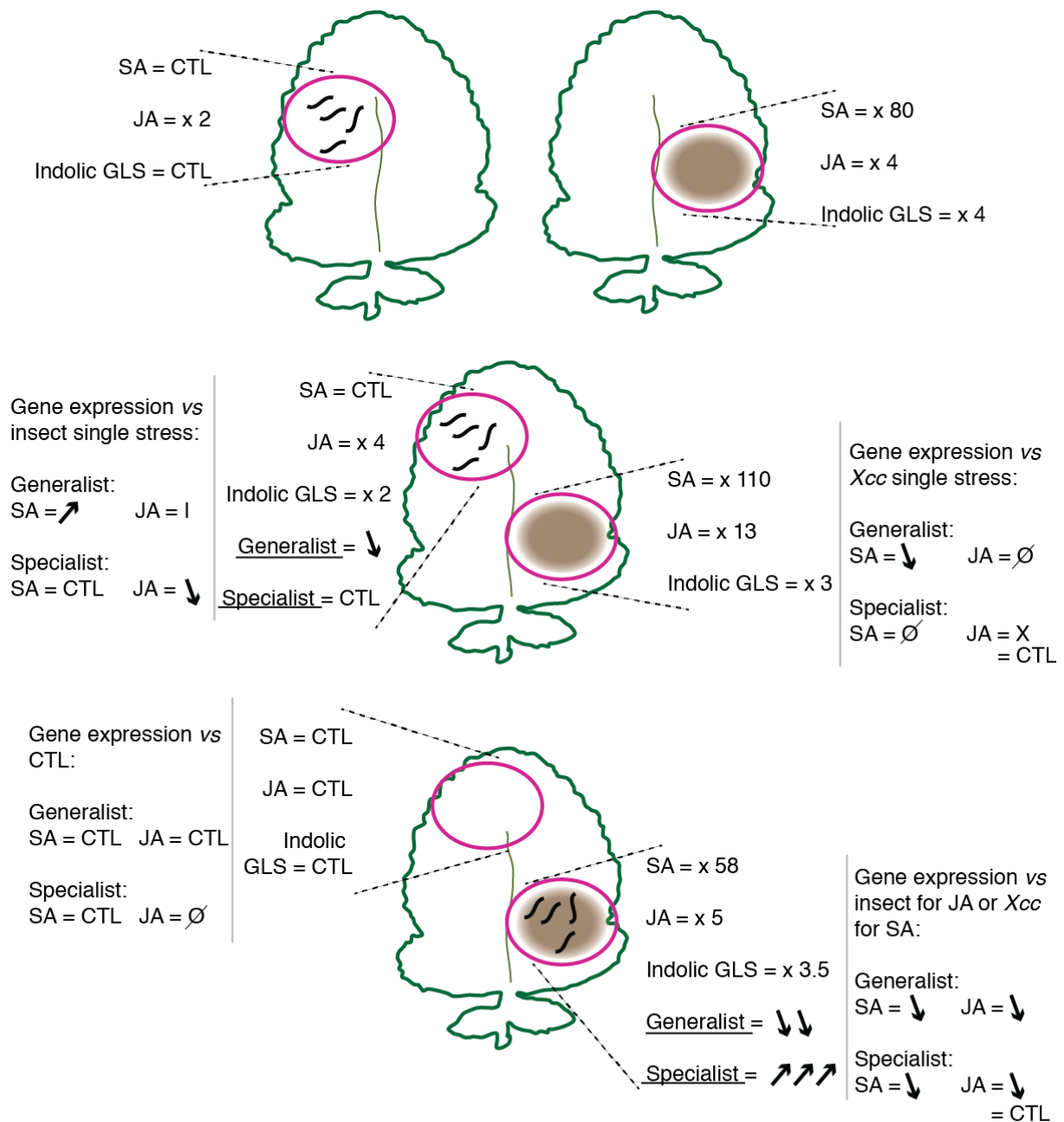
Moreover, the induction of camalexin biosynthesis genes is known to be strictly restricted to the tissue undergoing cell death in response to pathogen invasion (Schuegger *et al.*, 2007). Camalexin and indolic GS biosynthesis share common precursors and regulation steps. The observed rise in some indolic GS after *Xcc* treatment may indicate that the camalexin pathway is induced. The analysis of camalexin concentration or other phytoalexins derived from the tryptophan pathway in *Xcc* infected tissue of *B. nigra* may validate this hypothesis. This rise in indolic GS and potentially camalexin may participate in increased performance of the specialist and, inversely, a decreased performance of the generalist when they fed precisely on *Xcc* pretreated tissues. As previously described, the reduction of GS content in *Arabidopsis* increased the performance of *S. littoralis*, which does not contain the nitrile-specifier protein, a gut-specific enzyme that detoxifies GS (Wheat *et al.*, 2007; Schlaeppi *et al.*, 2008). On the other hand, Smallegange and coworkers previously showed that *P. brassicae* developed better on *B. nigra* tissue containing higher level of GS (Smallegange *et al.*, 2007). Nevertheless, these indolic GS changes cannot explain the performance of caterpillars feeding freely in entire leaves. Thus, we may postulate that a SA / JA crosstalk occurred in whole leaf after *Xcc* or egg extract pretreatment that triggered an increase of performance of the generalist and, inversely, a decreased performance of the specialist. This effect of the JA pathway inhibited by SA on insect performance could be specifically masked in *Xcc* infiltrated zone due to the increase of the camalexin through the indolic GS pathway.

In conclusion, GS, hormone concentration or SA / JA crosstalk cannot fully explain the difference in performance observed between specialist and generalist herbivores in plants challenged by *P. brassicae* eggs (Figure 16) or *Xcc* (Figure 17). We need to analyze more plant defense traits and nutritional values of treated sites to clearly understand how *B. nigra* deals with multiple attackers and why insect performance can be so different.



**Figure 16: Summary of plant defense in response to *P. brassicae* egg extract, insect feeding or the dual stress.**

The rise of SA, JA and indolic GS concentration is indicated in comparison to control (CTL) tissue. Expression changes of SA (*PR2* and *SAG13*) and JA marker genes (*MYC2* and *VSP2*) are mentioned for the generalist *S. littoralis* or the specialist *P. brassicae*. In case of distinct changes, for the same tissue, between marker genes belonging to the same pathway, no conclusion was done ( $\emptyset$ ), same gene expression ratio than CTL = CTL or insect single stress = I, decrease = ↙, increase = ↗. Generalist and Specialist insect performance is shown in comparison to insect feeding in untreated plants (same performance than insect feeding in CTL plant = CTL, slight decrease = ↘, decrease = ↙↙ and large decrease = ↘↘↘).



**Figure 17: Summary of plant defense in response to *Xcc*, insect feeding or the dual stress.**

The rise of SA, JA and indolic GS concentration is indicated in comparison to control (CTL) tissue. Expression changes of SA (*PR2* and *SAG13*) and JA marker genes (*MYC2* and *VSP2*) are mentioned for the generalist *S. littoralis* or the specialist *P. brassicae*. In case of distinct changes, for the same tissue, between marker genes, belonging to the same pathway, no conclusion was done (∅), same gene expression ratio than CTL = CTL or insect single stress = 1, decrease = ↘, increase = ↗. Generalist and Specialist insect performance is shown in comparison to insect feeding in untreated plants (same performance than insect feeding in CTL plant = CTL, slight decrease = ↘, decrease = ↘↘ and large decrease = ↘↘↘).



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## General discussion and perspectives

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Biotic and abiotic stresses are constantly threatening plants in nature. To adapt to their environment, plants have developed sophisticated mechanisms. Originally, plant defenses were extensively studied in response to one (a)biotic stress. For a few years now, research has focused more and more on plant defense responses after multiple attacks, to be closer to what is happening in fields or nature. During this work, my goals were to improve our knowledge on how *Brassica nigra*, the black mustard, defends itself against combined stresses. Whole-genome analyses revealed that a first stress, whether it was *Pieris brassicae* egg deposition, *Xanthomonas campestris* pv. *campestris* (*Xcc*) infection, *Brevicoryne brassicae* feeding or elevated ozone, had a very weak effect on subsequent *P. brassicae* herbivory. However, performance of both the specialist *P. brassicae* and the generalist *Spodoptera littoralis* was affected by a prior stress. Nevertheless, we showed that neither GS content, SA, JA and ABA phytohormones concentration, nor SA / JA crosstalk could entirely explain insect performance. Diverse traits that were not explored during this work could help to understand *B. nigra* defense responses in case of multiple stresses, and their impact on insect feeding.

First, a recent study showed that expression of some genes important for plant defense was really localized in cells where infection developed. Authors used laser microdissection to analyze the gene expression profile of *Vitis vinifera* at an early stage of infection by the oomycete *Plasmopara viticola* in stomata cells and surrounding areas. They identified new genes specifically localized in infected stomata cells and close to them, which were induced after colonization and totally masked in a whole-tissue analysis (Lenzi *et al.*, 2016). These findings validate what we observed during the analysis of SA and JA marker genes after either *Xcc* or *P. brassicae* egg extract pretreatment followed by insect feeding. It looks like the plant response can be different between the tissue exposed to a dual stress compared to an adjacent area that is still in the same leaf. During our whole-genome analysis, which was performed on entire leaves, this information was certainly lost. We can postulate that this phenomenon is not so marked in *Arabidopsis* due to the leaf scale. Furthermore, we demonstrated that BVOCs genes were not regulated at the transcriptional level. Whereas some studies showed an increase of a few genes important to BVOCs biosynthesis and emission (Clavijo McCormick *et al.*, 2014; Wei *et al.*, 2014), no clear evidence of a transcriptional regulation was observed. We cannot exclude either that BVOCs are regulated at a transcriptional level but specifically into challenged cells or close to them. Analysis of specific challenged cells using laser microdissection may help to answer this

## GENERAL DISCUSSION

question and to identify new players important for the regulation of *B. nigra* responses against multiple stresses.

Moreover, plant defense is not regulated only at a transcriptomic level. Some metabolites take part in plant response against stress, like for example reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals, and are known to be important players during plant defense against pathogens and insects (Maffei *et al.*, 2007; Miller *et al.*, 2009; Kerchev *et al.*, 2012). In plants, ROS oxidize lipids, proteins or nucleic acids and can initiate radical chain reactions. It is well characterized also that various signaling pathways are modulated by ROS but its major role is the initiation and execution of plant cell death (Overmyer *et al.*, 2003; Maffei *et al.*, 2007). For example, in Arabidopsis, *P. brassicae* eggs oviposition induced cell death, callose accumulation underneath batches and production of ROS on leaves (Little *et al.*, 2007; Gouhier-Darimont *et al.*, 2013). Moreover, ozone, *Xcc* and aphids are also known to induce cell death through an oxidative burst (Baier *et al.*, 2005; Qian *et al.*, 2005; Kerchev *et al.*, 2012). In our whole-genome analysis of *B. nigra* challenged by single or dual stress, we observed an induction of genes belonging to ROS biosynthesis. For example, after *P. brassicae* feeding during 24 hours, 8 % of the total induced genes are genes important for ROS, such as *ZAT12* (At5g59820). *ZAT12* is a zinc-finger protein that may control expression of some defense gene against diverse (a)biotic stresses through the ROS signaling pathway (Davletova *et al.*, 2005). Interestingly, in our whole-genome analysis, *ZAT12* was also induced after dual stresses, *Xcc* infection or *B. brassicae* followed by *P. brassicae* feeding. After *P. brassicae* egg extract followed by herbivory, *ZAT12* was induced but not confirmed statistically because of variation between replicates. The zinc-finger protein however is not induced after *Xcc* infiltration, *P. brassicae* egg extract application or aphid single stress. *ZAT12* is specifically induced after chewing insect herbivory. In Arabidopsis, the *zat12* mutant is more sensitive to the generalist *S. littoralis*. Authors hypothesized that plant defense response against herbivory can be modulated by the well-known JA signaling pathway and through another pathway involving *ZAT12* and the ROS signaling pathway (Schweizer *et al.*, 2013). Further experiments need to be done to clearly identify the impact of ROS production against herbivory and to understand how ROS signaling pathway could be involved in SA / JA crosstalk in the case of multiple stresses. During plant-insect interaction, ROS could add to the oxidation caused by the digestion of plants secondary metabolites such as glucosinolates (Barbehenn, 2003; Kerchev *et al.*, 2012). Interestingly, as demonstrated by Barbehenn (2003), the polyphagous *Melanoplus sanguinipes* maintains a higher level of antioxidants in its midgut than the specialist *Aulocara ellioti* which was best protected from oxidative stress when it fed on grasshoppers (Barbehenn, 2003). ROS production could explain our insect performance results, especially the difference after *Xcc* pretreatment between insects feeding freely into whole-leaf or constrained to feeding at the infiltrated zone. Analysis of

feeding site preferences of *S. littoralis* and *P. brassicae* and measurements of ROS could help to assess the importance of ROS in plant defense and insect fitness.

Furthermore, other minor or unknown secondary metabolites could be important in plant defense. As we demonstrated, the most important family of secondary metabolites in Brassicaceae, glucosinolates, were mostly not affected by any pretreatment applied followed by *P. brassicae* feeding. However, a recent study showed that an unknown metabolite was affected by the application of *P. brassicae* larvae after different concentrations of ozone fumigation in comparison to plants that were only fumigated. Moreover, *P. brassicae* larvae had a lower performance in plant pre-exposed to elevated ozone than in plants non-exposed (Khaling *et al.*, 2015). Unfortunately, the authors did not test the real impact of this new compound on insect performance. Furthermore, a metabolomics analysis of both the plant and the insect showed the importance to study each player. The interaction between *Brassica oleracea* and *Pieris rapae* was analyzed after pretreatment of the shoot or root with JA. The authors observed a correlation between the increase in the amount of three structurally related coumaroylquinic acids in plant and insect metabolomes. Coumaroylquinic acids enter the caterpillar through the metabolic interface after a feeding event. However, the impact of these metabolites on *P. rapae* performance was not tested (Jansen *et al.*, 2008). More efforts have to be made regarding metabolomics analyses in the case of dual stress. Some important plant defense metabolites could be regulated post-transcriptionally and biosynthesized in very low amounts but they could still affect insect fitness. Development of more sensitive techniques will help improve our knowledge in this direction (Shulaev *et al.*, 2008).

Plant arms are not just present inside tissues; indirect defense through airborne signals, BVOCs, is clearly important for plant defense against herbivores and pathogens (Dicke and Baldwin, 2010). To improve our knowledge on plant defense against combined stresses, this type of defense has to be better explored. Until now, most of the research has focused on the effect of BVOCs on the third trophic level, on an ecological point of view. In *B. nigra*, a study showed that parasitoid wasps did not discriminate dually infested or caterpillar-infested plants, when plants were pretreated with *P. brassicae* eggs, *B. brassicae* feeding or *Xcc* infection. The authors observed that the volatile blend emitted by the black mustard, after these single or dual stresses were similar between *P. brassicae* herbivory alone and dual stresses involving the larvae also, but different from *Xcc*, eggs or aphids single stress. Prior stress had no impact on *P. brassicae* BVOCs blend emitted or on *Cotesia glomerata* wasp attraction (Ponzio *et al.*, 2014). In contrast, dual infection by the leaf chewing feeder *S. littoralis* and the root herbivore *Diabrotica virgifera* in maize decreased the attraction of their respective parasitoids. Analysis of BVOCs emission showed that the major attractant emitted after *D. virgifera* feeding was decreased by the dual infection, whereas no changes were observed in volatiles emitted by challenged leaves



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(Rasmann and Turlings, 2007). Depending on stresses applied to the plant, the third trophic level seems to be negatively or not impacted by a dual stress. In our case, analysis of BVOCs emitted after a first stress and their impact on plant defense could be interesting. For example, a recent study showed that exposure of tomato plants to (Z)-3-hexenyl vicinioside, a volatile specifically emitted after *Spodoptera littura* feeding, had a negative impact on insect performance and survival rates independently of the JA signaling pathway (Sugimoto *et al.*, 2014). We could imagine pretreatment of *B. nigra* would trigger volatiles that trigger specific defense and impact insect performance, independently of SA and JA pathways. A further analysis of *B. nigra* BVOCs blend after *Xcc*, *P. brassicae* egg oviposition or aphids feeding and testing their effect on *P. brassicae* and *S. littoralis* performance could validate this hypothesis.

Finally, in order to clearly identify the impact of plant defense on herbivores, whether after single stress or after combined attacks, research has to focus on both sides of the interaction, from the plant side but also from the insect point of view. Detoxification of defense metabolites can really make a difference for specialist and generalist fitness, depending on plant defense strategies (Cornell and Hawkins, 2003; Ali and Agrawal, 2012; Heidel-Fisher and Vogel, 2015). Insects have to digest plant primary compounds and secondary metabolites. It is well characterized that some herbivores have developed methods to store and use plant toxic compounds for their own defense against predators (Simon *et al.*, 2015). For example, a recent study analyzed the ingestion of cyanogenic glucosides in insects with different feeding specializations. Authors compared three lepidopteran generalists, including *S. littoralis*, *Spodoptera exigua* and *Mamestra brassicae*, and three lepidopteran glucosinolates-specialists, including *Pieris rapae*, *P. brassicae* and *Plutella xylostella*, a generalist aphid, *Myzus persicae*, and finally an aphid glucosinolate-specialist, *Lipaphis erysimi*. They observed that all insect species were tolerant to cyanogenic plants. Depending on the herbivore species, diverse strategies were observed; some excrete intact compounds, others metabolize cyanogenic glucosides into conjugate breakdown products with amino acids similarly to the metabolism of benzylglucosinolates, or furthermore, aphids avoid the toxic compounds during their feeding. Finally, all insects tested, independently of their specialization, are enabled to overcome chemical plant defenses and to adapt to their host plants (Pentzold *et al.*, 2015). However, the performance of insects feeding on plants or diet containing cyanogenic glucosides was not compared. The authors did not evaluate the impact of the xenobiotics on insect development depending on their ability to detoxify the cyanogenic glucosides. Up to now, most studies were conducted on one insect adaptation to few plant toxins. Our knowledge is limited in terms of insect species and xenobiotics analyzed. Future experiments could help discover other mechanisms of insect adaptation to chemical plant defense (Heidel-Fisher and Vogel, 2015). Furthermore, recent studies have demonstrated that plant defense impact on the insect community has to be done on

a larger scale, to better represent natural conditions and avoid misunderstanding. For example, after a comparison of multiple studies, Agrawal and Weber (2015) concluded that secondary metabolites might well not be as important as expected during plant-insect interactions (Agrawal and Weber, 2015). A large scale analysis of correlations between plant traits and herbivory was done in 21 dominant tree and shrub species of a subtropical forest in China. Surprisingly, they observed that chemical plant traits were less important for defense against herbivores than morphological and plant-history traits, which explained 70 % of the interspecific variation of herbivory (Schuldt *et al.*, 2012). Moreover, Loranger and coworkers (2012) measured aboveground herbivore damage on 51 grassland species and collected 42 different plant traits regrouped into four groups: physiological, morphological, phenological and herbivore-related. The multivariable approach showed that the most important traits, which explained variation of herbivory, were leaf nitrogen and lignin concentration. In their model, secondary metabolites had a minor influence on insect feeding (Loranger *et al.*, 2012). Interestingly, foliar nitrogen concentration was also demonstrated to be important for the immune function of *Parospsis atomaria* Oliver feeding on *Eucalyptus tereticornis* seedlings (Gherlenda *et al.*, 2016). Thus, authors confirmed the importance of analyzing plant-insect interaction involving the community as a whole, and not specifically between a single plant and a single herbivore or for a specific compound (Loranger *et al.*, 2012; Schuldt *et al.*, 2012; Agrawal and Weber, 2015). We can postulate that the same conclusion can be reached with analysis of plant defense against single or multiple attacks that do not integrate insect adaptation mechanisms. By restraining our research to few aspects only, we may miss important determinants that could help explain the complexity of plant defense in nature and how plants can deal with all the members of their own phyllosphere and rhizosphere.

## Conclusion

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In conclusion, I demonstrated that pretreatments had a weak impact on the transcriptomic response to *Pieris brassicae* herbivory in *Brassica nigra*. However, *Xanthomonas campestris* pv. *campestris* infection or *P. brassicae* egg extract application affected *P. brassicae* and *Spodoptera littoralis* performance. Efforts have to be made to find plant defense traits that are triggered after this pretreatment and that affect insect weight. Interestingly, SA / JA crosstalk and the antagonistic effect of one pathway on the other seem to be dependent on the history of attack. Finally, other stresses and other plants species have to be analyzed to improve our knowledge on how plants deal with multiple stresses in a natural environment. At the present time, my work and previously published reports indicate that plants prioritize their own defense against the second stress or that combined stresses create a new plant defense response. Contradictory results were noted between plant species and stresses applied. A high-throughput analysis of the most damageable attackers will have to be done on economically important plants to increase our knowledge and to develop robust solutions for improving plant defenses in agriculture.

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## Acknowledgements

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I would like to thank my parents, Elisabeth and Luc, my sister Aurélie and my favorite brother in law, Mikael, for their enthusiastic support throughout my thesis but also during my all-day-life.

I would specially thank Gaël for his support and love not only during this work. With him all my days are a Sky Full of Stars.

This thesis has been a challenge for me, but Philippe Reymond convinced me to start the adventure! Thank you so much Philippe for being the person who gave me the possibility to do this step in such a nice environment. The Reymond's group is not only a group of workers, it is a nice team where people share experiences, knowledge and good times!

This work could not exist without the fabulous Steve Lassueur, thank you so (so so) much for all the time you spent with me in this project and for having always taken care of weekend experiments. Thank you for your personal coaching during the down-phase, your happiness and your kindness. Definitely, this thesis would not have been the same without you!

I want to thank all my lab colleagues, Fabian Schweizer and Friedericke Bruessow for their help during my first experiments, Olivier Hilfiker for his nights hospitality, his nice whisky tastings and for being an excellent (and patient) R-Studio and Illustrator teacher, André Schmiesing for his scientific help and for being crazy enough to follow me during the spinning sessions, Raphaël Groux (but not for the Framboisine). Thank you also to our new baby Esteban, welcome in the Reymond's club, enjoy your new life as PhD student. I could not forget Caroline Darimont for all the help she always provides us with a nice smile.

A special thanks to Jonatan Montpetit, we started together five years ago and you became more than a colleague: a really nice friend!

Thanks to my nice office mates, Ghazanfar Khan and Blaise Tissot, it was so nice to be with you and share this time together. Thanks to Pietro Cattaneo for our running sessions and the really good meals you prepared for me sometimes.

Thanks to all DBMV members and especially to Guillaume Fabre, Imène Garroum, Céline Zimmerli, Pasqualina Magliano, Fred Masclaux, Lothar Kalmbach, Marie Barberon, Aurore Lenglet, Aurore Chatelat and Laurence Cienciala.

Thanks to the CIG team, Erwan Gouranton and Nicolas Bonhoure for our running sessions, coffee breaks and good moments shared together.

I cannot forget the "Princess team", thanks to my friends for all our girl weekends! Carine Chaintreuil, Alice Indalecio (Indelachio), Elodie Jallifier-Verne, Julie Vallet and Perrine Viargues, I love you (I wrote the list by alphabetic order... no jealousy!).

I want also to thank the Saugere Family, Céline, Julien, Bastien and Maxence, your friendship is really important to me.

Thanks to my “Master” friends Noémie Fahr, Quentin Alvarez and Fabien Jonchère.

Thanks to my boat mates, Lou Götz for our good time spend together and our crazy running sessions, Jeanne, Stéphane and Fabrice for our “Apéro party”!

Finally, I would like to thank the thesis jury members John Pannell, Marcel Dicke and Ted Turlings.

I thank Gaëtan Glauser, Floriane Consales, Johann Weber and Robin Liechti for their collaboration and help during this thesis.

I would like to thank all the A-BIO-VOC members for their scientific interactions and collaborations during this work.

I would like to thank the European Science Foundation, the Swiss National Foundation and the University of Lausanne for financial support.

# Annexes

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**Annex 1: List of genes induced or repressed in at least one condition: *P. brassicae* (P), egg extract / *P. brassicae* (EP), *Xcc* / *P. brassicae* (XP), *B. brassicae* (50 aphids) / *P. brassicae* (50AP), *B. brassicae* (100 aphids) / *P. brassicae* (100AP), egg extract (E), *Xcc* (X), *B. brassicae* (50 aphids) (50A), *B. brassicae* (100 aphids) (100A).**

**Annex 2: List of genes induced or repressed in at least one condition: *P. brassicae* (Finland) (PFL), ozone / *P. brassicae* (OP), ozone (O).**



ANNEX 1

AGI code	P	P-value	q-value	EP	P-value	q-value	XP	P-value	q-value	100 AP	P-value	q-value	50 AP	P-value	q-value	E	P-value	q-value	X	P-value	q-value	100 A	p-value	q-value	50 A	p-value	q-value
At1g01090	0.576	0.127	0.295	0.390	0.266	0.396	1.081	0.042	0.260	0.837	0.079	0.291	0.561	0.112	0.298	0.017	0.971	0.581	-0.053	0.812	0.587	-0.276	0.610	0.449	0.209	0.374	0.415
At1g01140	0.632	0.013	0.176	0.732	0.124	0.395	-0.318	0.111	0.297	0.268	0.077	0.291	0.651	0.049	0.251	0.315	0.444	0.413	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g01230	0.240	0.298	0.314	0.089	0.850	0.559	-0.058	0.839	0.523	0.493	0.123	0.295	0.212	0.612	0.434	0.597	0.029	0.363	-0.070	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g01360	-0.127	0.531	0.397	0.288	0.326	0.408	-0.230	0.509	0.409	0.106	0.363	0.330	0.670	0.025	0.226	-0.328	0.553	0.454	-0.012	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g01550	0.623	0.019	0.203	0.739	0.063	0.395	0.336	0.234	0.311	0.764	0.051	0.274	-0.256	0.572	0.419	0.540	0.431	0.410	0.228	0.419	0.442	0.224	0.286	0.378	0.040	0.374	0.415
At1g01800	0.129	0.203	0.296	0.595	0.019	0.395	0.177	0.178	0.308	0.019	0.298	0.314	0.000	1.000	1.000	0.124	0.401	0.400	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g02660	0.743	0.016	0.193	0.337	0.588	0.481	-0.456	0.051	0.270	0.359	0.079	0.291	0.865	0.028	0.227	0.347	0.710	0.505	-0.040	0.374	1.000	0.062	0.374	0.392	0.000	1.000	1.000
At1g02710	1.249	0.001	0.084	1.337	0.002	0.395	1.071	0.013	0.221	-0.163	0.856	0.486	0.699	0.098	0.290	0.481	0.220	0.363	0.186	0.527	0.481	-0.414	0.254	0.378	-0.176	0.374	0.415
At1g02820	-0.167	0.352	0.330	1.178	0.044	0.395	-0.306	0.159	0.308	-0.496	0.036	0.258	-0.413	0.480	0.385	0.120	0.849	0.549	-0.091	0.374	1.000	-0.153	0.511	0.422	0.306	0.279	0.414
At1g02950	0.018	0.920	0.514	-0.789	0.043	0.395	-0.196	0.400	0.364	-0.189	0.181	0.295	-0.493	0.046	0.249	-0.768	0.021	0.363	0.187	0.374	1.000	-0.062	0.179	0.378	0.000	1.000	1.000
At1g03850	0.065	0.559	0.406	1.014	0.029	0.395	0.420	0.039	0.256	0.000	1.000	1.000	0.061	0.374	1.000	0.239	0.505	0.435	0.159	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g03860	-0.029	0.808	0.484	0.284	0.276	0.398	0.348	0.175	0.308	0.386	0.051	0.274	0.625	0.007	0.180	0.272	0.290	0.375	0.043	0.410	0.440	0.079	0.472	0.411	0.000	1.000	1.000
At1g03905	0.106	0.671	0.443	-0.488	0.145	0.395	0.327	0.461	0.389	-0.236	0.566	0.395	1.122	0.019	0.226	-0.040	0.930	0.571	-0.112	0.374	1.000	0.080	0.374	0.392	0.000	1.000	1.000
At1g04300	0.598	0.043	0.268	0.041	0.785	0.539	0.000	1.000	1.000	0.334	0.109	0.295	0.000	1.000	1.000	0.136	0.169	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g04430	0.430	0.008	0.158	0.180	0.696	0.515	-0.190	0.347	0.345	0.873	0.006	0.194	1.013	0.060	0.269	0.190	0.666	0.491	0.053	0.656	0.535	0.454	0.066	0.378	0.240	0.132	0.414
At1g04480	-0.081	0.688	0.449	0.536	0.061	0.395	0.654	0.004	0.177	0.243	0.238	0.297	0.468	0.004	0.145	0.070	0.732	0.513	-0.001	0.374	1.000	0.110	0.427	0.400	0.074	0.374	0.415
At1g04800	0.100	0.596	0.417	0.251	0.499	0.455	0.946	0.017	0.237	0.551	0.351	0.327	0.073	0.774	0.485	0.071	0.820	0.540	0.269	0.268	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g05135	0.791	0.009	0.160	1.247	0.024	0.395	0.339	0.092	0.289	0.377	0.287	0.311	0.753	0.023	0.226	0.844	0.019	0.363	0.000	1.000	1.000	0.140	0.266	0.378	0.000	1.000	1.000
At1g05500	-0.026	0.851	0.497	-0.397	0.257	0.396	-0.286	0.000	0.081	0.056	0.880	0.492	-0.341	0.072	0.284	-0.056	0.561	0.456	-0.623	0.003	0.418	0.274	0.087	0.378	0.049	0.374	0.415
At1g05680	0.310	0.029	0.240	0.780	0.193	0.395	0.000	1.000	1.000	0.146	0.314	0.319	0.015	0.656	0.448	0.711	0.007	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g06080	0.042	0.865	0.500	0.000	1.000	1.000	-0.339	0.176	0.308	-0.174	0.269	0.305	-0.627	0.048	0.250	-0.152	0.451	0.416	0.000	1.000	1.000	0.000	1.000	1.000	0.083	0.374	0.415
At1g06430	-0.640	0.020	0.207	-0.112	0.710	0.519	-0.948	0.157	0.308	0.820	0.270	0.305	0.130	0.840	0.505	0.154	0.828	0.543	-0.132	0.585	0.506	0.696	0.247	0.378	-0.005	0.374	0.415
At1g06620	0.381	0.091	0.295	0.800	0.096	0.395	0.361	0.035	0.254	0.620	0.036	0.258	0.653	0.043	0.247	0.255	0.185	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g06680	-0.007	0.980	0.529	-0.607	0.331	0.409	-0.642	0.032	0.249	-0.088	0.419	0.347	-0.258	0.096	0.290	-0.963	0.047	0.363	-0.022	0.929	0.614	0.082	0.723	0.483	-0.066	0.752	0.543
At1g07000	0.205	0.064	0.285	1.032	0.032	0.395	0.028	0.875	0.534	0.444	0.043	0.266	0.566	0.056	0.264	0.855	0.157	0.363	0.134	0.178	0.418	0.021	0.374	0.392	0.000	1.000	1.000
At1g07110	-0.586	0.022	0.215	-0.234	0.633	0.497	-0.399	0.307	0.331	0.163	0.640	0.422	-0.017	0.971	0.539	-0.363	0.527	0.444	0.004	0.374	1.000	0.062	0.374	0.392	0.000	1.000	1.000
At1g07600	1.081	0.006	0.143	1.472	0.024	0.395	0.810	0.039	0.256	1.366	0.018	0.247	1.980	0.000	0.059	0.231	0.686	0.498	-0.032	0.961	0.621	0.797	0.096	0.378	0.676	0.016	0.414
At1g07890	0.642	0.001	0.075	0.599	0.112	0.395	-0.003	0.992	0.562	1.201	0.037	0.259	1.265	0.000	0.046	0.114	0.756	0.521	-0.061	0.374	1.000	0.407	0.135	0.378	0.071	0.195	0.414
At1g07930	0.231	0.104	0.295	1.053	0.102	0.395	0.731	0.129	0.308	0.691	0.048	0.271	0.936	0.027	0.226	0.227	0.578	0.461	0.201	0.158	0.418	0.090	0.526	0.427	0.121	0.257	0.414
At1g08380	-0.794	0.049	0.274	-1.038	0.314	0.404	-1.176	0.012	0.216	-1.291	0.002	0.123	-1.113	0.010	0.201	-1.572	0.004	0.321	-1.030	0.103	0.418	-0.507	0.118	0.378	-0.294	0.542	0.476
At1g08450	0.281	0.265	0.305	0.374	0.046	0.395	-0.020	0.882	0.536	1.235	0.012	0.239	0.756	0.229	0.313	0.275	0.386	0.395	0.114	0.374	1.000	0.039	0.374	0.392	0.000	1.000	1.000
At1g08920	0.189	0.102	0.295	0.737	0.126	0.395	0.102	0.300	0.329	0.242	0.142	0.295	0.031	0.374	1.000	0.756	0.046	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g08930	1.068	0.011	0.169	1.409	0.193	0.395	-0.364	0.329	0.337	0.474	0.274	0.306	1.529	0.037	0.240	1.455	0.054	0.363	-0.440	0.331	0.423	0.034	0.106	0.378	-0.148	0.374	0.415
At1g09070	0.718	0.067	0.289	0.345	0.302	0.403	0.640	0.008	0.200	0.967	0.064	0.288	0.658	0.061	0.270	0.811	0.255	0.366	0.121	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g09180	0.911	0.016	0.193	0.445	0.361	0.415	0.495	0.486	0.399	-0.222	0.772	0.465	0.610	0.188	0.310	-0.653	0.285	0.374	0.203	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g09340	-0.469	0.022	0.214	-0.367	0.353	0.414	-0.718	0.023	0.242	-0.457	0.038	0.259	-0.881	0.007	0.180	-0.374	0.070	0.363	-0.187	0.033	0.418	-0.276	0.354	0.386	-0.356	0.272	0.414
At1g09480	0.164	0.095	0.295	0.673	0.033	0.395	0.072	0.178	0.308	0.000	1.000	1.000	-0.075	0.410	0.359	0.554	0.090	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g10270	0.003	0.983	0.530	0.218	0.486	0.451	-0.001	0.991	0.562	0.254	0.334	0.323	0.769	0.004	0.144	0.591	0.124	0.363	-0.092	0.396	0.436	0.229	0.330	0.385	0.344	0.193	0.414
At1g10500	0.340	0.018	0.197	-0.014	0.951	0.585	-0.034	0.774	0.501	0.294	0.021	0.249	0.489	0.003	0.138	0.436	0.005	0.350	-0.103	0.073	0.418	0.681	0.003	0.378	0.108	0.659	0.511
At1g10522	-0.259	0.034	0.257	0.079	0.693	0.514	0.056	0.363	1.000	-0.654	0.001	0.091	-0.100	0.651	0.446	-0.003	0.995	0.586	0.000	1.000	1.000	-0.039	0.679	0.467	0.000	1.000	1.000
At1g11410	-0.139	0.227	0.299	0.042	0.816	0.549	-0.054	0.506	0.408	-0.586	0.022	0.249	-0.400	0.014	0.224	0.068	0.343	0.384	-0.077	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g11580	0.040	0.859	0.499	-0.001	0.984	0.591	0.118	0.185	0.308	0.218	0.283	0.309	0.876	0.050	0.253	-0.118	0.165	0.363	0.000	1.000	1.000	-0.057	0.374	0.392	0.000		

At1g11910	-0.217	0.761	0.472	1.546	0.254	0.396	0.955	0.326	0.336	1.295	0.280	0.308	1.030	0.048	0.250	0.780	0.457	0.418	0.576	0.254	0.418	0.864	0.225	0.378	-0.209	0.391	0.421
At1g12040	0.843	0.001	0.075	0.683	0.036	0.395	0.700	0.010	0.208	0.402	0.061	0.287	0.603	0.033	0.235	0.191	0.122	0.363	0.326	0.245	0.418	-0.019	0.374	0.392	0.000	1.000	1.000
At1g12090	0.155	0.468	0.374	0.045	0.919	0.577	0.322	0.412	0.369	-0.188	0.348	0.326	0.146	0.368	0.349	-0.822	0.038	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.107	0.374	0.415
At1g12110	0.434	0.091	0.295	0.124	0.498	0.454	0.462	0.026	0.246	0.561	0.035	0.258	0.801	0.007	0.180	0.386	0.374	0.393	0.000	1.000	1.000	0.024	0.374	0.392	0.000	1.000	1.000
At1g12450	0.404	0.101	0.295	0.478	0.123	0.395	-0.048	0.869	0.533	0.106	0.585	0.403	0.605	0.017	0.226	0.134	0.494	0.432	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g12500	1.212	0.004	0.114	1.029	0.017	0.395	0.283	0.024	0.245	0.794	0.039	0.260	0.275	0.259	0.319	0.739	0.003	0.315	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g12900	-0.962	0.011	0.169	-0.288	0.769	0.535	0.131	0.730	0.487	-0.689	0.097	0.291	-0.049	0.937	0.531	-1.088	0.257	0.367	0.079	0.851	0.594	0.090	0.748	0.492	0.228	0.717	0.528
At1g12920	0.854	0.088	0.295	0.730	0.417	0.429	1.047	0.010	0.208	1.081	0.017	0.246	0.576	0.410	0.359	-0.220	0.681	0.496	0.093	0.444	0.453	-0.313	0.357	0.386	-0.038	0.374	0.415
At1g13020	0.450	0.017	0.193	0.770	0.068	0.395	0.561	0.007	0.200	0.630	0.000	0.091	0.438	0.057	0.266	0.415	0.287	0.375	0.121	0.416	0.441	-0.004	0.980	0.545	0.012	0.374	0.415
At1g13520	0.089	0.189	0.295	0.318	0.390	0.421	0.181	0.148	0.308	0.042	0.374	1.000	0.051	0.374	1.000	0.642	0.036	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g13870	-0.053	0.805	0.483	-0.387	0.322	0.407	0.183	0.255	0.314	0.335	0.302	0.315	0.253	0.626	0.439	0.593	0.046	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g14150	0.089	0.663	0.440	0.607	0.001	0.385	0.005	0.986	0.561	-0.544	0.051	0.274	-0.347	0.260	0.320	-0.672	0.141	0.363	0.175	0.468	0.463	-0.286	0.252	0.378	0.031	0.893	0.591
At1g14280	-0.064	0.754	0.469	-0.297	0.368	0.416	-0.555	0.049	0.268	-0.372	0.091	0.291	-0.884	0.025	0.226	-0.172	0.679	0.496	-0.296	0.198	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g14345	-0.208	0.077	0.295	-0.058	0.778	0.537	-0.300	0.039	0.256	-0.497	0.054	0.277	-0.511	0.002	0.116	-0.733	0.007	0.361	-0.250	0.130	0.418	-0.121	0.215	0.378	0.063	0.334	0.414
At1g14400	0.241	0.406	0.351	-0.657	0.127	0.395	-0.289	0.520	0.413	-0.570	0.097	0.291	0.201	0.374	1.000	0.920	0.045	0.363	-0.062	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g15390	0.502	0.156	0.295	0.614	0.115	0.395	-0.176	0.666	0.466	0.776	0.018	0.247	0.143	0.699	0.462	-0.203	0.615	0.475	0.079	0.374	1.000	-0.153	0.589	0.445	0.112	0.374	0.415
At1g15580	-0.063	0.268	0.306	-0.823	0.080	0.395	-0.226	0.149	0.308	-0.355	0.072	0.291	-0.287	0.195	0.310	-1.135	0.000	0.175	-0.229	0.315	0.421	0.000	1.000	1.000	-0.057	0.374	0.415
At1g15740	-0.073	0.360	0.333	0.676	0.046	0.395	0.058	0.652	0.461	0.000	1.000	1.000	0.000	1.000	1.000	0.269	0.109	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g15810	-0.637	0.080	0.295	-1.176	0.142	0.395	-1.084	0.022	0.241	-0.886	0.043	0.266	-0.218	0.459	0.378	-1.268	0.039	0.363	-0.649	0.082	0.418	0.318	0.226	0.378	-0.294	0.229	0.414
At1g15820	-0.153	0.491	0.382	-0.281	0.738	0.527	-0.631	0.020	0.241	-0.690	0.019	0.247	-0.554	0.044	0.247	-1.135	0.037	0.363	-0.386	0.069	0.418	-0.587	0.208	0.378	-0.185	0.380	0.418
At1g15830	-0.259	0.110	0.295	-0.513	0.325	0.408	-0.360	0.229	0.311	-0.224	0.160	0.295	-0.209	0.223	0.312	-1.216	0.005	0.345	-0.026	0.187	0.418	-0.003	0.374	0.392	0.000	1.000	1.000
At1g16720	0.295	0.415	0.355	0.375	0.016	0.395	-0.041	0.871	0.533	-0.573	0.331	0.322	-0.769	0.004	0.145	0.033	0.923	0.569	-0.071	0.650	0.534	-0.259	0.130	0.378	-0.043	0.374	0.415
At1g17180	0.648	0.056	0.277	1.767	0.007	0.395	0.515	0.101	0.291	0.434	0.119	0.295	0.183	0.699	0.462	0.060	0.916	0.567	0.145	0.576	0.504	-0.320	0.188	0.378	0.051	0.759	0.546
At1g17290	0.232	0.479	0.378	0.701	0.031	0.395	0.608	0.109	0.297	1.315	0.118	0.295	0.017	0.984	0.541	0.053	0.944	0.574	0.000	1.000	1.000	0.249	0.374	0.392	0.000	1.000	1.000
At1g17420	2.310	0.000	0.020	1.721	0.046	0.395	0.969	0.003	0.166	3.514	0.000	0.075	3.425	0.000	0.046	0.775	0.355	0.387	0.279	0.232	0.418	1.744	0.017	0.378	1.075	0.080	0.414
At1g17840	-0.227	0.287	0.311	-0.042	0.751	0.530	-0.182	0.305	0.330	-0.451	0.036	0.258	-0.123	0.269	0.321	-0.611	0.005	0.350	-0.087	0.377	0.428	-0.528	0.212	0.378	0.007	0.980	0.612
At1g18060	-0.066	0.747	0.467	0.783	0.295	0.402	-0.198	0.615	0.448	0.010	0.976	0.519	-0.759	0.119	0.301	0.036	0.925	0.569	-0.113	0.752	0.567	-0.661	0.042	0.378	0.001	0.976	0.612
At1g18070	0.202	0.548	0.403	0.218	0.576	0.478	0.666	0.159	0.308	0.701	0.048	0.271	0.350	0.281	0.325	0.692	0.228	0.364	0.258	0.063	0.418	0.299	0.208	0.378	0.040	0.374	0.415
At1g18890	0.315	0.106	0.295	0.293	0.139	0.395	0.271	0.082	0.286	0.601	0.019	0.247	0.542	0.305	0.330	0.281	0.454	0.417	0.050	0.374	1.000	0.081	0.374	0.392	0.000	1.000	1.000
At1g19150	-0.350	0.099	0.295	-0.533	0.554	0.473	0.154	0.421	0.372	-0.504	0.002	0.142	-0.492	0.029	0.230	-1.235	0.042	0.363	0.019	0.374	1.000	-0.002	0.994	0.548	0.094	0.374	0.415
At1g19180	0.075	0.117	0.295	0.806	0.043	0.395	0.080	0.180	0.308	0.326	0.043	0.266	0.305	0.184	0.310	0.243	0.173	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
19550 // At1g1	0.964	0.000	0.011	0.782	0.166	0.395	0.221	0.333	0.338	0.878	0.012	0.239	1.138	0.003	0.138	-0.063	0.640	0.483	0.255	0.009	0.418	0.105	0.071	0.378	-0.072	0.374	0.415
At1g19715	-0.247	0.283	0.310	-0.015	0.952	0.585	-0.081	0.633	0.456	0.115	0.606	0.410	0.152	0.456	0.377	-0.760	0.024	0.363	0.029	0.374	1.000	-0.117	0.120	0.378	0.000	1.000	1.000
At1g19770	0.945	0.029	0.240	1.464	0.069	0.395	0.158	0.238	0.311	0.090	0.222	0.295	1.079	0.071	0.283	0.487	0.093	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g19870	-0.090	0.575	0.411	-0.573	0.030	0.395	-0.347	0.036	0.254	-0.315	0.134	0.295	-0.661	0.007	0.180	-0.247	0.294	0.376	-0.480	0.043	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g20340	-0.477	0.219	0.297	0.188	0.799	0.544	0.499	0.462	0.389	0.483	0.437	0.353	-1.505	0.275	0.324	-1.486	0.044	0.363	-0.771	0.259	0.418	1.322	0.350	0.386	1.424	0.052	0.414
At1g20440	0.671	0.112	0.295	0.238	0.157	0.395	-0.133	0.191	0.308	1.404	0.010	0.235	0.523	0.098	0.290	0.396	0.290	0.375	0.038	0.241	0.418	0.095	0.374	0.392	0.000	1.000	1.000
At1g20620	-0.153	0.453	0.369	0.216	0.305	0.403	-0.286	0.109	0.297	-0.671	0.030	0.249	-0.190	0.444	0.372	0.369	0.172	0.363	-0.012	0.374	1.000	-0.054	0.363	0.389	0.000	1.000	1.000
At1g20850	-0.494	0.009	0.165	-0.602	0.132	0.395	-0.087	0.153	0.308	-0.732	0.006	0.195	-0.533	0.066	0.273	-1.059	0.006	0.359	-0.150	0.374	1.000	-0.376	0.146	0.378	0.000	1.000	1.000
At1g21000	0.698	0.007	0.151	-0.051	0.950	0.584	0.008	0.970	0.559	-0.002	0.997	0.524	-0.413	0.513	0.396	0.371	0.311	0.377	0.055	0.758	0.568	-0.251	0.267	0.378	0.062	0.374	0.415
At1g21130	1.021	0.008	0.154	1.343	0.098	0.395	0.468	0.133	0.308	0.371	0.109	0.295	0.648	0.010	0.199	1.557	0.091	0.363	0.207	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g21500	-0.065	0.745	0.466	-0.476	0.435	0.435	-0.186	0.504	0.407	-0.170	0.414	0.345	-0.090	0.867	0.511	-1.431	0.048	0.363	-0.040	0.849	0.594	0.034	0.891	0.523	-0.045	0.374	0.415
At1g21750	-0.117	0.614	0.424	-0.055	0.740	0.527	0.534	0.011	0.216	0.643	0.010	0.235	0.375	0.012	0.214	0.137	0.644	0.484	0.116	0.435	0.449	0.266	0.170				

At1g22840	-0.170	0.364	0.335	1.662	0.079	0.395	0.684	0.128	0.308	0.096	0.620	0.414	1.261	0.025	0.226	0.279	0.457	0.418	-0.062	0.760	0.569	0.027	0.695	0.475	0.000	1.000	1.000
At1g23730	1.594	0.046	0.274	1.060	0.506	0.457	0.390	0.523	0.414	2.070	0.014	0.241	0.327	0.275	0.324	-1.318	0.145	0.363	0.300	0.503	0.473	0.604	0.374	0.392	0.282	0.374	0.415
At1g23870	0.279	0.137	0.295	0.635	0.023	0.395	-0.479	0.096	0.290	0.000	0.374	1.000	0.069	0.494	0.390	-0.055	0.890	0.561	0.016	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g24560	0.324	0.017	0.197	-0.225	0.135	0.395	-0.668	0.010	0.208	0.037	0.499	0.374	-0.011	0.953	0.535	0.153	0.195	0.363	-0.084	0.811	0.587	-0.003	0.984	0.546	-0.034	0.346	0.414
At1g24909	1.065	0.005	0.130	1.563	0.019	0.395	0.936	0.050	0.270	1.468	0.014	0.239	1.634	0.015	0.226	0.437	0.541	0.449	0.328	0.240	0.418	1.059	0.094	0.378	0.363	0.110	0.414
At1g26210	-0.144	0.576	0.411	-0.452	0.512	0.460	-0.961	0.002	0.163	-0.960	0.031	0.250	-0.065	0.469	0.381	-0.235	0.505	0.435	-0.009	0.212	0.418	0.002	0.374	0.392	0.000	1.000	1.000
At1g27000	0.208	0.201	0.295	0.203	0.672	0.507	-0.245	0.210	0.309	-0.200	0.183	0.295	-0.219	0.215	0.311	0.869	0.039	0.363	0.005	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g27140	1.134	0.002	0.087	1.319	0.064	0.395	0.715	0.015	0.232	1.120	0.018	0.247	1.002	0.036	0.238	1.710	0.006	0.359	0.109	0.505	0.474	0.075	0.660	0.458	-0.015	0.374	0.415
At1g27695	0.338	0.183	0.295	0.693	0.153	0.395	1.032	0.032	0.249	-0.087	0.667	0.431	0.000	1.000	1.000	0.153	0.462	0.419	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g27730	0.323	0.081	0.295	1.399	0.034	0.395	1.063	0.031	0.249	0.219	0.210	0.295	0.535	0.095	0.290	0.868	0.223	0.363	0.000	1.000	1.000	0.000	1.000	1.000	-0.066	0.374	0.415
At1g27760	0.484	0.023	0.220	-0.083	0.858	0.561	0.636	0.022	0.241	0.763	0.091	0.291	1.263	0.024	0.226	0.407	0.322	0.380	0.081	0.652	0.534	0.343	0.044	0.378	0.338	0.374	0.415
At1g27770	0.630	0.010	0.166	0.778	0.246	0.395	-0.349	0.033	0.252	0.435	0.089	0.291	0.785	0.055	0.261	0.444	0.500	0.434	-0.143	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g27950	0.104	0.375	0.339	-0.054	0.801	0.544	0.000	1.000	1.000	0.588	0.046	0.266	0.023	0.967	0.538	-0.124	0.115	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g27980	0.486	0.062	0.284	1.037	0.145	0.395	0.829	0.009	0.207	0.556	0.171	0.295	0.138	0.354	0.345	0.825	0.010	0.363	0.032	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g28100	-0.143	0.148	0.295	-0.460	0.138	0.395	-0.096	0.176	0.308	-0.043	0.569	0.396	-0.172	0.126	0.305	-0.676	0.001	0.291	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g28110	-0.385	0.058	0.277	-1.411	0.104	0.395	-0.348	0.157	0.308	-0.283	0.272	0.305	-0.009	0.818	0.498	-1.096	0.041	0.363	0.030	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g28395	-0.124	0.016	0.193	-0.408	0.027	0.395	-0.079	0.315	0.333	0.000	1.000	1.000	0.000	1.000	1.000	-0.644	0.018	0.363	-0.538	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g28480	0.479	0.064	0.286	0.363	0.096	0.395	0.449	0.078	0.285	1.171	0.037	0.259	0.746	0.103	0.292	0.380	0.069	0.363	0.017	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g28490	0.318	0.233	0.300	0.824	0.158	0.395	0.537	0.094	0.289	0.933	0.017	0.246	0.477	0.049	0.252	0.395	0.058	0.363	-0.048	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g29470	0.305	0.040	0.266	-0.262	0.453	0.442	0.594	0.060	0.276	0.664	0.143	0.295	0.965	0.003	0.138	0.931	0.106	0.363	0.061	0.186	0.418	0.010	0.374	0.392	-0.046	0.374	0.415
At1g29670	0.380	0.028	0.240	-0.233	0.588	0.481	0.084	0.696	0.476	1.283	0.029	0.249	1.519	0.016	0.226	-0.247	0.689	0.499	0.298	0.374	1.000	0.019	0.374	0.392	0.000	1.000	1.000
At1g29680	0.350	0.100	0.295	0.000	1.000	1.000	-0.056	0.291	0.325	0.685	0.117	0.295	1.022	0.027	0.226	-0.020	0.921	0.568	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g29690	0.725	0.002	0.095	0.668	0.161	0.395	-0.288	0.054	0.273	0.450	0.176	0.295	1.075	0.013	0.221	0.981	0.152	0.363	-0.013	0.826	0.591	0.322	0.097	0.378	0.180	0.374	0.415
At1g29740	-0.417	0.016	0.193	-0.415	0.455	0.442	-0.178	0.064	0.278	-0.234	0.080	0.291	-0.402	0.007	0.180	-1.141	0.034	0.363	-0.073	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g29850	0.208	0.128	0.295	0.276	0.061	0.395	0.001	0.986	0.561	-0.011	0.929	0.505	0.282	0.118	0.300	0.602	0.043	0.363	-0.015	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g29930	-0.425	0.199	0.295	-0.740	0.536	0.467	-1.033	0.002	0.163	0.029	0.953	0.512	-0.895	0.059	0.268	-2.407	0.041	0.363	0.234	0.374	1.000	-0.251	0.357	0.386	-0.351	0.027	0.414
At1g30320	0.341	0.134	0.295	-0.061	0.770	0.535	-0.145	0.020	0.241	0.976	0.005	0.175	0.894	0.007	0.180	-0.159	0.076	0.363	-0.025	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g30360	-0.070	0.665	0.441	-0.449	0.049	0.395	-0.302	0.118	0.303	1.338	0.001	0.123	1.141	0.010	0.200	0.107	0.628	0.479	-0.018	0.875	0.601	1.351	0.002	0.378	0.412	0.068	0.414
At1g30380	-0.321	0.171	0.295	-0.477	0.219	0.395	-0.534	0.060	0.276	-0.256	0.229	0.295	-0.318	0.413	0.361	-0.885	0.019	0.363	-0.486	0.124	0.418	-0.185	0.690	0.473	-0.126	0.197	0.414
At1g30590	-0.208	0.429	0.361	-0.010	0.980	0.590	-0.064	0.363	1.000	-0.011	0.824	0.478	-0.086	0.374	1.000	-1.008	0.038	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g32450	0.281	0.063	0.285	0.488	0.268	0.396	1.219	0.008	0.200	0.112	0.547	0.390	-0.118	0.400	0.357	0.355	0.382	0.395	0.094	0.364	0.423	0.000	1.000	1.000	0.000	1.000	1.000
At1g32640	0.936	0.000	0.003	0.664	0.011	0.395	0.280	0.365	0.351	1.139	0.004	0.174	1.074	0.004	0.144	-0.069	0.804	0.535	0.034	0.891	0.606	0.489	0.148	0.378	0.055	0.374	0.415
At1g32920	1.090	0.004	0.121	1.650	0.031	0.395	0.605	0.060	0.276	0.175	0.520	0.381	0.499	0.016	0.226	0.513	0.417	0.405	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g33055	0.607	0.029	0.240	-0.094	0.567	0.475	-0.345	0.145	0.308	-0.061	0.374	1.000	0.085	0.390	0.354	-0.217	0.360	0.389	0.090	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g33360	0.517	0.011	0.170	0.418	0.108	0.395	0.947	0.004	0.177	0.995	0.019	0.247	0.199	0.682	0.457	0.528	0.199	0.363	0.339	0.172	0.418	0.087	0.178	0.378	-0.105	0.374	0.415
At1g34030	-0.093	0.608	0.422	0.585	0.015	0.395	0.136	0.373	0.354	-0.008	0.962	0.514	0.239	0.308	0.332	0.032	0.904	0.565	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g34130	0.106	0.772	0.476	0.187	0.563	0.475	0.599	0.036	0.254	0.134	0.788	0.469	-0.538	0.414	0.361	-0.297	0.284	0.374	0.121	0.250	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g35550	0.126	0.703	0.453	0.366	0.174	0.395	0.639	0.025	0.245	0.420	0.305	0.317	0.545	0.036	0.238	0.887	0.032	0.363	0.057	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g35620	-0.049	0.757	0.470	0.612	0.164	0.395	-0.020	0.875	0.534	0.592	0.030	0.249	0.279	0.136	0.309	0.056	0.780	0.528	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g42550	-0.256	0.011	0.169	-0.430	0.107	0.395	-0.321	0.034	0.254	-0.738	0.012	0.239	-0.586	0.022	0.226	-0.444	0.008	0.363	-0.134	0.271	0.418	-0.130	0.448	0.406	0.000	1.000	1.000
At1g42970	-0.266	0.184	0.295	0.345	0.565	0.475	-0.036	0.921	0.547	-1.101	0.019	0.247	-0.197	0.839	0.505	-0.411	0.108	0.363	-0.043	0.768	0.572	-0.304	0.494	0.416	-0.053	0.876	0.589
At1g43170	0.043	0.896	0.508	0.171	0.453	0.442	0.946	0.021	0.241	-0.135	0.117	0.295	0.012	0.986	0.542	0.057	0.775	0.526	-0.296	0.374	1.000	0.550	0.097	0.378	0.000	1.000	1.000
At1g43190	-0.599	0.031	0.247	0.116	0.588	0.481	-0.340	0.123	0.305	-0.316	0.545	0.389	-0.500	0.116	0.299	0.016	0.963	0.579	0.016	0.939	0.614	-0.168	0.551	0.			

At1g47710	0.081	0.192	0.295	-0.153	0.663	0.505	0.081	0.363	1.000	0.148	0.178	0.295	0.042	0.374	1.000	0.660	0.009	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g48030	-0.023	0.886	0.505	0.369	0.231	0.395	0.055	0.743	0.491	-0.630	0.014	0.241	-0.349	0.013	0.221	-0.109	0.505	0.435	0.023	0.374	1.000	-0.035	0.841	0.511	-0.314	0.081	0.414	0.000	1.000	1.000
At1g48760	-0.017	0.946	0.521	-0.341	0.488	0.452	0.895	0.031	0.249	0.053	0.592	0.405	-0.253	0.310	0.332	-0.647	0.075	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g48920	0.560	0.057	0.277	0.694	0.071	0.395	1.182	0.005	0.200	0.410	0.050	0.272	0.480	0.131	0.305	0.189	0.600	0.469	0.227	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g49050	0.288	0.019	0.204	0.065	0.703	0.517	0.443	0.072	0.285	0.980	0.038	0.259	0.659	0.064	0.273	0.440	0.384	0.395	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g49190	0.274	0.100	0.295	0.006	0.374	1.000	0.022	0.363	1.000	0.714	0.039	0.261	0.000	1.000	1.000	0.032	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g49510	0.124	0.529	0.396	-0.345	0.339	0.411	0.093	0.808	0.512	-0.193	0.448	0.356	0.403	0.237	0.313	0.586	0.050	0.363	0.321	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g49970	0.073	0.595	0.417	-0.389	0.246	0.395	-0.075	0.612	0.447	0.539	0.102	0.294	0.717	0.004	0.144	0.649	0.029	0.363	-0.146	0.200	0.418	0.035	0.647	0.454	0.000	1.000	1.000	0.000	1.000	1.000
At1g50900	-0.259	0.224	0.299	-0.444	0.239	0.395	-0.766	0.035	0.254	-0.380	0.038	0.259	-0.518	0.034	0.235	-0.576	0.002	0.307	-0.311	0.164	0.418	-0.098	0.271	0.378	0.134	0.374	0.415	0.000	1.000	1.000
At1g50920	0.124	0.467	0.374	0.416	0.041	0.395	0.087	0.271	0.319	0.157	0.306	0.317	0.680	0.034	0.235	0.404	0.019	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g50970	0.302	0.085	0.295	0.519	0.074	0.395	0.705	0.035	0.254	0.322	0.233	0.296	0.125	0.785	0.490	-0.033	0.915	0.567	0.057	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g51400	-0.011	0.938	0.519	-0.560	0.384	0.419	-0.374	0.028	0.248	-0.482	0.047	0.268	-0.487	0.016	0.226	-0.711	0.018	0.363	-0.094	0.347	0.423	-0.257	0.180	0.378	0.035	0.374	0.415	0.000	1.000	1.000
At1g51590	0.497	0.023	0.220	0.357	0.356	0.414	0.639	0.048	0.266	0.707	0.057	0.281	0.495	0.212	0.311	0.462	0.104	0.363	0.036	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g51760	1.213	0.009	0.159	1.044	0.143	0.395	0.301	0.313	0.332	2.190	0.018	0.247	1.940	0.046	0.248	-0.176	0.659	0.489	-0.056	0.394	0.435	0.920	0.167	0.378	0.254	0.166	0.414	0.000	1.000	1.000
At1g51940	-0.449	0.007	0.148	-0.293	0.374	0.416	-0.308	0.171	0.308	-0.887	0.005	0.182	-0.415	0.145	0.310	-0.265	0.355	0.387	-0.164	0.206	0.418	-0.089	0.367	0.391	0.000	1.000	1.000	0.000	1.000	1.000
At1g52040	0.947	0.011	0.169	0.913	0.018	0.395	0.256	0.532	0.418	0.643	0.032	0.252	0.247	0.093	0.289	0.143	0.288	0.375	0.254	0.319	0.421	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g52230	-0.216	0.266	0.306	-0.842	0.155	0.395	-0.516	0.112	0.297	-0.513	0.020	0.247	-0.378	0.389	0.354	-0.968	0.003	0.315	-0.140	0.487	0.467	-0.279	0.240	0.378	-0.193	0.523	0.473	0.000	1.000	1.000
At1g52400	2.594	0.000	0.003	2.260	0.001	0.385	1.317	0.016	0.234	2.910	0.000	0.091	3.181	0.000	0.046	-0.535	0.153	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g52880	0.098	0.254	0.303	0.060	0.663	0.505	0.126	0.236	0.311	0.125	0.526	0.383	0.389	0.148	0.310	0.620	0.004	0.321	0.000	1.000	1.000	0.037	0.082	0.378	-0.074	0.481	0.458	0.000	1.000	1.000
At1g53210	0.538	0.003	0.111	0.056	0.762	0.533	-0.106	0.432	0.377	1.368	0.011	0.239	1.386	0.004	0.144	0.547	0.302	0.376	-0.281	0.447	0.455	1.432	0.009	0.378	0.971	0.021	0.414	0.000	1.000	1.000
At1g53265	-0.273	0.035	0.259	0.028	0.949	0.584	-0.122	0.287	0.325	-0.352	0.169	0.295	-0.184	0.039	0.243	-0.944	0.020	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g53510	-0.026	0.859	0.499	0.727	0.029	0.395	0.026	0.363	1.000	-0.151	0.229	0.295	0.042	0.690	0.459	0.539	0.059	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g53580	0.658	0.092	0.295	0.209	0.651	0.501	0.115	0.837	0.523	-0.077	0.583	0.402	0.822	0.025	0.226	0.038	0.645	0.484	0.000	1.000	1.000	-0.091	0.374	0.392	0.000	1.000	1.000	0.000	1.000	1.000
At1g53610	1.426	0.007	0.148	1.698	0.062	0.395	1.163	0.132	0.308	0.830	0.275	0.306	0.704	0.248	0.315	1.569	0.190	0.363	-0.056	0.630	0.524	0.671	0.117	0.378	-0.268	0.374	0.415	0.000	1.000	1.000
At1g53800	-0.242	0.167	0.295	-0.055	0.453	0.442	0.048	0.748	0.492	-0.588	0.025	0.249	-0.235	0.428	0.366	-0.096	0.560	0.455	-0.146	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g54030	0.493	0.005	0.129	0.480	0.106	0.395	0.630	0.020	0.241	0.503	0.044	0.266	0.696	0.012	0.221	-0.168	0.238	0.364	0.008	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g54100	0.288	0.072	0.293	0.095	0.677	0.508	0.301	0.268	0.319	0.303	0.115	0.295	0.793	0.027	0.226	0.731	0.117	0.363	0.182	0.232	0.418	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g54570	0.271	0.097	0.295	0.713	0.036	0.395	-0.068	0.363	1.000	0.238	0.235	0.296	-0.041	0.374	1.000	0.015	0.812	0.537	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g54650	0.574	0.001	0.074	0.981	0.001	0.385	0.139	0.153	0.308	0.620	0.016	0.246	0.427	0.079	0.286	0.777	0.002	0.307	0.131	0.374	1.000	0.052	0.595	0.445	0.000	1.000	1.000	0.000	1.000	1.000
At1g54790	-0.367	0.377	0.340	2.332	0.043	0.395	-0.254	0.509	0.409	-0.444	0.601	0.408	0.586	0.324	0.335	0.062	0.857	0.550	0.295	0.374	1.000	-0.916	0.129	0.378	-0.329	0.374	0.415	0.000	1.000	1.000
At1g55020	0.983	0.004	0.113	0.716	0.208	0.395	0.160	0.235	0.311	1.026	0.034	0.258	0.542	0.026	0.226	0.212	0.654	0.488	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g55090	-0.693	0.000	0.040	-0.881	0.010	0.395	-0.941	0.001	0.132	-0.620	0.059	0.285	-1.441	0.000	0.055	-0.904	0.069	0.363	-0.448	0.089	0.418	-0.764	0.033	0.378	-0.095	0.606	0.501	0.000	1.000	1.000
At1g55580	-0.479	0.000	0.037	-0.390	0.199	0.395	-0.371	0.006	0.200	-0.286	0.043	0.266	-0.369	0.055	0.261	-0.589	0.015	0.363	-0.152	0.374	1.000	-0.252	0.247	0.378	0.026	0.374	0.415	0.000	1.000	1.000
At1g55710	0.297	0.269	0.306	0.035	0.099	0.395	0.871	0.018	0.237	0.741	0.202	0.295	0.408	0.243	0.314	-0.282	0.182	0.363	0.490	0.307	0.419	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g56660	0.592	0.028	0.239	0.000	1.000	1.000	0.000	1.000	1.000	1.132	0.020	0.249	0.526	0.064	0.273	0.786	0.127	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g57720	-0.038	0.902	0.509	0.851	0.042	0.395	0.591	0.004	0.185	-0.108	0.767	0.463	0.447	0.378	0.351	0.079	0.673	0.494	-0.022	0.374	1.000	-0.087	0.731	0.486	0.000	1.000	1.000	0.000	1.000	1.000
At1g58290	-0.345	0.104	0.295	0.133	0.809	0.546	-0.082	0.519	0.413	-0.142	0.333	0.323	0.051	0.629	0.440	-0.889	0.041	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g59820	0.388	0.022	0.213	-0.072	0.873	0.565	0.161	0.238	0.311	0.285	0.339	0.324	0.459	0.038	0.242	0.617	0.003	0.315	-0.008	0.374	1.000	0.095	0.383	0.395	0.026	0.374	0.415	0.000	1.000	1.000
At1g60740	0.730	0.003	0.112	0.953	0.117	0.395	0.473	0.034	0.254	0.398	0.004	0.171	0.703	0.016	0.226	-0.084	0.594	0.467	0.063	0.469	0.463	0.104	0.573	0.440	-0.08					

At1g64390	-0.345	0.104	0.295	-0.041	0.959	0.586	0.605	0.101	0.291	-0.498	0.064	0.288	-0.673	0.036	0.238	-0.535	0.342	0.384	0.022	0.374	1.000	-0.467	0.097	0.378	-0.187	0.291	0.414
At1g64560	0.197	0.231	0.300	0.250	0.152	0.395	0.178	0.086	0.288	0.340	0.043	0.266	0.227	0.090	0.289	0.670	0.009	0.363	-0.028	0.479	0.465	0.000	1.000	1.000	0.000	1.000	1.000
At1g64720	0.034	0.918	0.514	0.481	0.233	0.395	-0.831	0.030	0.248	-0.932	0.062	0.287	-0.499	0.111	0.297	-0.455	0.563	0.456	0.155	0.545	0.489	-0.268	0.211	0.378	0.000	1.000	1.000
At1g64860	-0.543	0.002	0.091	-0.771	0.217	0.395	0.037	0.789	0.506	-0.351	0.000	0.091	-0.534	0.014	0.224	-0.747	0.001	0.291	0.085	0.615	0.518	-0.178	0.431	0.402	-0.339	0.085	0.414
At1g65720	0.816	0.037	0.260	0.095	0.405	0.425	0.124	0.227	0.310	-0.384	0.312	0.318	0.191	0.618	0.436	0.152	0.344	0.384	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g65840	-0.184	0.416	0.355	0.284	0.223	0.395	0.000	1.000	1.000	0.223	0.485	0.369	0.039	0.930	0.528	-0.828	0.037	0.363	0.000	1.000	1.000	-0.002	0.374	0.392	0.037	0.374	0.415
At1g65980	0.625	0.001	0.083	0.635	0.055	0.395	0.356	0.058	0.276	0.335	0.059	0.286	0.589	0.054	0.258	0.087	0.585	0.464	0.091	0.374	1.000	-0.008	0.942	0.536	0.000	1.000	1.000
At1g66240	0.163	0.462	0.372	0.689	0.039	0.395	0.394	0.151	0.308	0.366	0.333	0.323	-0.070	0.834	0.503	0.147	0.542	0.450	0.067	0.374	1.000	-0.048	0.374	0.392	0.000	1.000	1.000
At1g66690	1.451	0.001	0.078	0.948	0.129	0.395	0.151	0.251	0.314	0.788	0.047	0.268	0.977	0.030	0.232	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g67230	0.027	0.879	0.504	-0.699	0.047	0.395	0.023	0.930	0.549	0.113	0.550	0.391	-0.016	0.949	0.534	-0.391	0.139	0.363	-0.264	0.377	0.428	-0.071	0.374	0.392	0.000	1.000	1.000
At1g67280	0.385	0.064	0.285	0.941	0.037	0.395	0.175	0.234	0.311	0.571	0.036	0.258	0.096	0.638	0.443	0.331	0.001	0.291	0.000	1.000	1.000	0.068	0.374	0.392	0.000	1.000	1.000
At1g67310	0.890	0.006	0.142	-0.078	0.906	0.572	-0.516	0.255	0.314	0.261	0.195	0.295	-0.160	0.822	0.499	1.799	0.019	0.363	-0.045	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g67360	-0.036	0.793	0.481	-0.864	0.045	0.395	0.099	0.599	0.442	0.429	0.106	0.295	0.315	0.119	0.301	0.163	0.574	0.459	-0.213	0.402	0.438	0.392	0.096	0.378	0.159	0.100	0.414
At1g67480	0.160	0.545	0.401	0.434	0.029	0.395	-0.246	0.314	0.333	-0.076	0.669	0.432	0.413	0.382	0.352	0.790	0.031	0.363	0.053	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g67590	0.614	0.003	0.112	0.691	0.166	0.395	0.530	0.027	0.246	0.317	0.277	0.307	0.424	0.073	0.284	0.508	0.047	0.363	0.284	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g67870	-0.182	0.459	0.371	-0.116	0.644	0.499	-0.709	0.046	0.266	-0.265	0.079	0.291	-0.420	0.118	0.300	-0.175	0.447	0.415	-0.346	0.130	0.418	-0.039	0.849	0.513	-0.160	0.691	0.519
At1g67930	0.084	0.712	0.456	-0.782	0.021	0.395	0.047	0.227	0.310	0.153	0.594	0.406	0.018	0.969	0.538	-0.174	0.533	0.447	0.089	0.374	1.000	0.226	0.199	0.378	0.000	1.000	1.000
At1g67980	0.714	0.330	0.323	1.192	0.352	0.414	0.748	0.241	0.311	0.937	0.099	0.293	0.662	0.099	0.290	1.885	0.012	0.363	0.123	0.259	0.418	0.218	0.374	0.392	0.000	1.000	1.000
At1g68100	0.091	0.355	0.331	0.585	0.039	0.395	0.347	0.150	0.308	0.184	0.370	0.332	0.049	0.902	0.520	0.474	0.118	0.363	0.018	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g69270	-0.132	0.392	0.346	-0.139	0.859	0.561	-0.374	0.090	0.289	-0.025	0.374	1.000	-0.154	0.211	0.311	0.780	0.022	0.363	-0.016	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g69800	0.033	0.818	0.487	0.449	0.334	0.410	0.119	0.531	0.418	-0.470	0.122	0.295	-0.679	0.043	0.247	-0.033	0.928	0.571	0.015	0.796	0.581	0.000	1.000	1.000	0.000	1.000	1.000
At1g70160	-0.058	0.401	0.350	0.346	0.503	0.456	0.284	0.310	0.331	0.097	0.533	0.384	0.522	0.092	0.289	0.644	0.050	0.363	0.029	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g70600	-0.814	0.035	0.258	0.090	0.573	0.477	0.223	0.105	0.295	-0.192	0.038	0.259	-0.423	0.027	0.226	-0.187	0.339	0.384	0.083	0.873	0.600	-0.145	0.418	0.400	-0.186	0.233	0.414
At1g70700	0.771	0.067	0.289	1.170	0.021	0.395	0.063	0.310	0.331	1.312	0.092	0.291	0.999	0.021	0.226	0.262	0.382	0.395	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g70810	0.524	0.030	0.244	0.309	0.024	0.395	0.414	0.002	0.163	0.241	0.511	0.378	0.989	0.022	0.226	0.650	0.029	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g70820	0.464	0.063	0.285	0.377	0.483	0.451	-0.094	0.422	0.373	-0.473	0.092	0.291	0.670	0.033	0.235	0.071	0.766	0.524	0.060	0.374	1.000	0.061	0.374	0.392	0.000	1.000	1.000
At1g71360	0.633	0.001	0.074	0.377	0.027	0.395	0.568	0.002	0.166	0.556	0.166	0.295	-0.197	0.757	0.480	0.394	0.050	0.363	0.562	0.161	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g71710	0.374	0.064	0.285	0.430	0.650	0.501	-0.363	0.194	0.308	0.314	0.111	0.295	0.141	0.619	0.437	0.652	0.013	0.363	0.098	0.277	0.418	0.350	0.072	0.378	-0.113	0.479	0.458
At1g71865	0.099	0.641	0.434	-0.119	0.811	0.547	0.288	0.034	0.254	0.316	0.199	0.295	0.652	0.028	0.227	-0.029	0.934	0.572	0.177	0.442	0.452	0.000	1.000	1.000	0.000	1.000	1.000
At1g71890	0.849	0.045	0.273	0.217	0.329	0.408	0.117	0.722	0.484	0.923	0.054	0.277	1.266	0.031	0.233	0.458	0.340	0.384	-0.135	0.374	1.000	-0.283	0.638	0.453	-0.196	0.374	0.415
At1g72020	0.023	0.891	0.507	0.130	0.785	0.539	0.248	0.422	0.372	0.691	0.010	0.235	0.407	0.185	0.310	-0.481	0.232	0.364	-0.063	0.709	0.550	-0.002	0.980	0.545	0.071	0.201	0.414
At1g72180	0.609	0.018	0.200	0.710	0.290	0.401	0.439	0.230	0.311	0.201	0.029	0.249	0.132	0.701	0.463	0.177	0.664	0.490	0.041	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g72260	1.196	0.011	0.169	1.578	0.074	0.395	0.683	0.100	0.291	1.078	0.026	0.249	1.035	0.018	0.226	-0.128	0.489	0.430	0.216	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g72290	2.513	0.001	0.080	3.084	0.027	0.395	1.903	0.056	0.276	3.451	0.000	0.091	4.299	0.000	0.047	-1.965	0.018	0.363	0.732	0.141	0.418	0.998	0.073	0.378	0.445	0.203	0.414
At1g72430	0.483	0.040	0.266	0.517	0.145	0.395	-0.768	0.155	0.308	0.568	0.100	0.293	0.880	0.043	0.247	0.006	0.976	0.582	-0.172	0.087	0.418	0.470	0.058	0.378	0.126	0.374	0.415
At1g72610	-0.505	0.073	0.294	-0.437	0.312	0.404	-1.045	0.068	0.283	0.072	0.505	0.376	-1.512	0.036	0.238	-1.189	0.002	0.307	0.049	0.374	1.000	-0.387	0.067	0.378	0.079	0.374	0.415
At1g72680	0.393	0.017	0.193	0.297	0.138	0.395	0.089	0.715	0.482	0.181	0.001	0.091	0.782	0.036	0.238	0.214	0.123	0.363	0.031	0.834	0.593	-0.118	0.228	0.378	0.000	1.000	1.000
At1g73030	0.074	0.723	0.460	-0.288	0.366	0.416	-0.652	0.009	0.208	0.273	0.155	0.295	0.617	0.088	0.289	0.028	0.855	0.550	-0.199	0.347	0.423	0.825	0.035	0.378	0.123	0.715	0.528
At1g73180	0.127	0.269	0.306	-0.185	0.531	0.466	-0.119	0.406	0.366	0.180	0.400	0.340	0.325	0.068	0.279	0.682	0.032	0.363	-0.030	0.717	0.554	0.000	1.000	1.000	0.000	1.000	1.000
At1g73250	-0.169	0.009	0.160	-0.598	0.007	0.395	-0.056	0.232	0.311	-0.030	0.679	0.436	-0.035	0.772	0.485	-0.252	0.184	0.363	0.047	0.674	0.539	0.055	0.545	0.432	0.000	1.000	1.000
At1g73260	0.000	1.000	1.000	1.552	0.096	0.395	2.493	0.001	0.163	0.000	1.000	1.000	0.000	1.000	1.000	1.724	0.003	0.315	0.470	0.179	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At1g73325	1.428	0.011	0.169	1.230	0.264	0.396	0.790	0.096	0.290	1.837	0.001	0.122	2.537	0.000	0.059	-1.318	0.000	0.180	0.426	0.457	0.460	1.034	0.004	0.378	0.761	0.079	0.414
At1g73630	0.997	0.004	0.114	0.774	0.053	0.395	-0.064	0.516	0.412	0.810	0.061	0.287	1.110	0.007	0.180	0.870	0.183	0.363	0.048	0.374	1.000	0.043	0.374	0.			

At1g75380	0.137	0.615	0.425	-0.411	0.396	0.421	-0.173	0.483	0.398	-0.884	0.028	0.249	-0.985	0.028	0.227	0.125	0.691	0.500	-0.239	0.478	0.465	-0.264	0.167	0.378	-0.137	0.374	0.415
At1g75550	0.369	0.008	0.157	0.459	0.140	0.395	0.647	0.007	0.200	0.326	0.192	0.295	0.101	0.425	0.366	0.353	0.033	0.363	0.066	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g75630	0.454	0.035	0.258	0.331	0.232	0.395	0.003	0.974	0.559	0.486	0.024	0.249	0.266	0.178	0.310	0.684	0.020	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g76010	0.225	0.677	0.445	1.499	0.038	0.395	-0.314	0.121	0.304	0.405	0.382	0.335	0.219	0.534	0.404	0.719	0.210	0.363	-0.016	0.980	0.626	0.053	0.938	0.536	0.283	0.229	0.414
At1g76180	0.946	0.041	0.266	-0.952	0.182	0.395	-0.238	0.753	0.494	1.672	0.004	0.174	0.991	0.024	0.226	1.535	0.020	0.363	0.053	0.846	0.594	0.021	0.374	0.392	0.000	1.000	1.000
At1g76520	0.684	0.013	0.176	0.226	0.586	0.481	0.243	0.597	0.441	1.052	0.025	0.249	0.359	0.328	0.336	-0.262	0.555	0.454	0.022	0.186	0.418	0.136	0.416	0.399	0.000	1.000	1.000
At1g76530	0.380	0.095	0.295	0.328	0.304	0.403	0.434	0.217	0.310	0.711	0.004	0.171	0.629	0.053	0.256	0.232	0.422	0.408	0.006	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g76650	0.551	0.011	0.170	0.399	0.158	0.395	0.627	0.024	0.245	0.125	0.393	0.338	0.248	0.089	0.289	0.498	0.170	0.363	0.037	0.627	0.523	0.000	1.000	1.000	0.000	1.000	1.000
At1g78080	0.216	0.342	0.327	0.324	0.212	0.395	0.012	0.915	0.546	0.000	1.000	1.000	0.000	1.000	1.000	1.001	0.013	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g78370	0.591	0.002	0.091	0.557	0.433	0.434	-0.263	0.422	0.372	0.234	0.111	0.295	0.231	0.145	0.310	-0.240	0.750	0.519	0.244	0.413	0.440	0.404	0.249	0.378	-0.130	0.374	0.415
At1g78410	0.349	0.089	0.295	1.212	0.060	0.395	0.744	0.004	0.187	0.246	0.459	0.360	0.276	0.451	0.375	1.231	0.044	0.363	-0.126	0.374	1.000	0.000	1.000	1.000	0.011	0.374	0.415
At1g78600	-0.561	0.000	0.067	-0.300	0.392	0.421	0.002	0.983	0.560	-0.358	0.002	0.123	-0.637	0.004	0.138	-0.405	0.183	0.363	-0.055	0.374	1.000	-0.097	0.471	0.410	0.000	1.000	1.000
At1g78900	0.210	0.051	0.276	0.194	0.771	0.535	0.419	0.256	0.314	1.048	0.045	0.266	-0.062	0.947	0.533	-0.035	0.969	0.580	-0.027	0.374	1.000	0.208	0.374	0.392	0.000	1.000	1.000
At1g79030	-0.157	0.349	0.329	-0.297	0.419	0.429	-0.283	0.552	0.425	-0.768	0.044	0.266	-0.214	0.641	0.444	-0.446	0.258	0.367	0.240	0.274	0.418	-0.192	0.396	0.397	0.000	1.000	1.000
At1g79340	-0.272	0.423	0.358	-0.214	0.859	0.561	-0.429	0.417	0.371	-0.129	0.217	0.295	0.467	0.286	0.326	1.420	0.036	0.363	-0.779	0.097	0.418	0.195	0.716	0.482	0.042	0.265	0.414
At1g79550	0.002	0.996	0.533	0.731	0.166	0.395	-0.183	0.680	0.471	0.963	0.050	0.273	1.117	0.113	0.298	0.139	0.815	0.538	-0.112	0.412	0.440	0.038	0.858	0.515	0.150	0.366	0.414
At1g79750	1.049	0.003	0.098	0.435	0.252	0.396	0.128	0.771	0.500	0.707	0.053	0.277	0.867	0.105	0.293	-0.111	0.695	0.501	0.223	0.374	1.000	-0.030	0.374	0.392	0.000	1.000	1.000
At1g79850	0.089	0.764	0.473	0.357	0.493	0.453	-0.592	0.102	0.293	-0.690	0.026	0.249	-0.604	0.098	0.290	-0.256	0.514	0.439	-0.070	0.793	0.581	-0.121	0.679	0.467	0.036	0.888	0.591
At1g79910	-0.357	0.089	0.295	0.208	0.750	0.530	-0.568	0.199	0.308	0.201	0.317	0.319	0.691	0.044	0.247	0.280	0.515	0.439	0.229	0.178	0.418	0.089	0.617	0.450	0.000	1.000	1.000
At1g80360	0.310	0.089	0.295	-0.230	0.204	0.395	0.164	0.371	0.353	0.354	0.212	0.295	1.011	0.012	0.221	-0.372	0.323	0.381	0.797	0.016	0.418	0.463	0.146	0.378	0.043	0.583	0.494
At1g80380	-0.009	0.973	0.528	1.397	0.053	0.395	0.120	0.799	0.509	-0.278	0.318	0.319	0.241	0.655	0.448	-1.463	0.001	0.232	-0.385	0.374	1.000	-0.585	0.179	0.378	0.018	0.374	0.415
At1g80840	1.414	0.012	0.170	1.771	0.039	0.395	0.578	0.065	0.278	1.153	0.112	0.295	1.145	0.086	0.288	0.006	0.994	0.586	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At1g80910	0.045	0.928	0.516	0.567	0.547	0.470	-0.979	0.076	0.285	-0.975	0.082	0.291	-0.754	0.059	0.268	0.455	0.515	0.439	-0.260	0.223	0.418	-0.219	0.600	0.447	-0.947	0.008	0.414
At2g01290	0.228	0.245	0.302	0.081	0.824	0.552	0.649	0.027	0.248	0.416	0.022	0.249	0.409	0.046	0.248	0.157	0.607	0.472	0.011	0.860	0.597	0.000	1.000	1.000	0.000	1.000	1.000
At2g01400	0.044	0.710	0.455	0.347	0.044	0.395	0.029	0.694	0.476	-0.034	0.857	0.486	0.706	0.023	0.226	0.103	0.424	0.408	-0.040	0.187	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g01540	0.587	0.004	0.114	1.107	0.027	0.395	0.124	0.157	0.308	0.644	0.123	0.295	0.547	0.082	0.286	0.506	0.106	0.363	0.054	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g01660	-0.177	0.041	0.266	-0.587	0.002	0.395	-0.332	0.012	0.216	-0.091	0.130	0.295	-0.007	0.198	0.310	-0.148	0.419	0.406	-0.255	0.043	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g01690	-0.223	0.005	0.134	-0.592	0.011	0.395	-0.273	0.003	0.166	-0.084	0.490	0.371	-0.165	0.337	0.339	0.038	0.602	0.470	-0.244	0.151	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g01850	0.412	0.116	0.295	1.120	0.049	0.395	-0.684	0.023	0.243	0.092	0.608	0.411	0.062	0.748	0.477	0.480	0.385	0.395	0.173	0.298	0.419	0.204	0.451	0.406	0.070	0.753	0.543
At2g01940	0.039	0.687	0.448	-0.501	0.101	0.395	-0.444	0.001	0.157	-0.348	0.094	0.291	-0.781	0.000	0.060	-0.249	0.178	0.363	-0.098	0.195	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g02050	0.034	0.722	0.460	0.839	0.013	0.395	0.131	0.182	0.308	0.129	0.159	0.295	0.139	0.094	0.290	0.580	0.089	0.363	0.087	0.225	0.418	0.175	0.094	0.378	0.059	0.374	0.415
At2g02120	-0.531	0.003	0.111	-0.417	0.578	0.478	0.006	0.919	0.546	-0.249	0.135	0.295	-0.428	0.022	0.226	-1.148	0.036	0.363	-0.037	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g02220	-1.258	0.002	0.092	-0.591	0.089	0.395	-1.124	0.002	0.166	-1.245	0.007	0.206	-2.101	0.000	0.046	-1.173	0.003	0.321	-1.913	0.003	0.418	-1.141	0.016	0.378	-1.041	0.009	0.414
At2g02230	0.807	0.009	0.165	0.143	0.482	0.451	0.123	0.795	0.508	-0.151	0.689	0.439	0.799	0.086	0.288	0.284	0.452	0.416	0.107	0.206	0.418	0.189	0.374	0.392	0.091	0.374	0.415
At2g02990	0.694	0.032	0.247	0.848	0.218	0.395	0.707	0.023	0.243	1.533	0.020	0.247	1.210	0.043	0.247	-0.013	0.936	0.573	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g03440	-0.179	0.225	0.299	-0.011	0.948	0.583	-0.521	0.001	0.157	-0.444	0.001	0.112	-0.705	0.023	0.226	-0.409	0.028	0.363	-0.155	0.222	0.418	-0.119	0.374	0.392	0.000	1.000	1.000
At2g03750	0.612	0.030	0.244	0.630	0.199	0.395	0.515	0.057	0.276	-0.040	0.374	1.000	-0.041	0.374	1.000	0.168	0.761	0.522	-0.029	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g03890	-0.375	0.114	0.295	-0.835	0.030	0.395	-0.203	0.232	0.311	-0.463	0.016	0.246	-0.276	0.125	0.305	-0.297	0.219	0.363	-0.046	0.295	0.419	-0.054	0.506	0.421	0.000	1.000	1.000
At2g04039	-0.129	0.573	0.410	-0.624	0.039	0.395	0.066	0.658	0.463	-0.010	0.970	0.517	0.238	0.489	0.388	0.034	0.917	0.567	0.108	0.374	1.000	-0.102	0.215	0.378	0.129	0.374	0.415
At2g04040	0.452	0.057	0.277	0.791	0.040	0.395	0.568	0.040	0.258	0.537	0.080	0.291	0.397	0.078	0.286	0.739	0.016	0.363	0.136	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g04220	0.727	0.009	0.160	1.302	0.037	0.395	0.684	0.006	0.200	0.776	0.035	0.258	0.294	0.183	0.310	1.037	0.007	0.363	0.266	0.002	0.418	0.247	0.214	0.378	0.087	0.374	0.415
At2g04390	-0.158	0.475	0.377	0.648	0.053	0.395	0.601	0.017	0.234	0.154	0.580	0.401	0.356	0.051	0.254	0.336	0.040	0.363	0.053	0.374	1.000	0.147	0.434	0.403	0.056	0.374	0.415
At2g04900	0.116	0.250	0.303	0.350	0.253	0.396	0.034	0.266	0.318	0.405	0.061	0.287	-0.026	0.722	0.469	0.805	0.016	0.363	0.000	1.000	1.000	0.000	1.000	1.			

At2g07696	-0.911	0.001	0.080	-0.086	0.851	0.559	-0.664	0.021	0.241	-0.835	0.013	0.239	-1.557	0.000	0.046	-1.114	0.018	0.363	-1.245	0.003	0.418	-0.798	0.065	0.378	-0.463	0.051	0.414
At2g07708	-0.484	0.007	0.150	-0.517	0.005	0.395	-0.626	0.007	0.200	-0.283	0.037	0.259	-0.806	0.155	0.310	-0.541	0.014	0.363	-0.376	0.052	0.418	-0.263	0.178	0.378	0.043	0.336	0.414
At2g07739	-0.187	0.438	0.364	0.082	0.769	0.535	-0.037	0.774	0.501	0.067	0.725	0.450	-0.048	0.920	0.526	-0.670	0.056	0.363	-0.102	0.755	0.568	-0.865	0.036	0.378	-0.274	0.251	0.414
At2g09970	0.303	0.393	0.346	0.244	0.230	0.395	-0.516	0.100	0.291	0.386	0.038	0.259	0.992	0.022	0.226	0.306	0.468	0.422	-0.023	0.374	1.000	0.154	0.374	0.392	0.000	1.000	1.000
At2g10270	-1.021	0.003	0.104	-0.762	0.049	0.395	-0.638	0.008	0.200	-0.805	0.008	0.220	-1.801	0.000	0.046	-1.143	0.019	0.363	-1.401	0.006	0.418	-0.927	0.059	0.378	-0.672	0.054	0.414
At2g10530	-0.096	0.252	0.303	-0.087	0.374	1.000	0.000	1.000	1.000	-0.191	0.103	0.295	-0.637	0.006	0.160	-0.270	0.067	0.363	-0.079	0.100	0.418	-0.258	0.151	0.378	-0.133	0.141	0.414
At2g12280	-0.335	0.435	0.363	0.072	0.875	0.565	0.213	0.211	0.309	0.584	0.177	0.295	-0.555	0.393	0.354	1.077	0.039	0.363	0.137	0.274	0.418	-0.015	0.374	0.392	0.000	1.000	1.000
At2g12405	0.604	0.049	0.274	0.577	0.020	0.395	0.248	0.219	0.310	0.281	0.088	0.291	0.044	0.314	0.334	0.117	0.560	0.455	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g13360	-0.301	0.037	0.260	-0.507	0.137	0.395	-0.439	0.050	0.270	-0.283	0.228	0.295	-0.773	0.026	0.226	-0.614	0.112	0.363	0.230	0.372	0.427	-0.243	0.453	0.407	-0.170	0.204	0.414
At2g14110	0.082	0.815	0.486	0.404	0.157	0.395	0.658	0.050	0.270	0.169	0.302	0.315	-0.365	0.424	0.365	-0.322	0.261	0.368	0.174	0.374	1.000	0.046	0.374	0.392	-0.002	0.374	0.415
At2g14580	0.219	0.162	0.295	1.169	0.110	0.395	0.856	0.050	0.270	0.478	0.162	0.295	0.897	0.101	0.291	0.735	0.168	0.363	0.108	0.233	0.418	-0.113	0.374	0.392	0.000	1.000	1.000
At2g14610	-0.039	0.928	0.516	2.626	0.001	0.385	2.319	0.000	0.081	1.072	0.062	0.287	0.669	0.307	0.331	1.677	0.045	0.363	1.711	0.002	0.418	0.000	1.000	1.000	0.003	0.374	0.415
At2g14680	0.574	0.016	0.192	-0.126	0.501	0.456	0.085	0.686	0.473	0.304	0.166	0.295	0.606	0.027	0.226	0.229	0.241	0.364	0.157	0.374	1.000	0.000	1.000	1.000	0.046	0.374	0.415
At2g15050	-0.652	0.040	0.266	-0.531	0.451	0.441	-0.362	0.112	0.297	-0.113	0.890	0.496	1.200	0.050	0.252	-0.976	0.328	0.382	0.000	1.000	1.000	0.631	0.168	0.378	0.361	0.181	0.414
At2g15440	1.108	0.020	0.207	-0.109	0.764	0.533	0.331	0.537	0.420	0.908	0.162	0.295	0.751	0.072	0.284	1.106	0.022	0.363	0.572	0.134	0.418	-0.141	0.374	0.392	0.000	1.000	1.000
At2g15570	0.392	0.023	0.220	0.176	0.328	0.408	-0.182	0.284	0.324	0.717	0.045	0.266	0.773	0.053	0.256	-0.244	0.277	0.372	-0.142	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g15900	1.039	0.019	0.204	0.127	0.523	0.463	-0.301	0.233	0.311	0.805	0.167	0.295	0.805	0.094	0.289	0.250	0.127	0.363	0.017	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g15910	0.551	0.093	0.295	0.644	0.150	0.395	0.380	0.437	0.379	0.148	0.531	0.384	0.346	0.468	0.381	0.737	0.021	0.363	0.088	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g15970	0.366	0.002	0.095	0.452	0.178	0.395	0.377	0.125	0.306	0.840	0.031	0.250	0.366	0.158	0.310	0.308	0.502	0.435	-0.025	0.374	1.000	0.187	0.178	0.378	0.000	1.000	1.000
At2g16260	0.365	0.023	0.220	0.454	0.360	0.415	0.469	0.142	0.308	0.254	0.020	0.249	0.104	0.767	0.483	0.926	0.007	0.363	0.093	0.374	1.000	0.104	0.407	0.399	-0.026	0.322	0.414
At2g16480	0.186	0.138	0.295	-0.804	0.047	0.395	-0.046	0.363	1.000	0.029	0.853	0.486	-0.127	0.599	0.430	0.331	0.102	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g16580	-0.324	0.010	0.166	-0.187	0.298	0.402	0.053	0.672	0.468	-0.486	0.004	0.171	-0.630	0.021	0.226	-0.192	0.455	0.418	-0.134	0.427	0.446	-0.008	0.374	0.392	0.000	1.000	1.000
At2g17190	0.294	0.484	0.380	0.464	0.160	0.395	0.136	0.612	0.447	-0.204	0.541	0.388	0.734	0.020	0.226	0.539	0.500	0.434	0.154	0.093	0.418	-0.050	0.826	0.508	-0.116	0.253	0.414
At2g17360	-0.091	0.465	0.373	0.709	0.015	0.395	0.158	0.240	0.311	0.357	0.008	0.223	0.097	0.791	0.491	0.146	0.298	0.376	-0.003	0.988	0.629	-0.019	0.700	0.476	0.015	0.374	0.415
At2g17760	0.297	0.316	0.320	-0.024	0.894	0.569	-0.155	0.484	0.398	0.074	0.772	0.465	0.139	0.565	0.416	1.020	0.036	0.363	0.124	0.401	0.438	0.056	0.626	0.452	0.000	1.000	1.000
At2g17770	0.255	0.143	0.295	0.185	0.592	0.483	0.411	0.346	0.344	0.585	0.032	0.252	-0.131	0.796	0.493	0.456	0.308	0.376	0.138	0.374	1.000	0.454	0.195	0.378	-0.089	0.374	0.415
At2g17930	-0.121	0.286	0.311	-0.493	0.005	0.395	-0.104	0.444	0.382	-0.222	0.107	0.295	-0.602	0.018	0.226	-0.061	0.518	0.440	-0.201	0.320	0.421	-0.054	0.374	0.392	0.000	1.000	1.000
At2g18050	-0.133	0.382	0.343	-1.111	0.035	0.395	-0.268	0.405	0.366	-0.834	0.076	0.291	-0.727	0.008	0.181	-0.006	0.991	0.585	-0.147	0.374	1.000	-0.233	0.211	0.378	-0.071	0.374	0.415
At2g18400	0.622	0.012	0.173	0.328	0.193	0.395	0.107	0.674	0.469	-0.109	0.513	0.379	0.229	0.552	0.410	-0.076	0.716	0.507	0.120	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g18420	0.386	0.151	0.295	0.169	0.798	0.543	-0.561	0.071	0.285	0.099	0.427	0.350	0.350	0.020	0.226	0.825	0.021	0.363	-0.502	0.084	0.418	0.197	0.332	0.385	-0.069	0.422	0.430
At2g18680	1.315	0.008	0.157	1.620	0.055	0.395	1.282	0.007	0.200	1.322	0.020	0.249	1.193	0.173	0.310	1.471	0.203	0.363	0.150	0.275	0.418	0.102	0.374	0.392	0.000	1.000	1.000
At2g18700	-0.017	0.905	0.510	1.279	0.024	0.395	-0.188	0.358	0.349	-0.045	0.374	1.000	0.000	1.000	1.000	0.154	0.803	0.535	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g18960	-0.200	0.067	0.288	-0.649	0.007	0.395	-0.182	0.155	0.308	-0.227	0.182	0.295	-0.103	0.538	0.405	-0.198	0.338	0.384	-0.129	0.476	0.465	0.126	0.351	0.386	-0.371	0.101	0.414
At2g19000	0.596	0.048	0.274	0.047	0.374	1.000	0.161	0.303	0.330	0.128	0.186	0.295	-0.070	0.433	0.368	0.026	0.237	0.364	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g19110	0.527	0.001	0.075	0.689	0.036	0.395	0.158	0.127	0.307	0.258	0.017	0.246	0.347	0.021	0.226	0.539	0.016	0.363	0.079	0.519	0.478	-0.071	0.479	0.411	-0.155	0.223	0.414
At2g20670	0.657	0.234	0.300	0.754	0.395	0.421	-1.294	0.011	0.216	-0.007	0.977	0.519	-0.298	0.371	0.350	-1.061	0.319	0.380	0.469	0.108	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g20750	-0.375	0.050	0.275	-0.433	0.162	0.395	-0.165	0.194	0.308	-0.429	0.184	0.295	-0.464	0.173	0.310	-0.816	0.049	0.363	0.000	1.000	1.000	0.000	1.000	1.000	-0.019	0.374	0.415
At2g20780	0.188	0.346	0.329	0.110	0.770	0.535	0.171	0.403	0.365	0.693	0.019	0.247	0.515	0.291	0.327	-0.293	0.294	0.376	0.123	0.355	0.423	0.080	0.095	0.378	0.060	0.227	0.414
At2g21200	-0.136	0.524	0.394	-0.302	0.363	0.416	-0.481	0.042	0.261	-0.969	0.039	0.261	-0.302	0.200	0.310	-0.150	0.641	0.483	0.159	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g21220	-0.981	0.003	0.101	-0.476	0.591	0.483	-0.095	0.866	0.532	-0.564	0.340	0.324	-0.408	0.613	0.434	-0.556	0.233	0.364	-0.056	0.374	1.000	-0.267	0.374	0.392	0.000	1.000	1.000
At2g21330	-0.071	0.731	0.462	-0.441	0.542	0.469	-0.418	0.197	0.308	-0.569	0.122	0.295	-0.493	0.109	0.296	-0.764	0.002	0.314	-0.145	0.093	0.418	-0.243	0.027	0.378	0.136	0.433	0.436
At2g21620	0.136	0.107	0.295	0.222	0.444	0.438	0.201	0.095	0.290	0.636	0.017	0.246	0.390	0.049	0.252	0.849	0.011	0.363	0.069	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g21890	-0.076	0.858	0.498	-0.617	0.379	0.417	0.137	0.362	0.351	0.807	0.023	0.249	0.364	0.318	0.334	-1.064	0.029	0.363	0.070	0.374	1.000	-0.004	0.953	0.			

At2g24060	0.384	0.012	0.170	0.352	0.180	0.395	0.155	0.059	0.276	0.162	0.563	0.394	-0.309	0.180	0.310	0.680	0.013	0.363	0.040	0.374	1.000	0.310	0.211	0.378	0.008	0.374	0.415
At2g24270	1.080	0.006	0.142	0.611	0.527	0.465	0.161	0.800	0.510	0.078	0.907	0.500	-0.717	0.217	0.311	0.366	0.528	0.444	-0.075	0.750	0.567	-0.610	0.218	0.378	-0.231	0.512	0.471
At2g24550	0.634	0.008	0.158	1.061	0.103	0.395	0.723	0.163	0.308	0.063	0.463	0.361	0.017	0.374	1.000	0.804	0.035	0.363	0.298	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g24590	0.297	0.375	0.339	0.402	0.163	0.395	0.595	0.029	0.248	0.331	0.102	0.294	0.602	0.121	0.301	0.051	0.911	0.566	-0.150	0.374	1.000	-0.171	0.374	0.392	0.000	1.000	1.000
At2g25000	1.083	0.011	0.169	1.054	0.173	0.395	0.590	0.030	0.248	0.932	0.021	0.249	0.959	0.045	0.247	0.751	0.334	0.384	0.138	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g25320	-0.136	0.071	0.293	-0.658	0.049	0.395	-0.084	0.530	0.417	-0.274	0.122	0.295	-0.022	0.245	0.314	0.143	0.420	0.407	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g25630	0.401	0.080	0.295	0.943	0.001	0.385	0.082	0.175	0.308	0.722	0.040	0.261	1.196	0.000	0.064	0.234	0.724	0.510	0.000	1.000	1.000	0.201	0.077	0.378	0.027	0.374	0.415
At2g26250	-0.286	0.231	0.300	-0.518	0.430	0.433	-1.246	0.012	0.218	-0.448	0.417	0.346	-0.182	0.670	0.453	-0.596	0.383	0.395	-0.168	0.374	1.000	-0.101	0.774	0.498	0.000	1.000	1.000
At2g26500	-0.246	0.152	0.295	-0.084	0.805	0.546	-0.027	0.827	0.519	-0.651	0.008	0.223	-0.242	0.380	0.351	-0.245	0.229	0.364	-0.006	0.965	0.621	0.159	0.226	0.378	0.171	0.342	0.414
At2g26670	0.282	0.086	0.295	-0.078	0.830	0.553	0.063	0.672	0.468	0.161	0.184	0.295	0.048	0.832	0.503	0.850	0.039	0.363	-0.114	0.654	0.534	0.000	1.000	1.000	0.000	1.000	1.000
At2g27050	-0.195	0.233	0.300	-0.269	0.528	0.465	-0.397	0.005	0.200	-0.346	0.175	0.295	-0.606	0.043	0.247	0.040	0.880	0.558	-0.235	0.082	0.418	-0.093	0.187	0.378	0.000	1.000	1.000
At2g27180	0.525	0.110	0.295	1.135	0.169	0.395	1.444	0.048	0.266	0.789	0.168	0.295	-0.024	0.976	0.540	-0.597	0.212	0.363	0.030	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g27380	0.473	0.019	0.203	0.251	0.360	0.415	0.608	0.038	0.256	0.222	0.144	0.295	0.000	1.000	1.000	0.201	0.281	0.373	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g27385	-0.387	0.048	0.274	-0.690	0.130	0.395	-0.057	0.280	0.322	-0.450	0.105	0.295	-0.707	0.030	0.230	-1.049	0.021	0.363	0.000	1.000	1.000	-0.063	0.374	0.392	0.000	1.000	1.000
At2g27420	0.335	0.352	0.330	0.555	0.179	0.395	1.411	0.048	0.266	0.579	0.060	0.286	0.569	0.123	0.303	0.812	0.073	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g27500	0.683	0.037	0.261	0.616	0.108	0.395	0.085	0.175	0.308	0.656	0.153	0.295	0.306	0.121	0.301	0.851	0.307	0.376	0.107	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g27510	0.090	0.664	0.441	0.569	0.053	0.395	0.423	0.289	0.325	-0.207	0.679	0.436	1.226	0.003	0.138	0.236	0.588	0.465	0.011	0.971	0.623	0.027	0.865	0.517	0.000	1.000	1.000
At2g27530	0.271	0.036	0.260	0.696	0.020	0.395	0.161	0.234	0.311	0.432	0.004	0.168	0.165	0.121	0.301	0.506	0.054	0.363	0.103	0.192	0.418	0.070	0.517	0.423	0.000	1.000	1.000
At2g27660	0.292	0.046	0.274	0.852	0.047	0.395	0.174	0.077	0.285	0.085	0.183	0.295	0.532	0.027	0.226	0.684	0.088	0.363	0.114	0.374	1.000	0.055	0.374	0.392	0.000	1.000	1.000
At2g27920	0.095	0.553	0.404	0.444	0.273	0.398	0.391	0.193	0.308	-0.472	0.182	0.295	0.227	0.529	0.403	0.698	0.035	0.363	0.043	0.374	1.000	0.139	0.374	0.392	0.000	1.000	1.000
At2g28305	0.267	0.081	0.295	-0.035	0.834	0.554	0.188	0.399	0.364	0.632	0.009	0.235	0.437	0.318	0.334	-0.064	0.689	0.499	0.024	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g28350	0.086	0.176	0.295	-0.679	0.022	0.395	-0.033	0.403	0.365	0.124	0.374	1.000	0.000	1.000	1.000	-0.192	0.209	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g28520	1.049	0.007	0.150	-0.037	0.852	0.560	0.083	0.171	0.308	0.838	0.319	0.319	-0.229	0.723	0.469	-0.094	0.882	0.559	-0.153	0.374	1.000	-0.191	0.374	0.392	0.000	1.000	1.000
At2g28570	1.034	0.045	0.271	1.037	0.184	0.395	0.165	0.247	0.313	0.170	0.690	0.439	0.342	0.062	0.272	0.588	0.314	0.378	0.155	0.374	1.000	-0.014	0.374	0.392	0.153	0.374	0.415
At2g28670	0.393	0.014	0.186	0.824	0.036	0.395	0.912	0.002	0.163	0.384	0.095	0.291	0.412	0.013	0.224	0.403	0.104	0.363	0.003	0.944	0.616	0.064	0.314	0.381	0.000	1.000	1.000
At2g28820	-0.714	0.006	0.143	-0.437	0.261	0.396	-0.522	0.052	0.270	-0.663	0.050	0.272	-0.434	0.012	0.221	-0.854	0.003	0.315	-0.420	0.106	0.418	-0.313	0.174	0.378	-0.161	0.374	0.415
At2g29150	0.207	0.265	0.305	1.414	0.039	0.395	1.591	0.004	0.185	-0.056	0.226	0.295	0.126	0.138	0.310	1.219	0.005	0.347	0.256	0.234	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g29170	0.166	0.567	0.408	1.037	0.100	0.395	1.861	0.003	0.166	-0.050	0.703	0.443	0.015	0.947	0.533	1.339	0.006	0.353	0.207	0.148	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g29290	0.181	0.049	0.274	1.056	0.014	0.395	0.302	0.038	0.255	-0.009	0.972	0.517	-0.017	0.863	0.510	0.484	0.025	0.363	0.029	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g29440	0.627	0.002	0.089	0.310	0.081	0.395	0.284	0.206	0.309	0.816	0.001	0.091	0.532	0.047	0.249	0.066	0.669	0.492	0.072	0.392	0.434	0.095	0.180	0.378	0.000	1.000	1.000
At2g29490	0.797	0.000	0.045	0.753	0.047	0.395	0.465	0.057	0.276	1.044	0.005	0.184	1.115	0.028	0.227	0.102	0.360	0.389	-0.080	0.758	0.568	0.522	0.088	0.378	0.162	0.374	0.415
At2g29510	-0.093	0.227	0.299	-1.250	0.044	0.395	-0.038	0.363	1.000	-0.218	0.374	1.000	-0.348	0.197	0.310	0.231	0.215	0.363	-0.211	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g29630	-0.582	0.026	0.229	-0.471	0.016	0.395	-0.369	0.006	0.200	-0.401	0.096	0.291	-0.723	0.007	0.180	-0.397	0.020	0.363	-0.804	0.020	0.418	-0.124	0.125	0.378	-0.411	0.565	0.484
At2g29740	0.434	0.061	0.283	-0.050	0.576	0.478	0.028	0.333	0.338	0.975	0.021	0.249	0.083	0.610	0.433	0.086	0.800	0.534	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g29980	-0.332	0.033	0.252	-0.793	0.068	0.395	-0.295	0.082	0.286	-0.659	0.001	0.122	-0.326	0.048	0.250	-0.447	0.036	0.363	-0.004	0.922	0.614	-0.398	0.027	0.378	-0.361	0.088	0.414
At2g30360	0.405	0.015	0.190	0.181	0.593	0.483	0.572	0.079	0.285	0.475	0.016	0.246	0.484	0.088	0.289	0.768	0.024	0.363	0.042	0.374	1.000	0.144	0.354	0.386	0.000	1.000	1.000
At2g30440	0.131	0.166	0.295	-0.074	0.377	0.417	-0.082	0.620	0.450	-0.113	0.600	0.408	0.243	0.334	0.338	0.670	0.045	0.363	0.020	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g30560	0.560	0.095	0.295	0.989	0.123	0.395	1.075	0.015	0.232	0.512	0.110	0.295	0.260	0.442	0.371	0.940	0.057	0.363	0.383	0.026	0.418	-0.628	0.107	0.378	-0.288	0.442	0.438
At2g30570	0.996	0.127	0.295	-0.254	0.717	0.521	1.427	0.235	0.311	1.943	0.014	0.239	0.491	0.310	0.332	2.511	0.006	0.350	1.228	0.170	0.418	0.048	0.374	0.392	0.036	0.374	0.415
At2g30790	-0.272	0.352	0.330	-0.418	0.437	0.435	-0.647	0.044	0.261	-0.572	0.097	0.291	-0.628	0.012	0.218	-0.238	0.402	0.400	-0.254	0.728	0.559	-0.086	0.431	0.402	-0.547	0.100	0.414
At2g31890	0.515	0.265	0.305	-0.105	0.528	0.465	0.149	0.439	0.380	0.969	0.027	0.249	0.922	0.075	0.285	0.304	0.467	0.422	0.561	0.087	0.418	1.204	0.001	0.378	0.608	0.030	0.414
At2g32150	0.800	0.006	0.141	-0.009	0.970	0.588	-0.707	0.046	0.265	1.348	0.002	0.133	1.039	0.050	0.252	-0.810	0.151	0.363	-0.433	0.200	0.418	0.436	0.071	0.378	0.003	0.869	0.589
At2g32180	0.518	0.021	0.208	0.495	0.343	0.412	0.155	0.415	0.370	-0.480	0.097	0.291	0.830	0.039	0.243	-0.107	0.745	0.517	0.421	0.235	0.418	-0.329	0.153	0.			



At2g34300	-0.010	0.948	0.521	-0.331	0.341	0.412	-0.424	0.010	0.208	0.155	0.162	0.295	-0.007	0.960	0.536	-0.415	0.043	0.363	-1.034	0.007	0.418	0.554	0.051	0.378	0.653	0.071	0.414
At2g34420	-0.605	0.099	0.295	-1.457	0.087	0.395	-1.112	0.007	0.200	-0.478	0.110	0.295	-1.344	0.073	0.284	-2.088	0.003	0.315	-1.025	0.054	0.418	0.251	0.742	0.490	-0.436	0.286	0.414
At2g34430	-0.173	0.511	0.389	0.149	0.901	0.571	-0.934	0.004	0.187	-0.651	0.075	0.291	-0.276	0.224	0.312	-1.055	0.041	0.363	-0.177	0.484	0.466	-0.527	0.238	0.378	-0.448	0.228	0.414
At2g34460	-0.293	0.054	0.277	-0.436	0.441	0.437	-0.298	0.033	0.253	-0.329	0.065	0.290	-0.445	0.083	0.286	-0.603	0.025	0.363	0.000	1.000	1.000	-0.114	0.178	0.378	0.000	1.000	1.000
At2g34480	-0.786	0.007	0.149	0.093	0.813	0.548	-0.204	0.315	0.333	-0.347	0.220	0.295	-0.052	0.653	0.447	-0.153	0.636	0.481	-0.338	0.262	0.418	0.050	0.841	0.511	0.118	0.801	0.562
At2g34585	0.162	0.228	0.299	-0.096	0.740	0.527	-0.413	0.030	0.249	0.446	0.323	0.320	0.806	0.002	0.122	-0.109	0.696	0.501	-0.054	0.266	0.418	0.071	0.178	0.378	0.025	0.374	0.415
At2g34590	-0.235	0.085	0.295	-0.335	0.276	0.398	-0.238	0.096	0.290	-0.240	0.141	0.295	-0.418	0.049	0.252	-0.757	0.009	0.363	-0.127	0.129	0.418	0.177	0.293	0.378	-0.024	0.896	0.591
At2g34620	-0.384	0.056	0.277	-0.538	0.373	0.416	-0.766	0.046	0.265	-1.126	0.015	0.246	-1.108	0.000	0.050	-1.396	0.001	0.232	-0.093	0.393	0.434	-0.266	0.551	0.432	-0.079	0.162	0.414
At2g34810	1.486	0.001	0.067	0.938	0.173	0.395	0.747	0.042	0.261	1.892	0.010	0.239	1.154	0.225	0.312	-0.431	0.149	0.363	-0.067	0.607	0.515	0.144	0.324	0.383	0.095	0.186	0.414
At2g34850	0.107	0.575	0.411	-0.741	0.070	0.395	-0.007	0.363	1.000	0.801	0.031	0.250	0.697	0.025	0.226	0.077	0.782	0.529	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g34930	0.153	0.143	0.295	0.277	0.292	0.401	0.000	1.000	1.000	0.307	0.090	0.291	1.381	0.040	0.244	-0.032	0.930	0.571	0.000	1.000	1.000	0.040	0.374	0.392	0.111	0.374	0.415
At2g35370	-0.192	0.626	0.429	-0.185	0.595	0.483	-0.108	0.848	0.526	0.784	0.142	0.295	-0.248	0.703	0.463	-1.362	0.030	0.363	-0.030	0.908	0.609	0.352	0.440	0.404	0.210	0.164	0.414
At2g35650	-0.134	0.582	0.413	-0.009	0.986	0.592	-1.033	0.038	0.255	0.189	0.291	0.312	-0.167	0.263	0.320	-0.079	0.437	0.411	-0.302	0.374	1.000	-0.085	0.374	0.392	0.000	1.000	1.000
At2g35800	-0.361	0.002	0.089	-0.208	0.385	0.419	-0.287	0.037	0.255	-0.837	0.047	0.268	-0.558	0.008	0.181	-0.760	0.055	0.363	-0.126	0.374	1.000	-0.151	0.289	0.378	-0.101	0.144	0.414
At2g36430	0.080	0.477	0.377	0.585	0.039	0.395	0.052	0.341	0.342	0.010	0.722	0.449	-0.028	0.910	0.523	0.093	0.674	0.494	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g36530	-0.716	0.060	0.281	0.913	0.190	0.395	0.572	0.217	0.310	0.765	0.018	0.247	0.014	0.944	0.533	0.217	0.574	0.459	0.603	0.155	0.418	0.307	0.325	0.384	0.000	0.999	0.617
At2g36630	0.463	0.095	0.295	0.542	0.227	0.395	0.609	0.011	0.216	0.702	0.076	0.291	0.818	0.174	0.310	0.051	0.857	0.550	0.325	0.130	0.418	0.203	0.319	0.382	-0.137	0.612	0.502
At2g37340	-0.409	0.165	0.295	-0.075	0.737	0.527	-0.971	0.020	0.241	-0.511	0.069	0.291	0.134	0.737	0.474	-0.600	0.054	0.363	-0.326	0.065	0.418	0.187	0.443	0.404	0.143	0.235	0.414
At2g37630	-0.363	0.030	0.244	-1.237	0.012	0.395	-0.093	0.642	0.458	-0.221	0.227	0.295	-0.063	0.762	0.482	-0.652	0.140	0.363	0.080	0.504	0.474	-0.147	0.084	0.378	0.004	0.374	0.415
At2g37700	0.661	0.036	0.260	0.382	0.126	0.395	0.161	0.326	0.336	0.877	0.030	0.249	0.696	0.034	0.235	-0.103	0.477	0.425	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g37710	0.348	0.127	0.295	-0.155	0.756	0.532	0.031	0.857	0.528	0.488	0.051	0.274	0.686	0.029	0.230	0.269	0.607	0.472	0.081	0.718	0.554	0.446	0.230	0.378	0.027	0.534	0.474
At2g37770	0.374	0.003	0.102	0.146	0.747	0.529	0.265	0.565	0.430	0.693	0.095	0.291	0.912	0.015	0.226	-0.120	0.760	0.522	0.183	0.184	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g37960	0.963	0.001	0.084	0.769	0.017	0.395	0.325	0.303	0.330	0.878	0.079	0.291	0.678	0.001	0.083	0.246	0.282	0.374	0.011	0.374	1.000	0.049	0.374	0.392	0.000	1.000	1.000
At2g38120	-0.318	0.061	0.283	-0.415	0.046	0.395	-0.111	0.609	0.446	-1.283	0.011	0.239	-0.713	0.014	0.224	-0.341	0.318	0.379	0.000	1.000	1.000	-0.215	0.231	0.378	0.000	1.000	1.000
At2g38240	1.314	0.001	0.077	1.690	0.020	0.395	0.231	0.255	0.314	1.298	0.008	0.223	1.385	0.032	0.233	0.044	0.346	0.385	0.057	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g38290	0.725	0.056	0.277	1.110	0.077	0.395	-0.185	0.295	0.327	0.519	0.083	0.291	1.344	0.019	0.226	1.129	0.046	0.363	0.068	0.374	1.000	0.216	0.179	0.378	0.000	1.000	1.000
At2g38470	0.485	0.058	0.277	1.012	0.034	0.395	0.032	0.820	0.517	0.000	1.000	1.000	0.000	1.000	1.000	0.342	0.650	0.486	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g38540	-0.285	0.250	0.303	-0.727	0.392	0.421	-0.588	0.043	0.261	0.286	0.382	0.335	0.194	0.109	0.297	-1.618	0.001	0.307	-0.222	0.342	0.423	0.021	0.891	0.523	0.176	0.300	0.414
At2g38640	0.147	0.238	0.301	-0.185	0.381	0.418	0.023	0.204	0.309	0.366	0.012	0.239	0.698	0.013	0.223	0.495	0.042	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g38750	1.118	0.005	0.129	1.019	0.093	0.395	0.432	0.235	0.311	1.114	0.015	0.246	1.571	0.002	0.122	-0.238	0.087	0.363	-0.136	0.374	1.000	0.078	0.196	0.378	0.000	1.000	1.000
At2g38790	1.688	0.003	0.111	1.276	0.239	0.395	0.569	0.133	0.308	0.398	0.192	0.295	1.589	0.007	0.180	1.121	0.408	0.402	0.271	0.374	1.000	0.262	0.225	0.378	0.000	1.000	1.000
At2g38870	0.000	1.000	1.000	0.805	0.034	0.395	1.215	0.035	0.254	-0.024	0.374	1.000	0.000	1.000	1.000	0.779	0.057	0.363	0.115	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g38940	-0.025	0.835	0.491	0.375	0.253	0.396	0.591	0.030	0.248	0.262	0.254	0.301	0.362	0.070	0.281	0.496	0.019	0.363	0.140	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g39010	-0.207	0.322	0.321	-0.364	0.353	0.414	-0.375	0.132	0.308	-0.515	0.183	0.295	-0.586	0.018	0.226	-0.751	0.017	0.363	0.026	0.374	1.000	-0.658	0.076	0.378	-0.085	0.374	0.415
At2g39520	0.815	0.043	0.268	0.321	0.468	0.446	-0.030	0.936	0.551	0.105	0.884	0.494	-0.087	0.737	0.474	0.171	0.231	0.364	-0.041	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g39720	-0.047	0.881	0.504	0.190	0.775	0.536	0.160	0.657	0.463	1.324	0.025	0.249	0.750	0.293	0.327	1.165	0.010	0.363	-0.001	0.999	0.631	0.107	0.578	0.442	0.283	0.374	0.415
At2g39730	-0.318	0.049	0.275	-0.428	0.369	0.416	-0.553	0.025	0.245	-0.332	0.245	0.298	-0.895	0.092	0.289	0.052	0.829	0.543	-0.815	0.048	0.418	-0.017	0.894	0.524	0.735	0.113	0.414
At2g39800	-0.253	0.028	0.240	-1.021	0.022	0.395	-0.076	0.367	0.352	-0.228	0.122	0.295	-0.162	0.107	0.294	-0.828	0.157	0.363	0.021	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g39805	0.114	0.491	0.382	0.069	0.739	0.527	0.204	0.045	0.262	0.777	0.005	0.176	0.378	0.048	0.250	-0.056	0.808	0.536	0.025	0.374	1.000	0.015	0.374	0.392	0.000	1.000	1.000
At2g40010	-0.118	0.525	0.395	-0.006	0.971	0.589	0.225	0.211	0.309	0.631	0.031	0.250	-0.060	0.765	0.483	0.074	0.764	0.523	0.155	0.308	0.419	-0.064	0.671	0.463	0.020	0.374	0.415
At2g40060	-0.088	0.628	0.430	0.121	0.771	0.535	0.316	0.408	0.366	0.099	0.866	0.490	-0.171	0.743	0.475	-0.011	0.985	0.584	-0.127	0.349	0.423	0.660	0.283	0.378	0.625	0.020	0.414
At2g40095	0.191	0.237	0.301	0.447	0.104	0.395	0.877	0.025	0.245	0.720	0.052	0.276	0.171	0.181	0.310	0.550	0.052	0.363	0.096	0.765	0.572	0.000	1.000	1.000	0.000	1.000	1.000
At2g40100	-0.366	0.073	0.294	-0.069	0.898	0.570	-0.497	0.163	0.308	-0.630	0.034	0.257	-0.209	0.481	0.385	-1.010	0.020	0.363	-0.392	0.031	0.418	-0.294	0.311	0.			

At2g43000	-0.289	0.378	0.341	0.629	0.157	0.395	1.152	0.008	0.200	-0.132	0.128	0.295	-0.289	0.250	0.316	1.161	0.016	0.363	0.851	0.049	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g43290	0.276	0.222	0.298	1.100	0.108	0.395	-0.201	0.527	0.416	-0.058	0.756	0.460	0.415	0.158	0.310	0.627	0.023	0.363	0.034	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g43360	-0.623	0.001	0.074	-0.406	0.096	0.395	-0.138	0.107	0.295	-0.300	0.030	0.250	-0.493	0.134	0.308	-0.509	0.103	0.363	-0.026	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g43460	-0.038	0.875	0.503	0.506	0.297	0.402	0.663	0.004	0.180	0.392	0.012	0.239	0.511	0.072	0.284	0.398	0.128	0.363	0.059	0.730	0.559	-0.044	0.296	0.378	-0.045	0.638	0.503
At2g43580	0.173	0.082	0.295	1.450	0.052	0.395	0.958	0.019	0.241	-0.036	0.502	0.375	0.101	0.182	0.310	1.281	0.056	0.363	0.262	0.179	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g43590	0.580	0.207	0.296	1.866	0.092	0.395	0.538	0.420	0.372	0.733	0.043	0.266	-0.008	0.984	0.541	1.581	0.041	0.363	0.524	0.207	0.418	0.271	0.412	0.399	0.000	1.000	1.000
At2g43640	-0.126	0.079	0.295	-0.615	0.041	0.395	0.004	0.974	0.559	-0.002	0.987	0.521	-0.011	0.729	0.471	0.504	0.120	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g44200	0.046	0.699	0.452	-0.626	0.006	0.395	-0.001	0.993	0.562	0.110	0.253	0.301	0.277	0.026	0.226	-0.006	0.950	0.575	-0.132	0.140	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g44490	0.749	0.023	0.220	1.693	0.049	0.395	-0.238	0.762	0.497	0.884	0.274	0.306	1.111	0.132	0.305	0.860	0.249	0.365	-0.005	0.986	0.629	0.268	0.585	0.443	0.168	0.374	0.415
At2g44530	-0.350	0.010	0.168	-0.743	0.048	0.395	-0.214	0.050	0.270	-0.091	0.657	0.428	0.044	0.793	0.492	0.208	0.434	0.410	-0.330	0.123	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At2g44970	-0.072	0.449	0.368	-0.631	0.014	0.395	-0.150	0.275	0.321	-0.176	0.205	0.295	-0.338	0.052	0.256	0.359	0.070	0.363	-0.118	0.322	0.421	0.159	0.451	0.406	-0.132	0.338	0.414
At2g45180	-0.120	0.717	0.458	0.050	0.836	0.554	-1.634	0.036	0.254	-0.436	0.350	0.327	-0.611	0.044	0.247	-1.443	0.060	0.363	-0.320	0.271	0.418	-0.219	0.756	0.494	0.454	0.231	0.414
At2g45290	-0.052	0.872	0.502	-0.209	0.683	0.511	-0.017	0.951	0.555	-0.413	0.277	0.307	-1.157	0.036	0.238	-0.080	0.885	0.560	-0.845	0.046	0.418	0.137	0.846	0.512	-0.604	0.387	0.419
At2g45340	-0.886	0.004	0.114	-0.704	0.150	0.395	-0.079	0.682	0.471	-0.515	0.028	0.249	-0.535	0.030	0.231	-0.614	0.065	0.363	0.041	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g45550	0.387	0.097	0.295	0.813	0.066	0.395	0.423	0.220	0.310	0.521	0.076	0.291	1.033	0.022	0.226	0.461	0.021	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g45820	0.082	0.375	0.339	0.527	0.076	0.395	-0.267	0.182	0.308	0.664	0.026	0.249	1.184	0.036	0.238	0.706	0.152	0.363	-0.002	0.374	1.000	0.143	0.374	0.392	0.000	1.000	1.000
At2g46080	0.622	0.002	0.089	0.623	0.108	0.395	0.180	0.574	0.433	1.203	0.042	0.266	0.696	0.093	0.289	0.401	0.169	0.363	-0.055	0.374	1.000	0.091	0.248	0.378	0.000	1.000	1.000
At2g46300	1.044	0.028	0.239	0.065	0.884	0.567	0.468	0.293	0.326	0.304	0.717	0.448	1.035	0.088	0.289	0.511	0.412	0.404	1.121	0.020	0.418	0.095	0.209	0.378	0.071	0.374	0.415
At2g46370	0.089	0.176	0.295	-0.109	0.507	0.458	-0.076	0.332	0.338	0.659	0.028	0.249	0.619	0.033	0.235	0.428	0.076	0.363	0.000	1.000	1.000	0.037	0.374	0.392	0.000	1.000	1.000
At2g46400	0.736	0.067	0.288	1.608	0.081	0.395	1.254	0.019	0.241	0.661	0.113	0.295	1.103	0.020	0.226	1.499	0.192	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g46600	0.786	0.010	0.166	0.093	0.898	0.570	-0.015	0.965	0.557	0.381	0.510	0.378	0.904	0.107	0.295	-1.343	0.017	0.363	-0.295	0.500	0.472	-0.118	0.757	0.494	0.000	1.000	1.000
At2g46650	1.104	0.000	0.013	0.951	0.157	0.395	0.661	0.021	0.241	0.988	0.014	0.239	1.080	0.006	0.160	0.098	0.786	0.530	0.218	0.374	1.000	0.063	0.374	0.392	0.000	1.000	1.000
At2g47380	0.363	0.033	0.252	0.479	0.197	0.395	0.601	0.004	0.177	0.267	0.250	0.299	-0.142	0.703	0.463	0.063	0.656	0.488	0.107	0.606	0.515	0.178	0.327	0.385	0.151	0.513	0.471
At2g47400	-0.910	0.002	0.087	-0.768	0.146	0.395	-0.743	0.005	0.200	-0.529	0.225	0.295	-0.863	0.043	0.247	-0.793	0.073	0.363	-0.707	0.096	0.418	-0.395	0.388	0.395	-0.014	0.401	0.425
At2g47600	0.577	0.003	0.111	0.755	0.059	0.395	0.889	0.007	0.200	0.731	0.003	0.166	0.591	0.059	0.268	-0.097	0.745	0.517	0.043	0.374	1.000	0.023	0.861	0.516	0.043	0.374	0.415
At2g47690	0.591	0.038	0.261	0.599	0.263	0.396	0.473	0.207	0.309	0.521	0.011	0.239	-0.143	0.532	0.403	0.747	0.086	0.363	0.270	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At2g47700	0.591	0.010	0.168	0.163	0.236	0.395	0.162	0.510	0.409	0.493	0.008	0.220	0.268	0.138	0.310	0.510	0.076	0.363	0.095	0.589	0.508	0.143	0.606	0.448	-0.048	0.374	0.415
At3g01440	-0.690	0.001	0.081	-0.264	0.611	0.489	-0.389	0.229	0.311	-0.351	0.270	0.305	-0.718	0.275	0.324	-0.195	0.621	0.477	-0.110	0.656	0.535	-0.291	0.276	0.378	-0.018	0.898	0.591
At3g01500	-0.515	0.038	0.261	-0.101	0.937	0.581	-0.576	0.343	0.342	-0.433	0.401	0.340	0.588	0.286	0.326	-1.307	0.046	0.363	0.175	0.625	0.522	0.432	0.394	0.397	0.744	0.244	0.414
At3g01670	0.029	0.940	0.519	-0.224	0.262	0.396	-0.045	0.167	0.308	-0.130	0.737	0.454	-0.485	0.242	0.314	-1.031	0.025	0.363	-0.022	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g01680	-0.620	0.007	0.153	-0.914	0.073	0.395	-0.600	0.149	0.308	-0.736	0.009	0.235	-1.205	0.002	0.120	-1.262	0.002	0.307	-0.045	0.735	0.562	-0.566	0.114	0.378	-0.236	0.316	0.414
At3g01950	1.096	0.025	0.225	0.104	0.234	0.395	0.415	0.527	0.416	1.498	0.062	0.288	0.076	0.851	0.507	0.626	0.181	0.363	-0.239	0.479	0.465	0.000	1.000	1.000	0.000	1.000	1.000
At3g01990	0.311	0.298	0.314	-0.612	0.153	0.395	0.009	0.960	0.556	-0.645	0.022	0.249	-0.539	0.033	0.235	0.304	0.521	0.441	-0.163	0.306	0.419	-0.010	0.374	0.392	0.000	1.000	1.000
At3g02230	-0.089	0.719	0.458	-0.020	0.958	0.586	0.164	0.422	0.372	0.788	0.017	0.246	0.697	0.086	0.288	0.209	0.425	0.408	-0.349	0.455	0.458	0.602	0.085	0.378	0.933	0.078	0.414
At3g02570	0.454	0.072	0.293	0.015	0.972	0.589	0.167	0.194	0.308	0.913	0.002	0.133	0.901	0.019	0.226	-0.268	0.469	0.422	0.047	0.374	1.000	0.140	0.374	0.392	0.000	1.000	1.000
At3g02660	-0.181	0.570	0.409	0.591	0.397	0.422	0.321	0.251	0.313	-0.420	0.174	0.295	-0.090	0.813	0.497	-0.608	0.039	0.363	-0.074	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g02730	0.016	0.930	0.517	0.685	0.252	0.396	-0.536	0.044	0.261	0.096	0.784	0.468	-0.606	0.048	0.250	0.025	0.943	0.574	-0.005	0.962	0.621	-0.210	0.081	0.378	-0.095	0.345	0.414
At3g02750	0.411	0.035	0.259	0.868	0.029	0.395	-0.437	0.228	0.311	0.943	0.009	0.232	0.561	0.108	0.295	0.538	0.217	0.363	-0.221	0.162	0.418	0.217	0.377	0.393	-0.209	0.376	0.416
At3g02770	0.154	0.202	0.295	0.352	0.064	0.395	0.207	0.225	0.310	0.334	0.138	0.295	0.260	0.196	0.310	0.657	0.033	0.363	0.088	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g03080	0.209	0.129	0.295	0.212	0.374	1.000	0.000	1.000	1.000	0.705	0.024	0.249	0.946	0.027	0.226	0.194	0.234	0.364	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g03270	0.426	0.041	0.266	0.343	0.430	0.433	0.536	0.051	0.270	0.312	0.137	0.295	0.697	0.022	0.226	0.416	0.317	0.379	0.127	0.607	0.515	0.000	1.000	1.000	0.000	1.000	1.000
At3g03290	0.974	0.019	0.205	0.347	0.199	0.395	0.256	0.231	0.311	0.541	0.063	0.288	0.535	0.111	0.297	0.524	0.300	0.376	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g03470	-0.067	0.781	0.478	0.664	0.117	0.395	0.888	0.031	0.249	-0.345	0.302	0.315	0.132	0.723	0.469	0.686	0.309	0.376	0.079	0.554	0.495	-0.271	0.525	0.			

At3g04260	-0.299	0.007	0.149	-0.515	0.054	0.395	-0.112	0.564	0.430	-0.605	0.021	0.249	-0.052	0.836	0.504	-0.426	0.157	0.363	-0.010	0.852	0.594	-0.239	0.232	0.378	0.000	1.000	1.000
At3g04717	1.056	0.036	0.260	3.874	0.002	0.395	2.621	0.008	0.200	1.134	0.021	0.249	0.938	0.059	0.268	3.376	0.011	0.363	1.233	0.134	0.418	0.019	0.374	0.392	0.000	1.000	1.000
At3g04730	0.059	0.748	0.467	-0.424	0.176	0.395	-0.699	0.005	0.200	-0.550	0.036	0.258	-0.277	0.329	0.337	-0.673	0.247	0.364	-0.133	0.571	0.501	-0.387	0.152	0.378	-0.048	0.356	0.414
At3g05100	-0.283	0.047	0.274	-0.489	0.051	0.395	-0.121	0.116	0.300	-0.007	0.975	0.518	-0.010	0.953	0.535	-0.626	0.023	0.363	-0.071	0.374	1.000	-0.056	0.374	0.392	0.000	1.000	1.000
At3g05760	0.152	0.644	0.434	0.322	0.342	0.412	0.487	0.087	0.288	1.002	0.044	0.266	0.116	0.432	0.368	0.315	0.440	0.412	0.210	0.286	0.419	0.000	1.000	1.000	0.000	1.000	1.000
At3g05960	0.151	0.291	0.312	0.498	0.100	0.395	0.611	0.000	0.057	0.164	0.449	0.356	0.354	0.111	0.297	0.029	0.819	0.540	0.137	0.318	0.421	0.000	1.000	1.000	0.000	1.000	1.000
At3g06070	0.137	0.612	0.424	0.520	0.302	0.403	-0.830	0.027	0.246	-0.191	0.362	0.330	-0.244	0.309	0.332	0.368	0.306	0.376	0.102	0.782	0.575	0.145	0.371	0.391	0.067	0.374	0.415
At3g06450	0.109	0.536	0.399	0.698	0.123	0.395	0.176	0.136	0.308	0.498	0.024	0.249	0.576	0.037	0.240	0.609	0.050	0.363	0.068	0.374	1.000	0.119	0.454	0.407	0.000	1.000	1.000
At3g06710	-0.243	0.026	0.232	-1.240	0.001	0.385	-0.239	0.089	0.289	-0.203	0.113	0.295	-0.255	0.087	0.289	-1.239	0.000	0.033	-0.187	0.242	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g07030	0.045	0.871	0.501	-0.651	0.265	0.396	0.305	0.101	0.291	0.225	0.421	0.348	0.225	0.211	0.311	-0.292	0.064	0.363	0.629	0.009	0.418	-0.113	0.416	0.399	-0.278	0.083	0.414
At3g07090	0.432	0.011	0.169	0.144	0.383	0.419	-0.130	0.468	0.392	0.993	0.006	0.189	1.005	0.053	0.256	0.286	0.462	0.419	-0.115	0.606	0.515	0.000	1.000	1.000	0.000	1.000	1.000
At3g07390	0.530	0.051	0.276	0.899	0.022	0.395	0.197	0.221	0.310	1.273	0.014	0.239	1.120	0.046	0.248	0.634	0.226	0.364	0.000	1.000	1.000	0.343	0.191	0.378	0.000	1.000	1.000
At3g07810	0.296	0.150	0.295	0.186	0.566	0.475	0.140	0.575	0.434	0.899	0.004	0.166	0.226	0.629	0.440	0.184	0.607	0.472	-0.296	0.374	1.000	0.167	0.293	0.378	0.000	1.000	1.000
At3g08510	0.876	0.002	0.087	-0.069	0.701	0.516	0.864	0.029	0.248	0.577	0.065	0.290	0.470	0.029	0.230	0.027	0.921	0.568	0.286	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g08520	-0.262	0.737	0.464	0.365	0.677	0.508	1.493	0.029	0.248	-0.718	0.317	0.319	0.209	0.672	0.454	0.688	0.154	0.363	0.357	0.448	0.455	-0.251	0.583	0.443	0.116	0.261	0.414
At3g08700	0.495	0.011	0.169	0.125	0.541	0.469	0.357	0.032	0.249	0.978	0.001	0.091	0.754	0.083	0.286	0.390	0.004	0.321	-0.193	0.427	0.446	0.238	0.132	0.378	-0.008	0.815	0.568
At3g08950	0.217	0.060	0.282	0.050	0.858	0.561	0.000	1.000	1.000	0.174	0.108	0.295	0.623	0.035	0.237	0.533	0.045	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g08970	0.195	0.052	0.276	0.204	0.374	1.000	0.066	0.363	1.000	0.349	0.061	0.287	0.980	0.039	0.242	-0.014	0.768	0.525	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g09210	0.047	0.867	0.500	0.347	0.180	0.395	0.648	0.020	0.241	0.177	0.430	0.351	0.258	0.264	0.320	0.396	0.321	0.380	-0.113	0.374	1.000	0.112	0.248	0.378	0.000	1.000	1.000
At3g09390	-0.115	0.544	0.401	-0.892	0.020	0.395	-0.538	0.024	0.245	-0.376	0.063	0.288	-0.219	0.170	0.310	-0.565	0.091	0.363	-0.039	0.374	1.000	-0.166	0.130	0.378	0.038	0.374	0.415
At3g09500	0.054	0.647	0.435	0.667	0.040	0.395	0.564	0.136	0.308	0.287	0.054	0.277	0.094	0.741	0.475	0.029	0.945	0.574	-0.042	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g09650	-0.286	0.012	0.171	-0.317	0.312	0.404	-0.363	0.131	0.308	-0.659	0.005	0.182	-0.591	0.019	0.226	-0.274	0.043	0.363	-0.123	0.210	0.418	-0.095	0.451	0.406	-0.186	0.185	0.414
At3g09770	0.470	0.019	0.203	0.654	0.020	0.395	0.042	0.220	0.310	0.603	0.076	0.291	0.161	0.181	0.310	0.060	0.749	0.519	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g09840	0.061	0.687	0.448	-0.266	0.020	0.395	0.159	0.164	0.308	0.647	0.002	0.133	0.193	0.091	0.289	0.117	0.618	0.476	0.000	1.000	1.000	0.152	0.185	0.378	0.000	1.000	1.000
At3g10815	-0.018	0.512	0.389	0.577	0.099	0.395	0.177	0.133	0.308	-0.097	0.374	1.000	0.017	0.374	1.000	0.701	0.025	0.363	-0.048	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g10920	0.607	0.015	0.188	-0.689	0.049	0.395	0.518	0.022	0.241	0.628	0.145	0.295	1.184	0.072	0.284	0.194	0.503	0.435	-0.215	0.374	1.000	0.189	0.227	0.378	0.007	0.374	0.415
At3g11030	0.771	0.037	0.260	0.074	0.627	0.495	0.099	0.378	0.356	0.017	0.983	0.520	0.350	0.273	0.323	-0.935	0.199	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g11120	0.347	0.599	0.418	0.464	0.263	0.396	1.114	0.075	0.285	-0.986	0.393	0.338	1.697	0.045	0.247	0.705	0.103	0.363	0.447	0.319	0.421	0.247	0.357	0.386	0.379	0.374	0.415
At3g11510	0.015	0.948	0.521	0.665	0.033	0.395	0.456	0.024	0.245	0.254	0.240	0.297	0.181	0.420	0.364	0.021	0.911	0.566	-0.182	0.447	0.455	0.050	0.594	0.445	0.000	1.000	1.000
At3g11590	-0.075	0.627	0.429	-0.600	0.027	0.395	-0.462	0.178	0.308	-0.466	0.019	0.247	-0.368	0.185	0.310	-0.163	0.614	0.474	-0.125	0.374	1.000	0.152	0.286	0.378	0.000	1.000	1.000
At3g12120	0.517	0.114	0.295	-0.400	0.425	0.431	-0.730	0.036	0.254	-0.503	0.380	0.334	0.187	0.874	0.513	-0.897	0.067	0.363	0.065	0.639	0.528	0.221	0.318	0.382	-0.055	0.374	0.415
At3g13080	0.318	0.121	0.295	1.033	0.025	0.395	0.551	0.099	0.291	0.928	0.042	0.266	1.154	0.044	0.247	1.085	0.024	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g13890	0.319	0.095	0.295	0.605	0.028	0.395	0.039	0.363	1.000	0.225	0.137	0.295	0.134	0.280	0.325	0.159	0.064	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g14067	-0.021	0.895	0.508	0.250	0.602	0.485	0.366	0.093	0.289	-0.416	0.122	0.295	-0.669	0.117	0.300	-0.030	0.934	0.572	-0.964	0.005	0.418	0.004	0.985	0.546	0.188	0.374	0.415
At3g14240	-0.159	0.293	0.313	-0.405	0.261	0.396	-0.056	0.363	1.000	-0.215	0.293	0.312	-0.348	0.128	0.305	-0.882	0.045	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g14350	0.416	0.042	0.267	0.072	0.771	0.535	0.058	0.175	0.308	0.615	0.023	0.249	0.892	0.032	0.233	0.335	0.257	0.367	0.171	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g14420	-0.177	0.702	0.453	0.376	0.370	0.416	-0.036	0.772	0.501	-0.155	0.208	0.295	-0.509	0.221	0.311	0.262	0.269	0.372	-0.716	0.050	0.418	-0.253	0.263	0.378	-0.861	0.316	0.414
At3g14990	0.360	0.218	0.297	0.598	0.182	0.395	0.505	0.230	0.311	0.279	0.084	0.291	0.788	0.003	0.138	0.522	0.119	0.363	0.150	0.133	0.418	-0.040	0.374	0.392	-0.137	0.374	0.415
At3g15450	0.878	0.139	0.295	1.424	0.073	0.395	-1.192	0.012	0.216	-0.822	0.073	0.291	-0.473	0.315	0.334	-0.578	0.565	0.457	0.130	0.726	0.559	0.060	0.640	0.453	-0.197	0.374	0.415
At3g15460	0.742	0.118	0.295	0.980	0.258	0.396	-1.136	0.002	0.166	-1.101	0.060	0.286	-0.348	0.536	0.404	-0.729	0.516	0.439	0.027	0.932	0.614	-0.032	0.374	0.392	0.000	1.000	1.000
At3g15520	-0.070	0.679	0.445	0.013	0.980	0.590	-0.037	0.260	0.315	-0.248	0.286	0.311	-0.028	0.656	0.448	-0.614	0.047	0.363	-0.066	0.374	1.000	-0.167	0.374	0.392	0.000	1.000	1.000
At3g15530	0.832	0.010	0.166	0.640	0.049	0.395	0.488	0.101	0.291	0.374	0.096	0.291	0.458	0.088	0.289	-0.081	0.783	0.529	0.076	0.673	0.539	0.000	1.000	1.000	0.000	1.000	1.000
At3g15680	-0.591	0.000	0.015	-0.663	0.102	0.395	-0.003	0.363	1.000	-0.347	0.083	0.291	-0.578	0.098	0.290	-0.834	0.038	0.363	-0.021	0.374	1.000	-0.067	0.374	0.			

At3g16415	0.596	0.042	0.268	0.000	1.000	1.000	0.422	0.115	0.297	0.421	0.241	0.297	0.100	0.265	0.320	0.000	1.000	1.000	0.089	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g16590	0.364	0.010	0.166	0.704	0.039	0.395	0.083	0.178	0.308	0.441	0.115	0.295	0.114	0.117	0.299	0.414	0.067	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g16830	-0.099	0.165	0.295	0.063	0.853	0.560	0.268	0.381	0.357	0.802	0.002	0.134	0.338	0.284	0.325	0.765	0.006	0.350	0.227	0.133	0.418	0.004	0.374	0.392	-0.137	0.374	0.415
At3g17110	0.336	0.094	0.295	0.317	0.488	0.452	1.022	0.005	0.193	0.172	0.504	0.376	0.427	0.129	0.305	0.353	0.419	0.407	0.131	0.123	0.418	0.134	0.374	0.392	0.064	0.219	0.414
At3g17210	0.235	0.081	0.295	0.158	0.616	0.491	0.222	0.105	0.295	0.433	0.069	0.291	0.286	0.292	0.327	0.593	0.042	0.363	-0.042	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g17440	-0.580	0.218	0.297	-1.157	0.048	0.395	-0.237	0.471	0.393	0.629	0.085	0.291	-0.550	0.203	0.311	0.060	0.939	0.573	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g17550	0.034	0.184	0.295	0.070	0.374	1.000	0.766	0.030	0.248	0.109	0.374	1.000	0.000	1.000	1.000	0.076	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g17810	0.359	0.012	0.173	0.106	0.777	0.537	0.421	0.124	0.305	0.328	0.257	0.301	0.689	0.002	0.122	0.392	0.129	0.363	0.000	0.994	0.631	0.056	0.272	0.378	0.074	0.374	0.415
At3g17930	0.439	0.010	0.166	0.952	0.072	0.395	-0.098	0.457	0.387	-0.304	0.285	0.311	0.253	0.531	0.403	0.761	0.037	0.363	0.186	0.187	0.418	-0.309	0.226	0.378	0.084	0.374	0.415
At3g18780	-0.358	0.243	0.302	1.099	0.011	0.395	-0.277	0.219	0.310	-0.354	0.127	0.295	-1.231	0.187	0.310	0.984	0.072	0.363	-0.102	0.555	0.496	-0.709	0.355	0.386	0.009	0.374	0.415
At3g19550	0.407	0.116	0.295	0.544	0.303	0.403	1.100	0.003	0.166	0.212	0.178	0.295	0.145	0.374	1.000	0.111	0.267	0.372	0.010	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g19710	1.468	0.000	0.011	1.697	0.072	0.395	0.597	0.002	0.163	0.870	0.025	0.249	0.845	0.076	0.286	0.095	0.855	0.550	0.199	0.227	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g19820	0.300	0.192	0.295	-0.426	0.353	0.414	-0.553	0.052	0.270	-0.115	0.796	0.471	-1.767	0.040	0.245	-1.426	0.030	0.363	-0.085	0.138	0.418	-0.309	0.098	0.378	-0.084	0.365	0.414
At3g19930	0.128	0.225	0.299	0.522	0.153	0.395	-0.042	0.834	0.522	0.070	0.860	0.488	0.285	0.091	0.289	0.946	0.029	0.363	0.290	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g20050	0.469	0.264	0.305	-0.890	0.038	0.395	-0.150	0.528	0.416	-0.620	0.131	0.295	-0.164	0.510	0.395	0.284	0.498	0.433	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g20370	0.628	0.010	0.166	1.070	0.053	0.395	0.629	0.267	0.318	0.282	0.140	0.295	0.571	0.021	0.226	-0.336	0.348	0.385	0.037	0.374	1.000	-0.028	0.374	0.392	0.000	1.000	1.000
At3g20390	0.643	0.001	0.075	0.821	0.063	0.395	0.707	0.040	0.256	0.374	0.409	0.343	1.347	0.010	0.196	0.023	0.962	0.579	-0.016	0.437	0.449	-0.474	0.194	0.378	-0.050	0.655	0.509
At3g20540	-0.493	0.190	0.295	-0.760	0.029	0.395	0.162	0.764	0.498	0.137	0.660	0.429	0.733	0.099	0.290	-0.675	0.074	0.363	0.007	0.374	1.000	-0.077	0.374	0.392	0.000	1.000	1.000
At3g20820	-0.168	0.356	0.331	-0.641	0.433	0.434	-0.466	0.202	0.309	-0.738	0.005	0.176	-1.088	0.000	0.060	-0.969	0.018	0.363	-0.140	0.359	0.423	-0.541	0.096	0.378	-0.135	0.179	0.414
At3g20910	-0.257	0.127	0.295	0.302	0.325	0.408	0.148	0.608	0.446	0.353	0.320	0.319	0.761	0.038	0.242	-0.002	0.995	0.586	0.041	0.374	1.000	0.078	0.789	0.502	0.000	1.000	1.000
At3g21055	-0.295	0.197	0.295	-0.286	0.708	0.519	-0.665	0.020	0.241	-0.791	0.019	0.247	-0.410	0.141	0.310	-1.068	0.040	0.363	-0.093	0.680	0.540	-0.252	0.642	0.453	0.111	0.679	0.516
At3g21070	0.479	0.002	0.093	0.141	0.382	0.418	0.121	0.242	0.312	0.853	0.036	0.258	1.118	0.006	0.167	0.120	0.856	0.550	0.070	0.250	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g22380	-0.386	0.093	0.295	-1.019	0.016	0.395	-0.244	0.073	0.285	-0.244	0.205	0.295	-0.104	0.431	0.367	-0.565	0.004	0.321	-0.135	0.370	0.427	-0.158	0.386	0.395	-0.058	0.629	0.503
At3g22800	0.590	0.006	0.135	0.270	0.533	0.466	0.638	0.008	0.200	0.512	0.028	0.249	0.291	0.200	0.310	0.316	0.289	0.375	0.322	0.350	0.423	0.161	0.271	0.378	0.000	1.000	1.000
At3g22890	0.528	0.024	0.221	0.663	0.077	0.395	-0.002	0.995	0.563	0.589	0.053	0.277	0.726	0.027	0.226	0.014	0.935	0.572	0.057	0.774	0.574	0.596	0.097	0.378	0.207	0.374	0.415
At3g22930	0.134	0.574	0.411	0.222	0.613	0.490	-0.245	0.180	0.308	1.255	0.029	0.249	1.017	0.021	0.226	0.187	0.669	0.492	-0.147	0.106	0.418	0.440	0.384	0.395	0.319	0.122	0.414
At3g22942	-0.368	0.321	0.321	0.577	0.566	0.475	-0.820	0.218	0.310	-1.201	0.012	0.239	-0.069	0.902	0.520	-0.735	0.165	0.363	-0.219	0.179	0.418	-0.566	0.072	0.378	0.086	0.867	0.589
At3g23245	0.416	0.022	0.214	0.398	0.045	0.395	0.613	0.001	0.157	0.832	0.029	0.249	0.335	0.153	0.310	1.152	0.090	0.363	0.053	0.463	0.462	0.000	1.000	1.000	0.000	1.000	1.000
At3g23820	0.323	0.009	0.165	-0.258	0.184	0.395	-0.167	0.026	0.246	0.625	0.013	0.239	0.890	0.008	0.180	0.027	0.867	0.554	-0.008	0.936	0.614	0.683	0.004	0.378	0.462	0.065	0.414
At3g23920	0.469	0.005	0.135	-0.475	0.119	0.395	0.625	0.038	0.255	1.337	0.000	0.049	1.228	0.003	0.138	0.479	0.017	0.363	0.211	0.245	0.418	0.155	0.471	0.410	0.000	1.000	1.000
At3g23990	0.224	0.163	0.295	0.489	0.157	0.395	0.624	0.003	0.166	0.697	0.013	0.239	0.583	0.002	0.122	0.560	0.010	0.363	0.083	0.563	0.498	-0.103	0.519	0.424	0.102	0.374	0.415
At3g24190	-0.943	0.044	0.271	-0.225	0.669	0.506	-0.013	0.971	0.559	0.316	0.375	0.333	0.234	0.230	0.313	0.355	0.090	0.363	0.089	0.657	0.535	0.000	1.000	1.000	0.230	0.374	0.415
At3g24420	-0.239	0.476	0.377	0.373	0.595	0.483	-0.412	0.198	0.308	0.443	0.073	0.291	0.174	0.347	0.342	0.341	0.384	0.395	-0.367	0.239	0.418	0.124	0.255	0.378	1.017	0.048	0.414
At3g24480	0.800	0.008	0.153	0.756	0.008	0.395	0.761	0.002	0.166	0.870	0.069	0.291	0.293	0.564	0.415	1.010	0.047	0.363	0.317	0.075	0.418	0.008	0.959	0.541	0.173	0.186	0.414
At3g24840	0.082	0.334	0.325	-0.602	0.038	0.395	-0.091	0.363	1.000	0.430	0.088	0.291	0.282	0.174	0.310	-0.422	0.062	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g25150	0.164	0.345	0.328	0.258	0.145	0.395	0.564	0.016	0.234	0.294	0.020	0.247	0.602	0.014	0.224	0.207	0.426	0.409	0.381	0.148	0.418	-0.186	0.282	0.378	0.112	0.374	0.415
At3g25220	0.090	0.633	0.431	0.088	0.833	0.553	0.729	0.025	0.245	0.564	0.185	0.295	1.211	0.047	0.249	0.217	0.303	0.376	0.214	0.220	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g25230	0.133	0.392	0.346	0.416	0.367	0.416	-0.030	0.861	0.530	0.391	0.292	0.312	0.781	0.035	0.236	-0.531	0.130	0.363	-0.167	0.374	1.000	0.062	0.490	0.415	0.044	0.374	0.415
At3g25730	0.751	0.037	0.260	1.175	0.016	0.395	0.301	0.308	0.331	0.553	0.085	0.291	1.055	0.008	0.181	0.299	0.222	0.363	0.204	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g25780	0.759	0.035	0.259	0.470	0.140	0.395	0.007	0.363	1.000	0.512	0.260	0.302	0.212	0.059	0.268	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g25882	-0.193	0.547	0.402	0.655	0.240	0.395	1.963	0.007	0.200	0.817	0.008	0.223	0.373	0.183	0.310	0.958	0.125	0.363	0.618	0.148	0.418	0.325	0.170	0.378	0.066	0.374	0.415
At3g25890	-0.042	0.747	0.467	-0.124	0.749	0.530	0.199	0.252	0.314	0.173	0.353	0.328	0.031	0.855	0.508	0.676	0.004	0.321	-0.062	0.374	1.000	0.015	0.374	0.392	0.000	1.000	1.000
At3g26060	-0.726	0.028	0.238	-0.006	0.995	0.594	-0.220	0.380	0.357	-0.635	0.037	0.259	0.596	0.361	0.346	-0.497	0.372	0.392	0.056	0.374	1.000	-0.041	0.859	0.			

At3g26980	0.962	0.008	0.157	0.773	0.133	0.395	-0.121	0.050	0.270	1.065	0.028	0.249	1.522	0.027	0.226	0.420	0.643	0.484	0.257	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g27140	1.186	0.003	0.103	0.892	0.284	0.399	1.011	0.010	0.208	0.668	0.009	0.231	1.217	0.038	0.242	0.593	0.383	0.395	0.030	0.407	0.439	0.055	0.374	0.392	0.000	1.000	1.000
At3g27210	0.949	0.004	0.114	0.568	0.390	0.421	1.055	0.046	0.265	1.452	0.055	0.279	1.056	0.072	0.284	1.168	0.080	0.363	0.096	0.374	1.000	0.617	0.097	0.378	0.009	0.374	0.415
At3g27510	-0.135	0.725	0.461	0.391	0.095	0.395	-0.840	0.046	0.266	0.478	0.126	0.295	-0.017	0.974	0.539	0.666	0.193	0.363	-0.390	0.299	0.419	0.090	0.374	0.392	0.000	1.000	1.000
At3g27690	-0.614	0.008	0.158	-0.060	0.964	0.587	-1.261	0.012	0.216	-0.441	0.101	0.293	-0.617	0.005	0.154	-1.520	0.041	0.363	-0.245	0.475	0.465	-0.371	0.394	0.397	0.181	0.558	0.482
At3g28450	0.853	0.001	0.074	0.657	0.200	0.395	0.277	0.149	0.308	0.535	0.049	0.271	0.570	0.068	0.278	0.393	0.183	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g28540	-1.843	0.000	0.016	-1.540	0.001	0.385	-1.813	0.000	0.070	-1.741	0.000	0.091	-2.556	0.000	0.046	-2.110	0.000	0.211	-2.290	0.000	0.072	-1.584	0.015	0.378	-1.723	0.001	0.220
At3g28630	0.561	0.008	0.153	0.710	0.085	0.395	0.831	0.003	0.166	0.288	0.087	0.291	0.556	0.103	0.292	0.529	0.000	0.083	0.533	0.070	0.418	0.100	0.430	0.401	0.000	1.000	1.000
At3g29034	0.109	0.134	0.295	0.456	0.208	0.395	0.179	0.229	0.311	0.604	0.023	0.249	0.105	0.210	0.311	0.421	0.089	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g29320	-0.048	0.826	0.488	-0.072	0.909	0.573	0.046	0.660	0.464	1.068	0.025	0.249	0.308	0.298	0.328	-0.653	0.156	0.363	0.005	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g32990	-0.528	0.260	0.304	-0.455	0.047	0.395	-0.797	0.023	0.243	-0.358	0.025	0.249	0.180	0.753	0.478	-0.480	0.151	0.363	-0.337	0.269	0.418	0.365	0.285	0.378	-0.144	0.798	0.561
At3g44190	0.208	0.044	0.271	0.693	0.021	0.395	0.038	0.707	0.480	0.589	0.029	0.249	0.316	0.186	0.310	0.213	0.603	0.470	0.007	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g45030	-0.013	0.953	0.523	0.602	0.010	0.395	0.292	0.117	0.301	0.286	0.018	0.247	0.595	0.024	0.226	0.161	0.555	0.454	0.014	0.374	1.000	0.112	0.471	0.410	0.000	1.000	1.000
At3g45140	1.559	0.000	0.018	2.528	0.021	0.395	2.004	0.031	0.249	2.372	0.002	0.133	2.002	0.000	0.046	-1.307	0.013	0.363	1.050	0.218	0.418	1.003	0.015	0.378	0.967	0.068	0.414
At3g45253	0.101	0.778	0.477	0.206	0.285	0.399	0.585	0.018	0.237	0.000	1.000	1.000	0.333	0.374	1.000	0.081	0.179	0.363	0.019	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g45640	0.957	0.004	0.114	0.003	0.997	0.595	0.559	0.160	0.308	0.676	0.256	0.301	0.930	0.085	0.288	1.146	0.096	0.363	-0.099	0.870	0.600	0.526	0.218	0.378	-0.460	0.243	0.414
At3g45980	-0.363	0.002	0.089	-0.704	0.032	0.395	-0.117	0.305	0.330	-0.212	0.170	0.295	-0.276	0.025	0.226	-0.153	0.164	0.363	-0.121	0.227	0.418	-0.230	0.164	0.378	-0.054	0.226	0.414
At3g46220	0.495	0.093	0.295	0.950	0.100	0.395	0.823	0.028	0.248	0.432	0.200	0.295	0.598	0.138	0.310	0.056	0.882	0.559	0.341	0.123	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g46780	-0.026	0.865	0.500	-0.134	0.836	0.554	-0.363	0.143	0.308	-0.325	0.244	0.298	-0.132	0.110	0.297	-1.031	0.050	0.363	-0.130	0.069	0.418	0.077	0.651	0.456	0.178	0.405	0.425
At3g46830	0.785	0.101	0.295	0.501	0.490	0.452	0.354	0.575	0.434	1.709	0.078	0.291	0.607	0.608	0.433	1.154	0.026	0.363	0.518	0.193	0.418	0.269	0.617	0.450	0.014	0.374	0.415
At3g47050	-0.024	0.878	0.503	0.163	0.619	0.492	-0.720	0.048	0.268	-0.104	0.386	0.336	0.000	1.000	1.000	0.160	0.683	0.497	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g47070	-0.478	0.044	0.270	-0.438	0.264	0.396	-0.422	0.174	0.308	-0.445	0.058	0.282	-0.727	0.041	0.246	-0.362	0.065	0.363	-0.489	0.084	0.418	-0.442	0.112	0.378	0.122	0.205	0.414
At3g47380	0.274	0.197	0.295	0.378	0.439	0.436	0.106	0.588	0.438	0.694	0.004	0.166	0.535	0.238	0.313	-0.687	0.081	0.363	-0.189	0.264	0.418	0.348	0.079	0.378	0.022	0.374	0.415
At3g47430	-0.199	0.268	0.306	-0.496	0.150	0.395	-0.408	0.121	0.304	-0.743	0.023	0.249	-0.732	0.015	0.226	-0.505	0.078	0.363	-0.124	0.182	0.418	-0.045	0.374	0.392	0.000	1.000	1.000
At3g47470	-0.806	0.052	0.276	-0.470	0.552	0.472	-1.686	0.003	0.166	-1.680	0.025	0.249	-0.486	0.118	0.300	-1.434	0.046	0.363	-0.729	0.229	0.418	0.034	0.781	0.500	-0.835	0.104	0.414
At3g47530	-0.122	0.595	0.417	-0.141	0.670	0.506	-0.239	0.094	0.289	-0.310	0.261	0.302	-0.408	0.177	0.310	-0.627	0.003	0.315	0.000	1.000	1.000	-0.013	0.374	0.392	0.000	1.000	1.000
At3g47950	0.563	0.069	0.290	-0.209	0.102	0.395	0.320	0.167	0.308	1.406	0.014	0.241	1.191	0.045	0.247	0.412	0.099	0.363	0.076	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g47960	0.620	0.001	0.074	0.418	0.065	0.395	0.129	0.307	0.331	0.712	0.013	0.239	0.946	0.009	0.189	0.231	0.324	0.381	0.106	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g48070	0.265	0.135	0.295	0.914	0.362	0.415	0.229	0.477	0.396	-0.440	0.319	0.319	1.284	0.036	0.238	-0.010	0.990	0.585	0.027	0.847	0.594	-0.032	0.184	0.378	0.000	1.000	1.000
At3g48080	-0.080	0.833	0.490	0.680	0.020	0.395	0.466	0.362	0.351	0.771	0.178	0.295	0.625	0.192	0.310	0.243	0.781	0.529	0.129	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g48180	0.839	0.056	0.277	0.089	0.850	0.559	0.927	0.099	0.291	0.453	0.149	0.295	1.890	0.022	0.226	0.662	0.289	0.375	0.706	0.229	0.418	0.021	0.374	0.392	0.000	1.000	1.000
At3g48800	0.506	0.045	0.271	0.345	0.108	0.395	0.072	0.110	0.297	0.282	0.259	0.302	0.323	0.281	0.325	0.657	0.020	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g49070	0.088	0.483	0.379	0.727	0.001	0.385	0.058	0.363	1.000	0.006	0.374	1.000	0.171	0.099	0.290	0.496	0.017	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g49220	0.202	0.297	0.314	-0.279	0.424	0.431	-0.011	0.898	0.540	0.255	0.094	0.291	0.039	0.853	0.508	-0.668	0.044	0.363	0.000	1.000	1.000	-0.011	0.374	0.392	0.011	0.374	0.415
At3g49260	-0.227	0.131	0.295	-0.468	0.019	0.395	-0.031	0.528	0.416	-0.619	0.040	0.262	-0.738	0.004	0.145	-0.545	0.020	0.363	-0.018	0.374	1.000	-0.204	0.202	0.378	0.000	1.000	1.000
At3g49300	0.600	0.002	0.091	0.881	0.063	0.395	0.304	0.089	0.288	0.531	0.052	0.276	0.407	0.182	0.310	0.842	0.033	0.363	0.169	0.475	0.465	0.049	0.613	0.450	0.120	0.268	0.414
At3g49720	0.801	0.025	0.228	0.627	0.118	0.395	-0.358	0.311	0.331	1.599	0.002	0.142	1.914	0.003	0.130	0.274	0.182	0.363	0.108	0.374	1.000	0.078	0.374	0.392	0.021	0.374	0.415
At3g50080	0.920	0.005	0.131	0.705	0.374	0.416	0.630	0.326	0.336	0.535	0.358	0.329	0.795	0.358	0.346	0.130	0.754	0.520	0.185	0.220	0.418	-0.188	0.374	0.392	0.000	1.000	1.000
At3g50130	0.411	0.337	0.326	-0.489	0.384	0.419	1.350	0.039	0.256	0.940	0.069	0.291	0.370	0.650	0.446	1.470	0.046	0.363	0.000	1.000	1.000	-0.035	0.787	0.502	0.000	1.000	1.000
At3g50685	0.175	0.347	0.329	0.226	0.256	0.396	0.291	0.206	0.309	-0.095	0.666	0.431	-0.200	0.546	0.409	-0.586	0.005	0.350	0.016	0.819	0.589	-0.564	0.107	0.378	-0.121	0.410	0.426
At3g50770	0.356	0.005	0.129	1.471	0.025	0.395	0.482	0.004	0.177	-0.034	0.762	0.462	0.287	0.009	0.192	0.505	0.169	0.363	0.113	0.178	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At3g50800	0.510	0.011	0.169	0.087	0.889	0.569	0.919	0.009	0.208	0.382	0.084	0.291	0.385	0.141	0.310	0.621	0.067	0.363	-0.096	0.724	0.557	-0.015	0.217	0.378	0.000	1.000	1.000
At3g50830	0.070	0.611	0.424	0.632	0.092	0.395	-0.081	0.749	0.493	-0.160	0.237	0.297	-0.037	0.739	0.474	0.718	0.003	0.321	-0.053	0.374	1.000	0.000	1.000	1.			

At3g52380	-0.063	0.454	0.369	0.754	0.008	0.395	0.212	0.134	0.308	-0.267	0.112	0.295	0.035	0.750	0.477	0.182	0.275	0.372	0.031	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g52740	-0.392	0.137	0.295	-1.090	0.026	0.395	-0.146	0.210	0.309	0.390	0.174	0.295	-0.172	0.425	0.366	-0.375	0.070	0.363	0.145	0.374	1.000	-0.081	0.278	0.378	0.152	0.329	0.414
At3g52840	0.244	0.062	0.284	0.799	0.042	0.395	-0.104	0.363	0.351	0.200	0.079	0.291	-0.053	0.754	0.479	0.055	0.709	0.505	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g52850	0.357	0.022	0.212	0.589	0.104	0.395	0.704	0.022	0.241	1.034	0.001	0.123	0.618	0.022	0.226	0.081	0.534	0.447	0.011	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g53010	0.670	0.017	0.193	0.105	0.327	0.408	-0.262	0.389	0.361	0.216	0.752	0.459	0.724	0.069	0.281	1.351	0.060	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g53460	0.679	0.009	0.165	0.875	0.056	0.395	1.022	0.005	0.200	0.189	0.212	0.295	0.425	0.009	0.189	0.939	0.013	0.363	0.330	0.212	0.418	0.095	0.380	0.395	-0.047	0.517	0.472
At3g53490	-2.515	0.000	0.000	-2.724	0.001	0.385	-3.100	0.000	0.030	-2.732	0.000	0.056	-2.641	0.000	0.031	-2.632	0.000	0.043	-3.305	0.000	0.072	-1.480	0.025	0.378	-2.701	0.001	0.220
At3g53540	-0.428	0.057	0.277	-1.134	0.044	0.395	0.010	0.358	0.349	-0.602	0.061	0.287	-0.463	0.095	0.290	-0.723	0.023	0.363	0.000	1.000	1.000	-0.048	0.374	0.392	0.000	1.000	1.000
At3g53720	-0.245	0.446	0.366	-0.945	0.042	0.395	0.246	0.365	0.351	-0.180	0.523	0.382	0.374	0.098	0.290	-0.043	0.941	0.574	0.028	0.853	0.594	-0.095	0.571	0.440	0.037	0.374	0.415
At3g54140	0.095	0.136	0.295	1.113	0.041	0.395	0.466	0.153	0.308	0.171	0.095	0.291	0.143	0.374	1.000	0.669	0.148	0.363	0.145	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g54150	0.546	0.048	0.274	1.015	0.005	0.395	0.422	0.137	0.308	0.243	0.158	0.295	0.323	0.208	0.311	0.847	0.117	0.363	0.142	0.481	0.465	0.000	1.000	1.000	0.000	1.000	1.000
At3g54400	-0.398	0.072	0.293	-0.519	0.487	0.452	-0.328	0.311	0.331	-0.357	0.079	0.291	-0.278	0.401	0.357	-1.243	0.043	0.363	0.046	0.374	1.000	-0.223	0.273	0.378	-0.094	0.374	0.415
At3g54440	-0.179	0.066	0.287	-0.731	0.049	0.395	-0.493	0.001	0.163	-0.761	0.003	0.155	-0.360	0.027	0.226	-0.127	0.351	0.385	-0.055	0.562	0.498	-0.175	0.310	0.380	-0.323	0.034	0.414
At3g54680	0.455	0.053	0.276	0.471	0.028	0.395	0.855	0.006	0.200	0.607	0.157	0.295	0.183	0.224	0.312	0.463	0.041	0.363	0.012	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g54890	-0.263	0.014	0.183	-0.428	0.593	0.483	-0.595	0.007	0.200	-0.497	0.049	0.271	-0.517	0.026	0.226	-1.354	0.025	0.363	-0.248	0.181	0.418	-0.361	0.268	0.378	-0.193	0.167	0.414
At3g55240	0.468	0.431	0.362	-0.874	0.040	0.395	-0.991	0.069	0.284	-0.703	0.073	0.291	-0.032	0.922	0.526	0.520	0.185	0.363	0.207	0.680	0.540	-0.122	0.415	0.399	-0.174	0.374	0.415
At3g55360	-0.413	0.002	0.087	-0.671	0.234	0.395	-0.484	0.032	0.249	-0.246	0.043	0.266	-0.418	0.028	0.227	-0.733	0.003	0.321	-0.058	0.374	1.000	0.087	0.364	0.390	0.112	0.374	0.415
At3g55430	0.476	0.016	0.192	0.582	0.023	0.395	0.024	0.715	0.482	0.397	0.091	0.291	0.632	0.029	0.230	0.385	0.293	0.375	0.039	0.374	1.000	0.011	0.374	0.392	0.000	1.000	1.000
At3g55480	0.222	0.103	0.295	-0.222	0.119	0.395	0.035	0.761	0.497	-0.232	0.163	0.295	0.647	0.045	0.248	-0.175	0.347	0.385	-0.031	0.194	0.418	-0.006	0.894	0.524	0.000	1.000	1.000
At3g55800	-0.658	0.014	0.186	-0.542	0.109	0.395	-0.885	0.001	0.132	-0.503	0.101	0.293	-0.265	0.532	0.403	-0.811	0.004	0.325	-0.484	0.066	0.418	-0.326	0.165	0.378	-0.509	0.176	0.414
At3g55880	0.255	0.068	0.289	0.674	0.046	0.395	0.367	0.046	0.266	0.255	0.364	0.330	0.125	0.628	0.440	0.407	0.237	0.364	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g56150	0.591	0.004	0.120	0.392	0.258	0.396	0.333	0.150	0.308	0.889	0.001	0.123	0.545	0.084	0.286	0.713	0.066	0.363	-0.105	0.259	0.418	0.002	0.374	0.392	0.000	1.000	1.000
At3g56800	0.077	0.500	0.386	-0.046	0.765	0.533	-0.491	0.058	0.276	1.500	0.001	0.123	0.392	0.247	0.315	-0.062	0.684	0.497	-1.112	0.189	0.418	1.263	0.029	0.378	1.107	0.009	0.414
At3g56900	-0.460	0.005	0.129	0.251	0.350	0.413	-0.238	0.323	0.336	-0.659	0.013	0.239	-0.407	0.045	0.247	-0.280	0.397	0.399	-0.088	0.628	0.523	-0.287	0.356	0.386	-0.006	0.974	0.612
At3g56940	-0.352	0.042	0.267	-0.372	0.153	0.395	-0.546	0.014	0.228	-0.316	0.261	0.302	-0.308	0.126	0.305	-1.075	0.018	0.363	-0.153	0.525	0.480	-0.176	0.464	0.409	0.155	0.622	0.502
At3g57050	0.296	0.181	0.295	0.420	0.130	0.395	0.365	0.083	0.286	0.639	0.002	0.133	0.183	0.344	0.340	-0.069	0.835	0.545	-0.072	0.180	0.418	0.053	0.544	0.432	0.006	0.374	0.415
At3g57450	1.568	0.001	0.081	1.789	0.085	0.395	0.592	0.006	0.200	0.943	0.065	0.290	1.009	0.043	0.247	0.694	0.419	0.406	0.051	0.374	1.000	0.019	0.196	0.378	0.033	0.374	0.415
At3g57530	0.351	0.006	0.148	0.694	0.032	0.395	0.021	0.363	1.000	0.181	0.180	0.295	0.398	0.049	0.251	0.323	0.369	0.391	-0.029	0.779	0.574	0.000	1.000	1.000	0.000	1.000	1.000
At3g57550	0.577	0.014	0.186	0.940	0.037	0.395	-0.083	0.401	0.365	0.492	0.128	0.295	0.545	0.025	0.226	0.544	0.274	0.372	0.124	0.232	0.418	-0.003	0.374	0.392	0.000	1.000	1.000
At3g57690	-0.059	0.340	0.326	0.509	0.158	0.395	0.000	1.000	1.000	0.002	0.374	1.000	0.000	1.000	1.000	0.812	0.025	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g57930	1.122	0.002	0.087	0.623	0.258	0.396	-0.133	0.690	0.474	0.542	0.042	0.266	1.074	0.026	0.226	0.888	0.286	0.374	-0.637	0.274	0.418	0.418	0.205	0.378	0.068	0.374	0.415
At3g58020	0.534	0.058	0.277	0.640	0.390	0.421	0.900	0.078	0.285	0.472	0.337	0.324	1.325	0.022	0.226	0.857	0.121	0.363	0.041	0.374	1.000	0.131	0.443	0.404	-0.236	0.374	0.415
At3g58490	0.314	0.302	0.316	0.048	0.941	0.582	0.441	0.213	0.309	1.010	0.048	0.270	0.433	0.293	0.327	0.057	0.927	0.570	0.207	0.374	1.000	0.119	0.504	0.420	0.181	0.374	0.415
At3g58700	-0.256	0.169	0.295	0.921	0.037	0.395	-0.043	0.761	0.497	0.310	0.206	0.295	0.207	0.257	0.319	0.638	0.105	0.363	-0.075	0.374	1.000	-0.045	0.420	0.400	0.094	0.212	0.414
At3g59400	-0.289	0.217	0.297	-0.444	0.234	0.395	-0.303	0.235	0.311	0.014	0.925	0.504	-0.199	0.553	0.411	-0.686	0.006	0.359	-0.267	0.315	0.421	0.305	0.480	0.411	-0.224	0.438	0.438
At3g59500	0.236	0.532	0.398	0.228	0.677	0.508	0.195	0.593	0.440	0.274	0.228	0.295	0.826	0.021	0.226	0.655	0.404	0.401	0.069	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g59900	0.193	0.186	0.295	1.406	0.055	0.395	0.891	0.021	0.241	0.000	1.000	1.000	0.202	0.374	1.000	1.355	0.130	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g60120	0.000	1.000	1.000	0.979	0.095	0.395	0.901	0.077	0.285	0.000	1.000	1.000	0.000	1.000	1.000	1.346	0.001	0.291	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g60130	0.043	0.541	0.400	0.516	0.091	0.395	0.906	0.025	0.245	0.000	1.000	1.000	0.000	1.000	1.000	-0.096	0.711	0.506	0.226	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g60245	0.027	0.903	0.509	0.610	0.060	0.395	0.719	0.013	0.224	0.273	0.115	0.295	0.336	0.140	0.310	0.572	0.108	0.363	0.038	0.834	0.593	0.098	0.396	0.397	-0.068	0.449	0.441
At3g60520	0.611	0.029	0.240	0.364	0.311	0.404	0.483	0.085	0.286	0.636	0.123	0.295	0.845	0.094	0.290	0.482	0.019	0.363	0.087	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At3g61050	0.525	0.036	0.260	0.872	0.012	0.395	0.024	0.908	0.542	0.121	0.623	0.415	-0.127	0.671	0.453	0.356	0.407	0.402	-0.066	0.513	0.478	-0.076	0.451	0.406	0.000	1.000	1.000
At3g61470	0.128	0.847	0.495	-0.194	0.496	0.454	-1.689	0.032	0.249	-0.543	0.247	0.299	0.604	0.470	0.381	-1.122	0.079	0.363	-0.092	0.827	0.591	-0.461	0.751	0.			

At3g63140	-0.635	0.019	0.201	0.196	0.766	0.534	0.284	0.442	0.381	-0.242	0.577	0.400	-0.046	0.888	0.517	-0.526	0.071	0.363	0.254	0.354	0.423	-0.297	0.453	0.407	0.616	0.107	0.414
At3g63500	-0.018	0.548	0.403	-0.852	0.016	0.395	0.000	1.000	1.000	-0.068	0.374	1.000	0.103	0.374	1.000	-0.234	0.571	0.458	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g00300	0.745	0.004	0.114	0.238	0.099	0.395	0.240	0.255	0.314	1.040	0.037	0.259	1.220	0.001	0.069	0.618	0.221	0.363	-0.115	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g00370	0.173	0.112	0.295	-0.332	0.431	0.434	-0.212	0.324	0.336	0.672	0.038	0.259	0.230	0.381	0.351	0.092	0.591	0.466	-0.129	0.374	1.000	0.000	0.374	0.392	0.030	0.374	0.415
At4g00830	-0.036	0.770	0.475	0.136	0.173	0.395	0.068	0.402	0.365	0.378	0.165	0.295	0.715	0.022	0.226	0.362	0.013	0.363	0.011	0.374	1.000	0.008	0.840	0.511	0.000	1.000	1.000
At4g00860	0.598	0.139	0.295	0.553	0.068	0.395	0.509	0.038	0.255	0.142	0.763	0.462	1.013	0.045	0.247	0.367	0.084	0.363	0.111	0.181	0.418	0.002	0.374	0.392	0.000	1.000	1.000
At4g01050	-0.170	0.097	0.295	-0.030	0.896	0.569	0.008	0.960	0.556	-0.293	0.066	0.291	-0.015	0.945	0.533	-0.671	0.036	0.363	0.015	0.908	0.609	-0.307	0.208	0.378	-0.065	0.628	0.503
At4g01100	0.237	0.328	0.323	0.659	0.030	0.395	0.109	0.386	0.360	-0.079	0.579	0.400	0.237	0.370	0.349	0.373	0.041	0.363	0.138	0.134	0.418	0.156	0.307	0.378	0.012	0.374	0.415
At4g01985	1.634	0.034	0.254	0.910	0.459	0.443	0.838	0.311	0.331	2.051	0.064	0.288	1.280	0.319	0.334	1.129	0.384	0.395	0.378	0.118	0.418	0.467	0.329	0.385	0.019	0.374	0.415
At4g02380	0.701	0.017	0.195	0.806	0.097	0.395	0.606	0.087	0.288	0.670	0.187	0.295	0.771	0.128	0.305	1.040	0.146	0.363	0.248	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g02440	-0.131	0.464	0.373	-0.178	0.534	0.466	-0.283	0.077	0.285	-0.328	0.112	0.295	-0.402	0.292	0.327	-0.907	0.028	0.363	-0.004	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g02450	0.077	0.623	0.428	0.694	0.103	0.395	0.427	0.053	0.270	-0.009	0.950	0.511	0.095	0.506	0.394	0.658	0.037	0.363	0.175	0.104	0.418	0.010	0.910	0.529	0.016	0.374	0.415
At4g02480	0.187	0.068	0.289	0.384	0.081	0.395	-0.180	0.563	0.430	0.318	0.200	0.295	0.120	0.721	0.469	0.896	0.032	0.363	0.105	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g02520	0.253	0.440	0.365	1.567	0.031	0.395	1.277	0.010	0.208	0.395	0.216	0.295	0.562	0.080	0.286	1.915	0.038	0.363	0.189	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g02770	-0.377	0.108	0.295	-0.527	0.192	0.395	-0.800	0.056	0.276	-0.485	0.008	0.220	-0.947	0.106	0.293	-0.402	0.142	0.363	-0.937	0.027	0.418	0.019	0.701	0.477	0.017	0.975	0.612
At4g02840	0.680	0.003	0.111	0.561	0.069	0.395	0.314	0.072	0.285	0.502	0.031	0.250	0.391	0.088	0.289	0.810	0.009	0.363	0.066	0.595	0.510	-0.005	0.897	0.524	0.000	1.000	1.000
At4g03000	0.012	0.905	0.510	-0.362	0.357	0.414	-0.545	0.002	0.166	-0.221	0.136	0.295	-0.047	0.262	0.470	-0.624	0.005	0.326	0.011	0.702	0.547	0.097	0.224	0.378	-0.077	0.296	0.414
At4g03190	-0.268	0.206	0.296	-1.058	0.001	0.385	0.079	0.260	0.315	-0.542	0.090	0.291	-0.867	0.010	0.196	-0.388	0.333	0.384	0.019	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g03280	-0.002	0.987	0.531	0.142	0.591	0.483	-0.096	0.233	0.311	-0.223	0.238	0.297	-0.265	0.056	0.264	-0.621	0.031	0.363	-0.436	0.049	0.418	-0.067	0.302	0.378	-0.211	0.591	0.498
At4g03400	0.493	0.108	0.295	0.296	0.052	0.395	0.234	0.227	0.310	1.587	0.004	0.171	1.565	0.008	0.181	-0.090	0.646	0.484	-0.014	0.833	0.593	0.989	0.011	0.378	0.561	0.160	0.414
At4g03560	0.192	0.243	0.302	0.367	0.225	0.395	0.117	0.717	0.483	-0.239	0.511	0.378	0.629	0.043	0.247	0.246	0.043	0.363	0.201	0.374	1.000	0.211	0.340	0.386	0.000	1.000	1.000
At4g04640	-0.192	0.175	0.295	-0.101	0.827	0.552	-0.162	0.535	0.419	-0.276	0.079	0.291	-0.230	0.265	0.320	-0.848	0.005	0.326	0.012	0.374	1.000	-0.498	0.146	0.378	-0.009	0.942	0.603
At4g05090	0.117	0.444	0.366	0.695	0.045	0.395	0.069	0.617	0.449	-0.166	0.360	0.330	-0.148	0.203	0.311	0.068	0.765	0.524	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g05180	-0.086	0.675	0.444	-0.442	0.454	0.442	-0.435	0.026	0.246	-0.144	0.522	0.382	-0.165	0.226	0.312	-0.730	0.044	0.363	-0.010	0.888	0.605	-0.012	0.885	0.522	0.105	0.374	0.415
At4g05230	0.428	0.040	0.266	0.228	0.188	0.395	-0.303	0.109	0.297	0.764	0.121	0.295	0.597	0.045	0.247	0.394	0.084	0.363	-0.156	0.737	0.563	-0.006	0.374	0.392	0.000	1.000	1.000
At4g08300	0.176	0.657	0.438	0.371	0.569	0.476	-0.666	0.050	0.270	-0.209	0.508	0.377	0.683	0.044	0.247	-0.002	0.996	0.586	-0.161	0.595	0.510	0.482	0.124	0.378	-0.123	0.497	0.466
At4g09320	-0.140	0.360	0.333	0.585	0.002	0.395	0.505	0.057	0.276	0.204	0.484	0.369	-0.071	0.743	0.475	0.252	0.641	0.483	0.009	0.184	0.418	0.109	0.333	0.385	0.084	0.374	0.415
At4g09650	-0.462	0.118	0.295	0.056	0.928	0.579	-0.738	0.061	0.277	0.310	0.476	0.365	-0.744	0.059	0.268	-1.028	0.019	0.363	0.370	0.255	0.418	0.068	0.861	0.516	0.268	0.604	0.501
At4g10120	0.184	0.303	0.316	-0.374	0.187	0.395	-0.279	0.366	0.352	-0.756	0.000	0.091	-0.762	0.003	0.138	0.014	0.924	0.569	-0.032	0.374	1.000	-0.534	0.019	0.378	-0.097	0.374	0.415
At4g10340	-0.314	0.124	0.295	-0.650	0.485	0.451	-1.253	0.010	0.214	-0.845	0.012	0.239	-0.856	0.007	0.177	-1.477	0.033	0.363	-0.414	0.052	0.418	-0.061	0.879	0.520	-0.095	0.616	0.502
At4g10450	0.445	0.327	0.322	1.267	0.100	0.395	0.126	0.721	0.484	-0.240	0.793	0.471	-0.528	0.328	0.336	0.775	0.031	0.363	0.000	1.000	1.000	0.169	0.374	0.392	0.000	1.000	1.000
At4g11175	0.187	0.026	0.229	0.144	0.494	0.453	0.460	0.055	0.275	0.369	0.297	0.313	0.986	0.031	0.233	0.749	0.106	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g11310	0.988	0.010	0.166	0.945	0.328	0.408	0.149	0.777	0.502	-0.629	0.365	0.331	-0.674	0.332	0.338	-0.314	0.343	0.384	-0.384	0.384	0.430	-0.001	0.374	0.392	0.000	1.000	1.000
At4g11530	0.278	0.156	0.295	0.427	0.367	0.416	-0.131	0.675	0.469	0.095	0.374	1.000	0.206	0.289	0.326	1.008	0.050	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g11600	0.146	0.407	0.352	0.085	0.793	0.542	0.450	0.061	0.277	0.623	0.011	0.239	0.855	0.017	0.226	0.644	0.137	0.363	-0.049	0.374	1.000	0.315	0.162	0.378	0.004	0.374	0.415
At4g12790	0.101	0.437	0.364	-0.099	0.544	0.469	-0.183	0.125	0.305	0.684	0.035	0.258	0.505	0.026	0.226	0.154	0.494	0.432	-0.067	0.374	1.000	0.031	0.374	0.392	0.019	0.374	0.415
At4g13170	-0.182	0.391	0.345	0.303	0.322	0.407	0.710	0.178	0.308	0.184	0.575	0.399	0.723	0.106	0.293	0.595	0.037	0.363	0.064	0.374	1.000	0.114	0.374	0.392	0.052	0.374	0.415
At4g13850	0.459	0.156	0.295	0.641	0.408	0.426	0.586	0.123	0.305	-0.237	0.459	0.360	0.416	0.320	0.334	1.194	0.023	0.363	0.525	0.220	0.418	0.832	0.145	0.378	0.458	0.252	0.414
At4g13930	-0.286	0.104	0.295	-0.343	0.529	0.465	0.724	0.024	0.243	-0.030	0.847	0.484	0.354	0.322	0.334	-0.478	0.115	0.363	0.225	0.429	0.446	0.450	0.132	0.378	0.516	0.069	0.414
At4g14320	-0.064	0.713	0.456	0.672	0.009	0.395	0.267	0.024	0.245	0.122	0.271	0.305	0.154	0.428	0.366	0.431	0.036	0.363	-0.148	0.260	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g14365	0.519	0.182	0.295	1.390	0.093	0.395	0.436	0.586	0.437	1.393	0.035	0.258	2.256	0.000	0.046	0.720	0.370	0.391	-0.020	0.963	0.621	0.238	0.374	0.392	0.236	0.374	0.415
At4g14540	0.833	0.003	0.110	0.828	0.109	0.395	0.608	0.015	0.232	0.530	0.003	0.155	0.594	0.114	0.298	0.613	0.128	0.363	0.369	0.031	0.418	0.297	0.179	0.378	0.000	1.000	1.000
At4g14570	0.852	0.003	0.102	0.960	0.012	0.395	0.327	0.435	0.378	0.201	0.544	0.389	0.198	0.335	0.339	0.482	0.219	0.363	-0.130	0.579	0.505	0.370	0.228	0.			

At4g16240	0.464	0.002	0.091	1.142	0.101	0.395	0.524	0.021	0.241	0.845	0.044	0.266	0.249	0.170	0.310	-0.293	0.327	0.381	0.181	0.375	0.427	-0.072	0.674	0.465	0.000	1.000	1.000
At4g16390	-0.492	0.058	0.277	-0.173	0.359	0.415	-0.046	0.804	0.511	0.322	0.154	0.295	-0.643	0.015	0.226	0.082	0.568	0.457	-0.072	0.374	1.000	-0.045	0.374	0.392	0.062	0.647	0.505
At4g16410	-0.255	0.051	0.276	-0.152	0.563	0.475	-0.666	0.022	0.241	-0.817	0.007	0.206	-0.475	0.149	0.310	-0.103	0.711	0.506	-0.296	0.012	0.418	0.527	0.312	0.380	-0.035	0.882	0.591
At4g16490	-0.126	0.265	0.305	-0.629	0.044	0.395	-0.040	0.269	0.319	-0.313	0.062	0.287	-0.108	0.259	0.319	-0.149	0.440	0.412	0.000	1.000	1.000	-0.087	0.374	0.392	0.000	1.000	1.000
At4g16500	0.345	0.159	0.295	0.041	0.844	0.557	0.648	0.261	0.316	-0.093	0.826	0.479	0.799	0.049	0.252	0.734	0.078	0.363	0.340	0.314	0.421	0.029	0.841	0.511	-0.022	0.374	0.415
At4g16660	0.238	0.214	0.297	0.053	0.857	0.561	0.437	0.194	0.308	0.619	0.025	0.249	0.858	0.029	0.230	-0.024	0.911	0.566	0.107	0.181	0.418	-0.090	0.803	0.505	0.099	0.374	0.415
At4g17230	0.674	0.044	0.271	1.123	0.110	0.395	0.321	0.010	0.208	0.192	0.116	0.295	1.210	0.038	0.242	1.102	0.092	0.363	-0.078	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g17370	-0.076	0.575	0.411	0.170	0.167	0.395	0.000	1.000	1.000	-0.024	0.374	1.000	-0.003	0.374	1.000	0.585	0.015	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g17440	0.275	0.038	0.261	0.203	0.295	0.402	0.617	0.027	0.246	0.092	0.718	0.448	0.387	0.060	0.268	0.449	0.175	0.363	-0.058	0.160	0.418	-0.133	0.582	0.443	0.021	0.374	0.415
At4g17510	0.084	0.449	0.368	0.000	1.000	1.000	0.000	1.000	1.000	0.887	0.023	0.249	0.908	0.090	0.289	0.000	1.000	1.000	0.000	1.000	1.000	0.269	0.374	0.392	0.000	1.000	1.000
At4g18220	0.052	0.830	0.489	0.825	0.285	0.400	-0.308	0.454	0.386	-0.354	0.107	0.295	0.161	0.618	0.436	1.098	0.021	0.363	0.492	0.159	0.418	0.080	0.401	0.397	0.000	1.000	1.000
At4g18320	0.179	0.146	0.295	0.696	0.026	0.395	-0.213	0.461	0.389	-0.118	0.625	0.416	0.168	0.069	0.281	0.761	0.026	0.363	0.013	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g18480	-0.014	0.957	0.524	0.480	0.378	0.417	0.248	0.274	0.320	-0.695	0.022	0.249	-0.251	0.530	0.403	-0.333	0.215	0.363	0.264	0.180	0.418	0.203	0.556	0.433	0.212	0.179	0.414
At4g18530	0.345	0.142	0.295	0.128	0.585	0.481	0.451	0.090	0.289	0.424	0.073	0.291	1.006	0.001	0.106	0.449	0.338	0.384	0.157	0.270	0.418	0.117	0.374	0.392	0.000	1.000	1.000
At4g18620	0.239	0.795	0.481	1.303	0.031	0.395	1.828	0.012	0.216	1.225	0.152	0.295	-0.664	0.311	0.332	1.307	0.128	0.363	-0.021	0.353	0.423	0.000	1.000	1.000	0.000	1.000	1.000
At4g18950	0.512	0.023	0.220	1.127	0.039	0.395	0.067	0.521	0.413	0.520	0.234	0.296	0.534	0.184	0.310	0.348	0.174	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g19200	0.858	0.011	0.169	-0.220	0.662	0.505	0.016	0.960	0.556	1.510	0.018	0.247	1.401	0.016	0.226	1.290	0.052	0.363	-0.229	0.231	0.418	0.598	0.179	0.378	0.110	0.783	0.558
At4g19820	0.152	0.117	0.295	1.162	0.052	0.395	0.713	0.035	0.254	-0.015	0.374	1.000	0.108	0.374	1.000	1.431	0.004	0.321	0.184	0.070	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g19860	1.069	0.008	0.155	-0.028	0.945	0.583	0.247	0.692	0.475	0.416	0.312	0.318	0.188	0.709	0.465	0.130	0.581	0.462	0.270	0.063	0.418	-0.064	0.374	0.392	-0.039	0.374	0.415
At4g20260	-0.051	0.690	0.449	0.203	0.455	0.442	-0.086	0.317	0.334	-0.137	0.098	0.293	0.033	0.856	0.509	0.789	0.045	0.363	0.003	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g20390	0.811	0.002	0.091	0.470	0.238	0.395	0.347	0.321	0.335	0.477	0.034	0.257	0.559	0.092	0.289	1.058	0.016	0.363	0.035	0.374	1.000	0.161	0.388	0.395	0.074	0.374	0.415
At4g20760	-1.316	0.000	0.003	-1.660	0.001	0.385	-1.582	0.001	0.157	-1.128	0.002	0.123	-1.495	0.000	0.046	-1.299	0.001	0.307	-1.045	0.051	0.418	-0.258	0.082	0.378	-0.074	0.224	0.414
At4g20840	0.548	0.001	0.074	0.784	0.003	0.395	0.006	0.925	0.548	0.423	0.055	0.280	1.288	0.006	0.160	0.416	0.373	0.393	0.055	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g20890	-0.461	0.002	0.087	-1.204	0.034	0.395	-0.732	0.002	0.166	-0.716	0.019	0.247	-0.510	0.018	0.226	-0.818	0.012	0.363	-0.921	0.021	0.418	-0.360	0.132	0.378	-0.435	0.046	0.414
At4g20960	-0.620	0.024	0.224	0.039	0.909	0.573	0.029	0.892	0.538	-0.116	0.718	0.448	-0.018	0.958	0.536	0.050	0.792	0.531	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g21120	-0.043	0.737	0.464	0.214	0.574	0.477	0.488	0.146	0.308	0.487	0.063	0.288	0.819	0.000	0.064	0.589	0.050	0.363	-0.078	0.351	0.423	0.000	1.000	1.000	0.000	1.000	1.000
At4g21570	0.394	0.127	0.295	-0.374	0.131	0.395	0.000	1.000	1.000	0.809	0.044	0.266	0.626	0.178	0.310	-0.036	0.910	0.566	0.000	1.000	1.000	0.013	0.374	0.392	0.000	1.000	1.000
At4g21960	-0.368	0.121	0.295	-1.562	0.037	0.395	-0.277	0.599	0.442	-1.300	0.005	0.182	-0.534	0.152	0.310	-0.302	0.695	0.501	-0.116	0.181	0.418	-0.409	0.199	0.378	-0.005	0.374	0.415
At4g22190	-0.118	0.538	0.399	-0.774	0.152	0.395	-0.352	0.198	0.308	-0.574	0.027	0.249	-0.708	0.005	0.151	-0.658	0.062	0.363	-0.205	0.089	0.418	-0.116	0.091	0.378	0.022	0.566	0.484
At4g22330	-0.010	0.903	0.509	-0.194	0.194	0.395	-0.203	0.192	0.308	0.603	0.002	0.123	0.398	0.101	0.291	-0.326	0.455	0.418	-0.047	0.334	0.423	0.408	0.097	0.378	0.205	0.148	0.414
At4g22710	1.206	0.005	0.134	0.927	0.131	0.395	0.669	0.072	0.285	-0.225	0.670	0.432	0.517	0.233	0.313	1.608	0.051	0.363	0.714	0.027	0.418	0.004	0.374	0.392	0.000	1.000	1.000
At4g22780	0.022	0.799	0.482	0.523	0.046	0.395	0.011	0.931	0.550	0.051	0.691	0.439	0.079	0.374	1.000	0.677	0.000	0.083	0.081	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g23010	0.275	0.042	0.267	0.777	0.183	0.395	0.762	0.067	0.281	0.428	0.056	0.281	0.408	0.034	0.235	1.024	0.000	0.211	-0.104	0.743	0.564	0.000	1.000	1.000	0.000	1.000	1.000
At4g23040	0.566	0.110	0.295	0.079	0.777	0.537	0.532	0.106	0.295	0.380	0.458	0.360	0.896	0.019	0.226	0.206	0.013	0.363	0.511	0.192	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g23100	1.182	0.001	0.074	0.728	0.321	0.407	0.391	0.085	0.286	0.392	0.401	0.340	0.025	0.963	0.537	1.231	0.056	0.363	-0.216	0.374	1.000	0.184	0.198	0.378	0.000	1.000	1.000
At4g23600	1.697	0.001	0.070	2.195	0.017	0.395	0.561	0.205	0.309	1.894	0.000	0.091	2.433	0.000	0.059	-0.287	0.223	0.363	0.085	0.374	1.000	0.647	0.086	0.378	0.468	0.163	0.414
At4g23880	1.008	0.007	0.149	1.078	0.039	0.395	1.049	0.014	0.228	1.087	0.010	0.239	0.883	0.061	0.269	0.808	0.077	0.363	0.128	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g23890	-0.192	0.141	0.295	-0.592	0.312	0.404	-0.871	0.011	0.216	-0.617	0.002	0.133	-0.452	0.105	0.293	-0.532	0.074	0.363	-0.206	0.296	0.419	0.435	0.455	0.407	-0.147	0.021	0.414
At4g24190	-0.236	0.160	0.295	0.296	0.629	0.495	0.722	0.022	0.241	0.596	0.012	0.239	1.218	0.054	0.256	0.091	0.755	0.521	0.586	0.109	0.418	0.902	0.190	0.378	0.689	0.185	0.414
At4g24260	0.145	0.559	0.406	1.195	0.077	0.395	1.230	0.010	0.208	0.775	0.122	0.295	0.108	0.310	0.332	1.004	0.038	0.363	0.167	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g24380	0.742	0.002	0.091	0.989	0.026	0.395	0.490	0.001	0.163	1.311	0.018	0.247	1.257	0.009	0.189	0.029	0.890	0.561	-0.033	0.374	1.000	0.148	0.178	0.378	0.062	0.374	0.415
At4g24690	0.099	0.555	0.405	0.644	0.158	0.395	0.241	0.544	0.422	0.160	0.640	0.422	-0.060	0.832	0.503	0.984	0.038	0.363	-0.460	0.105	0.418	0.245	0.654	0.457	-0.033	0.671	0.515
At4g24780	-0.721	0.035	0.260	-0.661	0.342	0.412	-0.410	0.109	0.297	-0.637	0.093	0.291	0.211	0.598	0.429	-0.361	0.505	0.435	0.000	1.000	1.000	0.000	1.000	1.			



At4g27070	0.182	0.491	0.383	0.585	0.038	0.395	0.056	0.838	0.523	0.676	0.155	0.295	0.867	0.069	0.281	0.008	0.968	0.580	0.000	1.000	1.000	0.647	0.141	0.378	0.163	0.198	0.414
At4g27130	0.199	0.367	0.336	0.378	0.077	0.395	-0.089	0.499	0.405	0.289	0.326	0.321	0.248	0.239	0.313	0.593	0.020	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g27280	1.527	0.001	0.080	1.077	0.044	0.395	0.355	0.214	0.309	1.139	0.043	0.266	1.149	0.049	0.252	0.252	0.573	0.459	0.164	0.314	0.421	0.001	0.374	0.392	0.059	0.374	0.415
At4g27440	-0.612	0.048	0.274	0.080	0.923	0.578	0.264	0.083	0.286	-0.596	0.010	0.237	-0.602	0.079	0.286	-1.478	0.027	0.363	-0.070	0.374	1.000	-0.329	0.303	0.378	0.000	1.000	1.000
At4g27520	0.613	0.054	0.277	0.181	0.555	0.473	0.019	0.363	1.000	1.223	0.005	0.184	0.559	0.066	0.274	0.059	0.805	0.535	0.000	1.000	1.000	0.192	0.374	0.392	0.000	1.000	1.000
At4g27600	-0.181	0.560	0.406	0.437	0.068	0.395	-0.136	0.416	0.370	-0.344	0.118	0.295	-0.979	0.003	0.138	-0.546	0.325	0.381	0.126	0.574	0.503	-0.074	0.647	0.454	-0.101	0.374	0.415
At4g27640	0.158	0.132	0.295	0.601	0.018	0.395	0.365	0.072	0.285	-0.030	0.875	0.491	0.186	0.478	0.384	0.127	0.747	0.517	0.003	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g27740	0.583	0.064	0.286	1.408	0.010	0.395	0.729	0.007	0.200	0.214	0.280	0.308	0.322	0.276	0.324	0.310	0.382	0.395	0.959	0.021	0.418	0.017	0.858	0.515	-0.038	0.870	0.589
At4g28060	-0.042	0.592	0.416	0.778	0.028	0.395	0.167	0.200	0.309	0.140	0.099	0.293	0.235	0.165	0.310	0.706	0.018	0.363	0.055	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g28070	-0.643	0.006	0.142	0.594	0.161	0.395	-0.227	0.322	0.335	-0.963	0.007	0.208	-0.417	0.279	0.325	-0.230	0.114	0.363	0.072	0.423	0.444	-0.001	0.374	0.392	0.000	1.000	1.000
At4g28080	-0.523	0.028	0.238	-1.433	0.103	0.395	-0.612	0.062	0.278	-0.845	0.004	0.171	-0.845	0.057	0.264	-0.559	0.046	0.363	0.019	0.269	0.418	-0.522	0.105	0.378	-0.356	0.241	0.414
At4g28400	0.065	0.348	0.329	0.683	0.125	0.395	0.058	0.180	0.308	0.088	0.345	0.325	0.076	0.374	1.000	0.878	0.004	0.325	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g28490	-0.077	0.797	0.482	-0.413	0.343	0.412	0.348	0.052	0.270	0.349	0.028	0.249	0.329	0.152	0.310	0.619	0.008	0.363	0.021	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g28880	0.128	0.070	0.291	0.404	0.249	0.396	0.022	0.721	0.484	0.082	0.224	0.295	0.189	0.065	0.273	0.674	0.044	0.363	-0.025	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g29010	0.767	0.027	0.234	0.413	0.390	0.421	0.711	0.025	0.245	0.083	0.824	0.478	0.385	0.444	0.372	0.253	0.167	0.363	-0.027	0.374	1.000	-0.035	0.374	0.392	-0.138	0.374	0.415
At4g29270	0.688	0.038	0.261	0.194	0.275	0.398	0.004	0.363	1.000	0.777	0.068	0.291	0.234	0.265	0.320	-0.407	0.161	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g29520	0.160	0.170	0.295	-0.285	0.214	0.395	0.091	0.232	0.311	0.646	0.049	0.272	0.518	0.005	0.158	-0.157	0.272	0.372	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g29780	0.593	0.013	0.175	0.069	0.829	0.552	0.144	0.377	0.356	0.464	0.196	0.295	0.495	0.205	0.311	-0.222	0.670	0.493	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g29900	0.219	0.123	0.295	0.257	0.498	0.454	-0.465	0.003	0.166	0.210	0.050	0.273	0.246	0.392	0.354	0.646	0.015	0.363	-0.022	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g30120	0.042	0.784	0.478	0.360	0.353	0.414	0.134	0.243	0.312	-0.136	0.615	0.412	0.285	0.175	0.310	1.217	0.027	0.363	0.064	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g30410	-0.367	0.052	0.276	-0.679	0.057	0.395	-0.066	0.211	0.309	-0.647	0.016	0.246	-0.884	0.002	0.122	-0.659	0.024	0.363	-0.085	0.374	1.000	-0.175	0.229	0.378	0.000	1.000	1.000
At4g30460	-0.084	0.910	0.512	-0.106	0.885	0.567	1.571	0.018	0.237	1.129	0.124	0.295	1.347	0.050	0.252	1.382	0.040	0.363	0.450	0.058	0.418	-0.256	0.175	0.378	0.068	0.894	0.591
At4g30530	0.850	0.099	0.295	0.270	0.738	0.527	0.138	0.192	0.308	1.798	0.037	0.259	1.317	0.077	0.286	-0.897	0.113	0.363	0.029	0.374	1.000	-0.010	0.374	0.392	0.000	1.000	1.000
At4g30600	0.469	0.000	0.040	0.287	0.349	0.412	-0.247	0.281	0.323	0.756	0.030	0.250	0.959	0.004	0.138	0.597	0.176	0.363	-0.143	0.719	0.555	0.728	0.030	0.378	0.357	0.025	0.414
At4g30650	0.279	0.108	0.295	-1.138	0.046	0.395	0.207	0.326	0.336	0.749	0.045	0.266	1.153	0.080	0.286	0.020	0.955	0.576	0.077	0.374	1.000	0.427	0.084	0.378	0.390	0.285	0.414
At4g30660	0.289	0.158	0.295	-1.046	0.036	0.395	-0.113	0.406	0.366	0.858	0.014	0.240	0.430	0.405	0.358	-0.397	0.311	0.377	0.000	1.000	1.000	0.639	0.094	0.378	0.175	0.374	0.415
At4g30910	0.217	0.237	0.301	1.106	0.044	0.395	0.838	0.039	0.256	0.633	0.040	0.261	0.068	0.879	0.514	0.636	0.141	0.363	0.478	0.124	0.418	0.285	0.173	0.378	-0.164	0.244	0.414
At4g30920	1.072	0.127	0.295	0.944	0.235	0.395	1.739	0.002	0.166	2.570	0.006	0.189	0.890	0.121	0.302	1.343	0.090	0.363	0.297	0.569	0.500	0.248	0.374	0.392	0.000	1.000	1.000
At4g30950	-0.245	0.016	0.192	-0.068	0.886	0.568	-0.010	0.969	0.558	-0.132	0.388	0.337	0.021	0.786	0.490	-0.707	0.030	0.363	-0.397	0.052	0.418	0.025	0.921	0.530	-0.093	0.288	0.414
At4g30996	0.625	0.109	0.295	0.402	0.093	0.395	-0.171	0.404	0.365	0.562	0.178	0.295	0.591	0.045	0.247	-0.225	0.526	0.443	0.105	0.374	1.000	0.001	0.374	0.392	0.000	1.000	1.000
At4g31040	0.132	0.495	0.384	0.802	0.042	0.395	-0.079	0.728	0.486	0.111	0.682	0.436	0.202	0.355	0.345	-0.067	0.831	0.543	-0.016	0.374	1.000	-0.150	0.374	0.392	0.000	1.000	1.000
At4g31500	0.426	0.065	0.286	0.609	0.315	0.404	0.715	0.028	0.248	1.057	0.011	0.239	0.529	0.259	0.320	0.722	0.198	0.363	0.023	0.892	0.606	0.267	0.195	0.378	0.200	0.517	0.472
At4g31560	-0.041	0.658	0.438	0.307	0.207	0.395	-0.616	0.027	0.247	-0.408	0.014	0.239	-0.064	0.875	0.513	0.185	0.709	0.505	-0.276	0.066	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g31800	1.542	0.000	0.015	1.740	0.037	0.395	1.203	0.064	0.278	1.413	0.028	0.249	1.271	0.019	0.226	1.972	0.016	0.363	0.215	0.306	0.419	0.000	1.000	1.000	0.000	1.000	1.000
At4g31870	0.267	0.059	0.278	0.096	0.533	0.466	0.169	0.469	0.392	0.261	0.593	0.405	0.712	0.040	0.244	0.360	0.020	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g32060	0.331	0.069	0.290	0.798	0.006	0.395	0.163	0.343	0.343	0.280	0.173	0.295	0.655	0.010	0.199	-0.482	0.125	0.363	0.077	0.374	1.000	0.177	0.290	0.378	0.043	0.374	0.415
At4g32070	0.221	0.152	0.295	0.285	0.089	0.395	0.668	0.021	0.241	0.605	0.096	0.291	0.529	0.036	0.238	0.201	0.289	0.375	0.505	0.107	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g32175	-0.043	0.593	0.416	0.191	0.224	0.395	0.041	0.657	0.463	0.267	0.215	0.295	0.597	0.017	0.226	0.139	0.281	0.373	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g32410	0.672	0.019	0.205	-0.191	0.586	0.481	0.221	0.493	0.402	0.270	0.488	0.370	0.166	0.767	0.483	-0.050	0.902	0.564	0.479	0.181	0.418	-0.002	0.988	0.546	0.010	0.374	0.415
At4g33030	-0.004	0.974	0.528	0.526	0.108	0.395	0.646	0.046	0.266	-0.269	0.502	0.375	-0.103	0.750	0.477	0.142	0.814	0.538	0.371	0.181	0.418	-0.484	0.169	0.378	-0.056	0.374	0.415
At4g33040	0.137	0.308	0.317	-1.155	0.006	0.395	0.122	0.552	0.425	-0.228	0.464	0.361	-0.006	0.986	0.542	0.149	0.833	0.544	-0.417	0.056	0.418	0.033	0.848	0.513	-0.080	0.374	0.415
At4g33300	0.499	0.044	0.270	0.602	0.178	0.395	0.019	0.881	0.535	0.585	0.043	0.266	0.761	0.034	0.235	0.672	0.135	0.363	0.054	0.689	0.543	0.167	0.374	0.392	0.040	0.374	0.415
At4g33550	0.263	0.132	0.295	-0.508	0.227	0.395	-0.063	0.363	1.000	-0.064	0.867	0.490	0.446	0.379	0.351	-0.653	0.008	0.363	0.000	1.000	1.000	0.000	1.000	1.			

At4g36220	0.124	0.486	0.381	0.111	0.635	0.497	0.036	0.872	0.533	0.190	0.435	0.352	0.693	0.002	0.122	-0.018	0.959	0.578	-0.253	0.169	0.418	0.592	0.105	0.378	0.318	0.229	0.414
At4g36390	-0.210	0.242	0.302	-0.123	0.767	0.534	0.013	0.922	0.547	-0.268	0.327	0.321	-0.871	0.000	0.047	-0.026	0.937	0.573	-0.034	0.374	1.000	-0.013	0.374	0.392	0.000	1.000	1.000
At4g36500	0.257	0.053	0.276	1.521	0.047	0.395	0.154	0.466	0.391	0.106	0.206	0.295	0.035	0.374	1.000	0.849	0.217	0.363	-0.023	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g36530	-0.834	0.072	0.293	0.498	0.312	0.404	0.061	0.895	0.539	-0.605	0.028	0.249	0.283	0.763	0.482	-0.787	0.046	0.363	-0.369	0.674	0.539	0.216	0.336	0.385	-0.265	0.366	0.414
At4g37030	0.205	0.120	0.295	0.101	0.706	0.518	0.741	0.022	0.242	0.000	1.000	1.000	0.157	0.360	0.346	0.613	0.230	0.364	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At4g37270	0.813	0.002	0.087	1.603	0.002	0.395	-0.400	0.404	0.365	-0.091	0.645	0.424	0.789	0.057	0.264	0.242	0.664	0.491	-0.122	0.184	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At4g37370	0.696	0.001	0.075	0.905	0.016	0.395	0.451	0.038	0.256	0.287	0.524	0.382	0.183	0.667	0.452	0.403	0.233	0.364	0.099	0.374	1.000	0.004	0.374	0.392	0.000	1.000	1.000
At4g37470	-0.231	0.207	0.296	-0.328	0.221	0.395	-0.301	0.096	0.290	-0.196	0.204	0.295	-0.788	0.003	0.138	0.164	0.456	0.418	-0.057	0.374	1.000	-0.078	0.642	0.453	0.040	0.374	0.415
At4g37790	0.457	0.044	0.271	0.345	0.283	0.399	0.806	0.001	0.132	0.417	0.001	0.091	0.154	0.476	0.383	0.514	0.032	0.363	0.336	0.108	0.418	0.024	0.374	0.392	0.000	1.000	1.000
At4g37920	0.156	0.441	0.365	0.329	0.594	0.483	-0.289	0.266	0.318	0.180	0.313	0.318	-0.668	0.033	0.234	-0.293	0.228	0.364	0.173	0.126	0.418	-0.047	0.867	0.517	0.008	0.788	0.560
At4g38430	0.129	0.339	0.326	-0.842	0.025	0.395	0.003	0.993	0.562	-0.558	0.295	0.312	-0.113	0.725	0.469	-0.027	0.842	0.548	0.007	0.960	0.621	0.193	0.252	0.378	-0.004	0.374	0.415
At4g38710	0.717	0.033	0.254	0.906	0.036	0.395	0.329	0.121	0.304	0.448	0.214	0.295	0.284	0.259	0.319	0.218	0.592	0.466	0.057	0.613	0.518	0.316	0.179	0.378	0.000	1.000	1.000
At4g38770	-0.194	0.028	0.239	-0.702	0.170	0.395	0.038	0.843	0.524	-0.186	0.183	0.295	-0.025	0.868	0.511	-0.835	0.000	0.212	0.063	0.429	0.446	-0.061	0.073	0.378	0.003	0.992	0.614
At4g38810	0.052	0.563	0.407	-0.884	0.037	0.395	-0.006	0.986	0.561	-0.095	0.059	0.285	-0.123	0.684	0.458	0.125	0.710	0.505	-0.091	0.335	0.423	-0.030	0.320	0.382	-0.027	0.374	0.415
At4g38970	-0.623	0.021	0.207	-0.366	0.312	0.404	-1.262	0.027	0.247	-0.173	0.376	0.333	-0.705	0.078	0.286	-0.552	0.157	0.363	-0.512	0.052	0.418	0.265	0.698	0.476	0.202	0.406	0.425
At4g39260	0.082	0.878	0.503	0.988	0.212	0.395	1.952	0.002	0.163	1.548	0.080	0.291	1.202	0.130	0.305	0.961	0.368	0.391	0.546	0.374	1.000	0.230	0.621	0.451	-0.058	0.258	0.414
At4g39730	0.357	0.303	0.316	1.185	0.045	0.395	0.067	0.596	0.441	1.517	0.011	0.239	0.691	0.104	0.293	0.220	0.558	0.455	0.604	0.357	0.423	0.375	0.037	0.378	0.378	0.374	0.415
At4g39940	1.087	0.019	0.204	0.277	0.386	0.420	0.422	0.299	0.329	0.758	0.147	0.295	0.234	0.368	0.349	-0.264	0.507	0.436	0.291	0.390	0.432	-0.118	0.374	0.392	0.000	1.000	1.000
At5g01090	-0.172	0.315	0.319	-0.508	0.052	0.395	-0.253	0.028	0.248	-0.474	0.069	0.291	-0.655	0.014	0.224	-0.533	0.043	0.363	-0.334	0.265	0.418	-0.332	0.150	0.378	-0.018	0.374	0.415
At5g01530	-0.273	0.233	0.300	-0.342	0.673	0.507	-0.619	0.012	0.216	-0.705	0.095	0.291	-0.781	0.013	0.224	-2.184	0.002	0.314	-0.289	0.031	0.418	0.059	0.896	0.524	0.014	0.925	0.597
At5g01600	0.433	0.078	0.295	-0.362	0.455	0.442	-0.045	0.894	0.539	0.677	0.034	0.258	1.025	0.002	0.120	0.580	0.217	0.363	-0.061	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g01710	0.933	0.012	0.170	0.243	0.236	0.395	-0.339	0.085	0.287	0.465	0.001	0.091	0.512	0.359	0.346	0.125	0.807	0.536	-0.210	0.232	0.418	0.047	0.190	0.378	0.000	1.000	1.000
At5g01740	-0.121	0.811	0.485	-0.496	0.701	0.516	-1.258	0.023	0.243	0.209	0.457	0.360	0.036	0.847	0.506	-0.103	0.664	0.491	-0.501	0.213	0.418	0.000	1.000	1.000	-0.026	0.374	0.415
At5g02270	-0.324	0.204	0.296	-0.094	0.661	0.504	0.234	0.053	0.270	0.630	0.039	0.261	0.300	0.020	0.226	0.007	0.813	0.538	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g02540	-0.236	0.294	0.313	-0.217	0.145	0.395	-0.248	0.115	0.297	-0.061	0.891	0.496	-0.716	0.004	0.144	-0.206	0.316	0.378	0.000	1.000	1.000	-0.262	0.213	0.378	0.000	1.000	1.000
At5g02580	0.799	0.041	0.266	1.060	0.087	0.395	0.185	0.679	0.470	0.790	0.106	0.295	0.536	0.408	0.359	0.351	0.424	0.408	-0.025	0.837	0.594	0.321	0.248	0.378	0.000	1.000	1.000
At5g03210	0.390	0.187	0.295	-0.105	0.846	0.558	1.422	0.021	0.241	0.590	0.236	0.297	0.238	0.527	0.403	0.878	0.212	0.363	0.922	0.140	0.418	1.182	0.072	0.378	-0.280	0.228	0.414
At5g03280	-0.477	0.003	0.100	-0.728	0.142	0.395	-0.114	0.231	0.311	-0.245	0.053	0.277	-0.627	0.005	0.146	-0.365	0.235	0.364	0.002	0.987	0.629	-0.107	0.644	0.453	-0.044	0.259	0.414
At5g03300	-0.289	0.204	0.296	-0.154	0.587	0.481	-0.192	0.533	0.418	0.441	0.190	0.295	0.776	0.038	0.241	-0.358	0.624	0.477	0.057	0.187	0.418	-0.042	0.858	0.515	0.030	0.374	0.415
At5g03455	0.357	0.060	0.282	0.779	0.044	0.395	-0.137	0.685	0.472	0.319	0.298	0.313	0.272	0.586	0.423	0.716	0.115	0.363	0.042	0.374	1.000	0.092	0.374	0.392	0.000	1.000	1.000
At5g03610	0.327	0.036	0.260	-0.058	0.844	0.557	0.000	1.000	1.000	0.365	0.115	0.295	0.698	0.019	0.226	0.011	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g03660	0.247	0.316	0.320	0.361	0.156	0.395	0.668	0.118	0.303	0.309	0.516	0.379	0.786	0.033	0.234	0.824	0.273	0.372	-0.350	0.119	0.418	-0.056	0.346	0.386	-0.258	0.560	0.482
At5g03710	0.552	0.026	0.229	0.416	0.370	0.416	0.400	0.047	0.266	0.694	0.020	0.247	0.236	0.624	0.438	0.979	0.017	0.363	0.634	0.102	0.418	-0.057	0.754	0.494	0.257	0.214	0.414
At5g03760	0.084	0.689	0.449	-0.060	0.926	0.579	0.582	0.030	0.248	0.180	0.490	0.371	0.271	0.383	0.352	-1.244	0.045	0.363	-0.137	0.355	0.423	-0.108	0.374	0.392	0.080	0.374	0.415
At5g04040	0.096	0.314	0.319	0.247	0.623	0.494	-0.938	0.024	0.243	0.000	1.000	1.000	0.000	1.000	1.000	-0.169	0.612	0.473	-0.003	0.666	0.538	0.000	1.000	1.000	0.000	1.000	1.000
At5g04140	-0.190	0.210	0.296	-0.026	0.958	0.586	-0.093	0.416	0.370	-0.237	0.249	0.299	-0.711	0.030	0.230	-0.189	0.378	0.394	-0.039	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g04590	-0.021	0.837	0.492	0.032	0.808	0.546	-0.321	0.007	0.200	-0.325	0.054	0.279	-0.592	0.026	0.226	-0.577	0.003	0.315	-0.420	0.051	0.418	-0.179	0.563	0.437	-0.286	0.116	0.414
At5g04810	-0.675	0.010	0.166	0.180	0.258	0.396	-0.298	0.307	0.331	-0.224	0.384	0.336	-0.506	0.083	0.286	-0.072	0.426	0.409	0.071	0.290	0.419	-0.138	0.196	0.378	0.000	1.000	1.000
At5g04930	0.678	0.010	0.166	0.131	0.758	0.532	0.525	0.023	0.242	0.344	0.091	0.291	0.264	0.303	0.330	0.373	0.458	0.418	0.254	0.374	1.000	0.051	0.374	0.392	0.000	1.000	1.000
At5g05170	-0.076	0.387	0.344	-0.792	0.112	0.395	-0.319	0.173	0.308	-0.164	0.210	0.295	-0.004	0.991	0.543	-0.626	0.044	0.363	-0.351	0.083	0.418	-0.326	0.080	0.378	-0.069	0.781	0.557
At5g05600	1.101	0.004	0.114	1.323	0.028	0.395	0.928	0.003	0.171	2.381	0.003	0.155	2.313	0.003	0.134	0.094	0.551	0.453	0.000	1.000	1.000	0.326	0.195	0.378	0.045	0.374	0.415
At5g05850	0.093	0.310	0.318	0.036	0.934	0.580	0.103	0.495	0.403	-0.130	0.249	0.299	0.142	0.441	0.371	0.689	0.022	0.363	0.187	0.179	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g06250	-0.134	0.492	0.383	0.236	0.163	0.395	-0.049	0.662	0.465	0.185	0.335	0.324	-0.065	0.178	0.310	0.675	0.019	0.363	0.006	0.374	1.000	0.000	1.000	1.			

At5g07270	0.364	0.030	0.244	0.187	0.534	0.466	0.466	0.012	0.216	0.129	0.758	0.461	0.821	0.016	0.226	0.185	0.246	0.364	0.079	0.374	1.000	0.072	0.623	0.452	0.000	1.000	1.000
At5g07350	0.172	0.482	0.379	0.774	0.099	0.395	0.791	0.064	0.278	1.198	0.160	0.295	0.919	0.032	0.233	0.253	0.716	0.507	-0.129	0.374	1.000	-0.067	0.374	0.392	0.000	1.000	1.000
At5g07650	0.675	0.018	0.198	0.603	0.254	0.396	0.971	0.002	0.163	0.896	0.013	0.239	0.632	0.198	0.310	0.526	0.260	0.368	0.230	0.306	0.419	0.006	0.962	0.542	0.072	0.331	0.414
At5g08290	-0.438	0.249	0.303	-0.058	0.899	0.570	-0.108	0.606	0.445	0.051	0.925	0.504	0.627	0.227	0.313	0.914	0.043	0.363	0.191	0.653	0.534	0.241	0.200	0.378	0.092	0.589	0.497
At5g08300	-0.025	0.867	0.500	0.473	0.211	0.395	0.515	0.026	0.246	0.655	0.017	0.246	0.364	0.004	0.138	0.484	0.163	0.363	-0.003	0.959	0.621	-0.111	0.437	0.404	0.008	0.374	0.415
At5g08380	0.063	0.506	0.387	0.681	0.082	0.395	0.718	0.005	0.200	0.059	0.197	0.295	0.342	0.080	0.286	0.381	0.202	0.363	0.077	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g08520	0.043	0.849	0.496	-0.010	0.973	0.589	-0.480	0.004	0.187	-1.078	0.012	0.239	-0.622	0.025	0.226	0.353	0.175	0.363	-0.125	0.358	0.423	-0.009	0.374	0.392	-0.054	0.374	0.415
At5g08560	0.713	0.048	0.274	0.276	0.801	0.544	-0.234	0.591	0.440	0.437	0.359	0.329	0.220	0.701	0.463	0.295	0.750	0.519	-0.011	0.374	1.000	-0.153	0.387	0.395	0.000	1.000	1.000
At5g09290	0.750	0.038	0.261	-0.151	0.638	0.497	0.553	0.149	0.308	0.775	0.106	0.295	0.230	0.660	0.450	0.297	0.182	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g09490	-0.320	0.416	0.355	1.574	0.033	0.395	1.132	0.041	0.259	0.490	0.544	0.389	0.719	0.278	0.324	0.771	0.119	0.363	0.563	0.263	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g09960	0.664	0.000	0.034	0.435	0.047	0.395	-0.433	0.026	0.246	0.439	0.079	0.291	0.593	0.075	0.285	0.587	0.220	0.363	-0.202	0.210	0.418	0.320	0.217	0.378	0.058	0.414	0.426
At5g10410	0.353	0.083	0.295	0.578	0.043	0.395	0.676	0.035	0.254	0.599	0.101	0.293	0.514	0.131	0.305	1.155	0.245	0.364	0.006	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g10450	-0.312	0.164	0.295	-0.096	0.655	0.502	-0.557	0.015	0.232	-0.045	0.781	0.467	-0.650	0.037	0.241	0.091	0.701	0.503	0.064	0.775	0.574	0.060	0.601	0.447	-0.266	0.368	0.415
At5g11430	0.092	0.292	0.313	0.549	0.005	0.395	0.911	0.038	0.256	1.012	0.029	0.249	1.025	0.001	0.083	0.462	0.207	0.363	-0.014	0.947	0.617	0.269	0.376	0.393	-0.013	0.631	0.503
At5g11650	0.352	0.119	0.295	-0.566	0.231	0.395	-0.249	0.250	0.313	0.032	0.831	0.480	0.726	0.039	0.243	0.187	0.178	0.363	0.109	0.055	0.418	-0.017	0.374	0.392	0.012	0.374	0.415
At5g11970	0.530	0.000	0.002	0.528	0.161	0.395	0.112	0.501	0.406	0.566	0.044	0.266	0.428	0.040	0.244	0.889	0.006	0.359	0.223	0.207	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g12020	0.423	0.004	0.114	0.893	0.077	0.395	0.925	0.004	0.187	0.367	0.034	0.258	0.784	0.019	0.226	-0.018	0.935	0.572	0.398	0.270	0.418	0.342	0.470	0.410	0.327	0.374	0.415
At5g12140	0.723	0.000	0.035	0.130	0.366	0.416	0.207	0.292	0.325	0.297	0.008	0.221	0.104	0.680	0.456	0.006	0.988	0.585	-0.162	0.620	0.521	0.256	0.293	0.378	0.082	0.351	0.414
At5g12190	0.237	0.051	0.276	0.414	0.044	0.395	0.178	0.030	0.248	0.127	0.390	0.337	0.181	0.084	0.286	0.628	0.046	0.363	0.096	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g13110	0.287	0.146	0.295	0.867	0.006	0.395	0.021	0.948	0.554	0.224	0.498	0.374	-0.024	0.953	0.535	0.585	0.002	0.307	-0.013	0.875	0.601	0.106	0.285	0.378	0.000	1.000	1.000
At5g13180	0.299	0.033	0.252	0.145	0.628	0.495	-0.327	0.468	0.392	0.929	0.031	0.250	0.063	0.870	0.512	0.158	0.764	0.524	-0.152	0.583	0.506	0.108	0.259	0.378	-0.046	0.234	0.414
At5g13190	1.082	0.003	0.111	1.678	0.050	0.395	0.251	0.421	0.372	0.544	0.016	0.246	0.526	0.041	0.246	0.936	0.271	0.372	0.004	0.374	1.000	-0.011	0.374	0.392	0.000	1.000	1.000
At5g13420	0.332	0.063	0.285	1.251	0.040	0.395	-0.181	0.357	0.349	-0.169	0.746	0.457	-0.209	0.470	0.382	-0.122	0.741	0.516	0.163	0.578	0.505	-0.020	0.374	0.392	0.313	0.374	0.415
At5g13630	-0.703	0.010	0.166	0.205	0.793	0.542	-0.119	0.830	0.520	-0.813	0.225	0.295	-2.219	0.016	0.226	-0.230	0.684	0.497	-0.908	0.154	0.418	0.636	0.396	0.397	1.494	0.045	0.414
At5g13690	0.490	0.036	0.260	0.426	0.180	0.395	0.098	0.148	0.308	0.756	0.035	0.258	0.695	0.051	0.254	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g13770	-0.032	0.736	0.463	-0.394	0.637	0.497	-0.248	0.198	0.308	-0.482	0.134	0.295	-0.134	0.584	0.423	-1.158	0.016	0.363	-0.013	0.927	0.614	-0.252	0.178	0.378	0.192	0.374	0.415
At5g13930	-1.246	0.024	0.222	-1.373	0.209	0.395	0.390	0.491	0.401	-0.690	0.105	0.295	-1.401	0.043	0.247	-1.333	0.151	0.363	-0.736	0.049	0.418	-0.370	0.159	0.378	0.015	0.901	0.591
At5g15410	-0.518	0.011	0.169	-1.014	0.031	0.395	-0.324	0.003	0.166	-0.269	0.083	0.291	-0.428	0.081	0.286	-0.164	0.480	0.426	-0.253	0.095	0.418	-0.062	0.546	0.432	0.038	0.374	0.415
At5g15440	-0.640	0.036	0.260	0.119	0.531	0.466	-0.038	0.860	0.529	-0.152	0.340	0.324	0.126	0.639	0.443	0.159	0.562	0.456	0.228	0.135	0.418	0.103	0.578	0.442	-0.185	0.298	0.414
At5g15490	0.431	0.121	0.295	0.205	0.337	0.411	0.426	0.109	0.297	0.959	0.075	0.291	1.330	0.009	0.189	0.137	0.727	0.511	0.000	1.000	1.000	0.345	0.059	0.378	0.031	0.374	0.415
At5g15540	-0.048	0.818	0.487	-0.898	0.024	0.395	-0.163	0.349	0.345	-0.173	0.142	0.295	-0.633	0.021	0.226	-0.085	0.672	0.493	-0.170	0.518	0.478	-0.040	0.390	0.396	0.000	1.000	1.000
At5g15820	0.406	0.316	0.320	-0.099	0.775	0.536	0.869	0.013	0.224	0.572	0.116	0.295	0.092	0.374	1.000	0.774	0.095	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g15880	0.175	0.122	0.295	0.397	0.197	0.395	0.765	0.020	0.241	-0.318	0.175	0.295	0.094	0.334	0.338	0.459	0.110	0.363	-0.046	0.703	0.548	0.000	1.000	1.000	0.000	1.000	1.000
At5g16310	0.097	0.472	0.375	0.019	0.961	0.586	0.664	0.017	0.236	0.549	0.053	0.277	0.712	0.031	0.233	0.161	0.545	0.451	0.000	1.000	1.000	0.120	0.368	0.391	0.000	1.000	1.000
At5g16930	0.231	0.225	0.299	-0.154	0.772	0.535	0.224	0.040	0.256	0.336	0.132	0.295	0.661	0.046	0.248	-0.066	0.800	0.534	0.132	0.500	0.472	0.000	1.000	1.000	0.000	1.000	1.000
At5g17000	0.808	0.002	0.091	-0.070	0.902	0.571	0.411	0.012	0.216	1.567	0.000	0.024	1.899	0.001	0.075	-0.064	0.749	0.519	-0.028	0.374	1.000	0.414	0.080	0.378	0.350	0.124	0.414
At5g17670	-0.588	0.003	0.112	-0.978	0.212	0.395	-0.415	0.157	0.308	-0.305	0.016	0.246	-0.483	0.033	0.233	-0.816	0.046	0.363	-0.118	0.134	0.418	-0.057	0.828	0.508	-0.012	0.890	0.591
At5g17870	-0.398	0.001	0.083	-0.038	0.887	0.568	-0.087	0.288	0.325	-0.111	0.178	0.295	-0.152	0.356	0.345	-0.691	0.012	0.363	-0.083	0.211	0.418	-0.101	0.453	0.407	0.004	0.374	0.415
At5g17920	-0.614	0.005	0.130	-0.537	0.195	0.395	-0.165	0.076	0.285	-0.068	0.702	0.443	-0.311	0.027	0.226	-0.610	0.036	0.363	-0.449	0.008	0.418	-0.077	0.635	0.453	-0.325	0.192	0.414
At5g17990	0.612	0.004	0.114	0.332	0.012	0.395	0.334	0.042	0.261	0.223	0.537	0.386	0.262	0.129	0.305	0.167	0.317	0.379	0.030	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g18050	0.212	0.612	0.424	-0.660	0.013	0.395	-0.698	0.167	0.308	-0.849	0.199	0.295	-0.753	0.118	0.300	-0.401	0.632	0.480	-0.207	0.185	0.418	-0.319	0.402	0.397	0.010	0.961	0.609
At5g18600	0.020	0.859	0.498	0.780	0.049	0.395	-0.033	0.661	0.465	0.270	0.443	0.355	0.547	0.066	0.273	0.321	0.464	0.420	-0.015	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g18640	0.162	0.582	0.413	0.696	0.046	0.395	0.108	0.744	0.491	-0.282	0.405	0.342	0.278	0.509	0.395	0.274	0.465	0.421	0.740	0.026	0.418	-0.077	0.374	0.			

At5g19690	0.197	0.274	0.307	0.030	0.949	0.584	0.663	0.026	0.246	0.691	0.074	0.291	0.835	0.014	0.224	0.284	0.618	0.476	0.182	0.374	1.000	-0.059	0.374	0.392	0.000	1.000	1.000
At5g20230	1.660	0.000	0.067	1.738	0.076	0.395	0.341	0.259	0.315	1.056	0.189	0.295	0.640	0.023	0.226	0.900	0.241	0.364	0.094	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g20280	-0.330	0.072	0.293	-0.597	0.004	0.395	-0.219	0.385	0.359	-0.386	0.035	0.258	-0.357	0.011	0.213	-0.320	0.102	0.363	-0.267	0.187	0.418	-0.117	0.390	0.396	-0.173	0.374	0.415
At5g20700	-0.218	0.289	0.312	-0.972	0.013	0.395	-0.596	0.070	0.285	0.099	0.759	0.461	-0.097	0.791	0.491	-0.617	0.201	0.363	-0.948	0.035	0.418	0.025	0.915	0.529	-0.258	0.548	0.477
At5g20720	0.488	0.210	0.296	0.198	0.765	0.533	0.442	0.450	0.385	0.952	0.007	0.211	0.692	0.157	0.310	0.373	0.090	0.363	0.038	0.374	1.000	0.283	0.309	0.379	0.028	0.374	0.415
At5g20840	-0.003	0.988	0.531	-0.408	0.267	0.396	-0.082	0.746	0.492	0.336	0.080	0.291	0.117	0.591	0.426	0.831	0.034	0.363	0.171	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g21940	0.827	0.011	0.169	0.329	0.424	0.431	-0.397	0.117	0.301	0.019	0.889	0.495	-0.031	0.374	1.000	-0.319	0.611	0.473	-0.046	0.849	0.594	0.000	1.000	1.000	0.000	1.000	1.000
At5g22270	0.172	0.085	0.295	0.735	0.027	0.395	0.163	0.086	0.288	0.064	0.225	0.295	0.000	1.000	1.000	0.767	0.023	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g23130	0.499	0.078	0.295	0.056	0.543	0.469	0.040	0.662	0.465	0.185	0.426	0.350	0.670	0.004	0.140	-0.063	0.717	0.507	0.023	0.247	0.418	0.017	0.374	0.392	0.000	1.000	1.000
At5g23340	-0.288	0.172	0.295	-1.193	0.020	0.395	-0.253	0.203	0.309	-0.810	0.005	0.182	-0.748	0.020	0.226	-0.287	0.447	0.415	-0.178	0.264	0.418	-0.044	0.374	0.392	0.000	1.000	1.000
At5g23920	-0.061	0.776	0.477	-0.399	0.448	0.440	-0.313	0.276	0.321	-0.446	0.004	0.171	-0.267	0.115	0.298	-0.724	0.026	0.363	-0.122	0.224	0.418	-0.120	0.528	0.427	0.122	0.374	0.415
At5g23950	0.551	0.024	0.222	0.634	0.265	0.396	0.207	0.158	0.308	0.707	0.009	0.225	1.203	0.001	0.081	0.272	0.594	0.467	0.141	0.374	1.000	0.834	0.075	0.378	0.300	0.310	0.414
At5g24313	0.905	0.015	0.188	0.489	0.071	0.395	0.920	0.064	0.278	-0.109	0.489	0.371	0.575	0.077	0.286	0.605	0.234	0.364	0.044	0.934	0.614	0.010	0.917	0.529	0.096	0.374	0.415
At5g24316	0.316	0.013	0.182	-0.092	0.661	0.504	0.599	0.017	0.235	-0.113	0.755	0.460	0.484	0.059	0.268	0.383	0.050	0.363	-0.511	0.254	0.418	0.119	0.374	0.392	-0.039	0.374	0.415
At5g24490	0.169	0.498	0.385	0.614	0.260	0.396	0.426	0.246	0.313	-0.390	0.539	0.387	-1.079	0.190	0.310	-1.164	0.026	0.363	0.137	0.705	0.549	-0.290	0.285	0.378	-0.039	0.199	0.414
At5g24530	0.325	0.401	0.350	0.345	0.253	0.396	0.812	0.041	0.259	1.486	0.040	0.263	1.310	0.024	0.226	0.341	0.347	0.385	0.040	0.374	1.000	0.213	0.374	0.392	0.000	1.000	1.000
At5g24770	1.877	0.000	0.045	1.533	0.150	0.395	1.115	0.043	0.261	1.519	0.014	0.241	2.520	0.017	0.226	-0.072	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g24810	0.233	0.535	0.399	0.158	0.749	0.530	-0.252	0.146	0.308	0.618	0.005	0.182	0.679	0.016	0.226	0.385	0.481	0.426	0.055	0.374	1.000	0.064	0.374	0.392	0.000	1.000	1.000
At5g25130	0.879	0.008	0.158	0.182	0.666	0.505	0.679	0.078	0.285	0.533	0.128	0.295	0.490	0.316	0.334	-0.099	0.642	0.484	0.085	0.279	0.418	-0.112	0.189	0.378	0.000	1.000	1.000
At5g25320	0.360	0.136	0.295	0.933	0.021	0.395	0.042	0.930	0.549	0.422	0.085	0.291	-0.092	0.513	0.396	-0.370	0.225	0.364	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g25350	-0.224	0.308	0.317	-0.743	0.017	0.395	-0.442	0.169	0.308	-0.485	0.037	0.259	-0.374	0.303	0.330	-0.943	0.018	0.363	-0.559	0.184	0.418	0.139	0.499	0.419	-0.465	0.188	0.414
At5g25610	-0.684	0.007	0.149	-1.323	0.106	0.395	-0.764	0.007	0.200	-0.423	0.113	0.295	-0.735	0.002	0.122	-1.027	0.073	0.363	-0.756	0.020	0.418	-0.159	0.312	0.380	-0.650	0.182	0.414
At5g25630	-0.013	0.924	0.515	-0.325	0.297	0.402	-0.425	0.004	0.188	-0.866	0.026	0.249	-1.014	0.003	0.138	-0.399	0.102	0.363	-0.374	0.299	0.419	-0.520	0.034	0.378	0.007	0.938	0.602
At5g26000	0.531	0.081	0.295	0.106	0.859	0.561	0.331	0.309	0.331	0.415	0.238	0.297	0.965	0.009	0.189	-1.146	0.017	0.363	0.079	0.374	1.000	0.011	0.374	0.392	0.000	1.000	1.000
At5g26667	0.301	0.501	0.386	-0.289	0.331	0.409	-0.226	0.748	0.492	-1.325	0.228	0.295	2.379	0.008	0.180	-0.081	0.915	0.567	0.000	1.000	1.000	0.486	0.268	0.378	0.000	1.000	1.000
At5g26960	0.509	0.030	0.244	0.277	0.082	0.395	0.995	0.055	0.274	0.300	0.600	0.408	1.003	0.042	0.247	0.783	0.014	0.363	0.420	0.374	1.000	0.040	0.374	0.392	0.000	1.000	1.000
At5g27380	0.659	0.004	0.114	1.232	0.013	0.395	0.275	0.123	0.305	0.405	0.134	0.295	1.017	0.000	0.064	0.817	0.104	0.363	0.306	0.466	0.462	0.181	0.445	0.404	0.214	0.209	0.414
At5g28237	0.406	0.016	0.192	1.412	0.061	0.395	0.067	0.700	0.478	0.868	0.044	0.266	0.138	0.789	0.491	0.001	0.990	0.585	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g28540	0.751	0.006	0.138	1.548	0.020	0.395	1.709	0.003	0.166	1.925	0.001	0.123	1.777	0.003	0.138	0.426	0.361	0.389	0.012	0.374	1.000	0.674	0.124	0.378	0.220	0.242	0.414
At5g28610	0.749	0.028	0.239	-0.042	0.930	0.580	0.012	0.971	0.559	0.226	0.600	0.408	-0.439	0.192	0.310	0.824	0.103	0.363	0.345	0.484	0.466	-0.033	0.912	0.529	-0.034	0.374	0.415
At5g28740	0.290	0.163	0.295	0.266	0.449	0.440	0.395	0.140	0.308	0.165	0.823	0.478	1.210	0.017	0.226	-0.002	0.977	0.582	0.062	0.374	1.000	0.151	0.374	0.392	-0.045	0.374	0.415
At5g28830	0.195	0.016	0.193	-0.064	0.668	0.506	0.163	0.232	0.311	0.326	0.378	0.334	0.656	0.034	0.235	0.231	0.127	0.363	0.025	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g33290	-0.033	0.179	0.295	0.107	0.690	0.514	-0.179	0.111	0.297	0.000	1.000	1.000	0.000	1.000	1.000	0.784	0.012	0.363	-0.085	0.358	0.423	0.000	1.000	1.000	0.000	1.000	1.000
At5g35160	0.551	0.063	0.285	0.503	0.191	0.395	0.020	0.754	0.495	0.712	0.034	0.257	0.502	0.094	0.290	0.576	0.148	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g35180	-0.122	0.538	0.399	-0.182	0.443	0.437	0.029	0.943	0.553	-0.598	0.039	0.261	-0.499	0.165	0.310	0.024	0.881	0.559	-0.205	0.374	1.000	-0.102	0.201	0.378	0.114	0.374	0.415
At5g35416	0.659	0.036	0.260	0.318	0.128	0.395	0.247	0.175	0.308	0.464	0.180	0.295	0.151	0.374	1.000	0.014	0.374	1.000	0.366	0.178	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g35910	-0.086	0.747	0.467	0.006	0.981	0.591	-0.288	0.389	0.361	0.199	0.346	0.326	0.054	0.848	0.506	0.728	0.019	0.363	-0.251	0.204	0.418	-0.063	0.374	0.392	0.000	1.000	1.000
At5g36700	0.268	0.408	0.352	-0.344	0.773	0.536	0.235	0.131	0.308	0.009	0.979	0.519	-0.091	0.718	0.467	0.627	0.014	0.363	-1.174	0.074	0.418	-0.389	0.068	0.378	0.466	0.382	0.418
At5g36910	0.957	0.008	0.153	0.505	0.397	0.422	0.016	0.363	1.000	0.347	0.057	0.281	0.340	0.063	0.273	-0.230	0.195	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g36950	-0.184	0.200	0.295	-1.252	0.001	0.385	0.063	0.805	0.511	0.000	1.000	1.000	-0.001	0.374	1.000	-1.108	0.001	0.248	-0.235	0.287	0.419	0.000	1.000	1.000	0.000	1.000	1.000
At5g37070	0.735	0.004	0.114	0.871	0.091	0.395	0.337	0.100	0.291	0.688	0.032	0.252	0.452	0.118	0.300	1.141	0.035	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g37600	0.317	0.065	0.287	0.312	0.232	0.395	0.424	0.254	0.314	-0.048	0.742	0.456	0.495	0.032	0.233	0.701	0.011	0.363	-0.079	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g37740	0.218	0.360	0.333	0.373	0.322	0.408	0.534	0.024	0.245	-0.258	0.067	0.291	0.300	0.399	0.356	0.963	0.000	0.033	0.099	0.374	1.000	0.000	1.000	1.			

At5g40770	0.097	0.794	0.481	0.232	0.094	0.395	0.610	0.360	0.350	1.027	0.027	0.249	0.533	0.296	0.328	-0.208	0.489	0.430	-0.252	0.454	0.458	-0.291	0.435	0.403	0.280	0.233	0.414
At5g41460	-0.082	0.586	0.415	-0.828	0.121	0.395	0.190	0.172	0.308	0.066	0.658	0.428	0.036	0.863	0.510	-0.688	0.050	0.363	0.000	1.000	1.000	0.022	0.633	0.453	0.000	1.000	1.000
At5g41520	0.279	0.020	0.207	0.467	0.037	0.395	0.651	0.000	0.034	0.283	0.195	0.295	0.347	0.007	0.180	0.521	0.030	0.363	0.108	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g41790	-0.781	0.035	0.259	-0.240	0.666	0.505	-0.495	0.428	0.375	-0.107	0.309	0.318	-1.169	0.221	0.311	0.085	0.882	0.559	-0.638	0.253	0.418	-0.468	0.339	0.386	0.456	0.190	0.414
At5g41950	0.037	0.774	0.476	0.172	0.551	0.472	-0.109	0.607	0.445	0.188	0.402	0.340	0.176	0.260	0.320	0.658	0.022	0.363	-0.118	0.105	0.418	0.084	0.602	0.447	-0.016	0.374	0.415
At5g42635	1.114	0.004	0.119	1.019	0.038	0.395	1.119	0.000	0.070	0.889	0.004	0.171	1.022	0.009	0.192	0.445	0.135	0.363	0.206	0.406	0.439	0.083	0.414	0.399	-0.108	0.374	0.415
At5g42680	-0.083	0.255	0.303	-0.654	0.033	0.395	-0.159	0.302	0.330	-0.486	0.074	0.291	-0.077	0.676	0.455	-0.477	0.216	0.363	0.000	1.000	1.000	-0.096	0.374	0.392	0.000	1.000	1.000
At5g42765	0.516	0.045	0.271	0.655	0.118	0.395	1.043	0.016	0.234	0.388	0.214	0.295	-0.231	0.546	0.409	0.024	0.925	0.570	0.070	0.191	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g43060	-0.041	0.714	0.456	-0.358	0.438	0.436	0.106	0.705	0.479	-0.225	0.161	0.295	-1.005	0.040	0.244	-0.377	0.383	0.395	0.039	0.374	1.000	0.000	1.000	1.000	-0.120	0.374	0.415
At5g43210	0.008	0.961	0.525	-0.537	0.324	0.408	-0.019	0.929	0.549	-0.079	0.856	0.486	-0.637	0.029	0.230	0.397	0.344	0.384	0.066	0.685	0.542	0.028	0.837	0.511	0.000	1.000	1.000
At5g43830	0.380	0.189	0.295	0.939	0.041	0.395	-0.118	0.460	0.389	0.448	0.040	0.261	0.331	0.047	0.249	-0.306	0.454	0.417	0.235	0.316	0.421	0.266	0.330	0.385	0.089	0.283	0.414
At5g43880	1.167	0.004	0.114	0.594	0.335	0.410	-0.779	0.067	0.281	0.856	0.002	0.133	0.170	0.729	0.470	0.672	0.129	0.363	-0.600	0.082	0.418	0.279	0.339	0.386	0.135	0.184	0.414
At5g43970	-0.143	0.481	0.378	0.448	0.248	0.395	-0.048	0.863	0.530	0.290	0.186	0.295	0.838	0.016	0.226	0.184	0.446	0.414	-0.208	0.488	0.468	0.173	0.214	0.378	0.029	0.374	0.415
At5g44005	1.554	0.000	0.027	0.876	0.218	0.395	0.359	0.221	0.310	0.651	0.049	0.271	1.020	0.032	0.233	0.699	0.384	0.395	-0.022	0.950	0.618	0.022	0.374	0.392	0.000	1.000	1.000
At5g44250	-0.136	0.167	0.295	-0.594	0.013	0.395	-0.250	0.097	0.290	-0.337	0.135	0.295	-0.109	0.143	0.310	-0.045	0.919	0.568	0.182	0.374	1.000	0.029	0.374	0.392	0.000	1.000	1.000
At5g44420	0.241	0.351	0.330	1.202	0.076	0.395	1.478	0.007	0.200	0.250	0.530	0.384	0.478	0.134	0.307	1.615	0.054	0.363	0.994	0.048	0.418	0.177	0.436	0.403	0.242	0.093	0.414
At5g44680	-0.373	0.118	0.295	-0.132	0.692	0.514	-0.121	0.562	0.429	-0.770	0.027	0.249	-0.946	0.013	0.223	-0.344	0.130	0.363	0.010	0.374	1.000	-0.071	0.374	0.392	0.000	1.000	1.000
At5g45280	0.353	0.054	0.277	0.086	0.799	0.543	-0.109	0.061	0.277	0.730	0.010	0.239	1.006	0.001	0.083	-0.281	0.486	0.428	-0.008	0.374	1.000	0.132	0.080	0.378	0.000	1.000	1.000
At5g45340	1.477	0.002	0.087	1.390	0.131	0.395	-0.236	0.526	0.415	0.169	0.173	0.295	1.085	0.031	0.233	0.600	0.522	0.441	0.002	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g45410	0.527	0.009	0.165	0.137	0.789	0.540	1.027	0.007	0.200	1.110	0.012	0.239	0.566	0.230	0.313	0.005	0.976	0.582	-0.056	0.374	1.000	-0.071	0.569	0.440	0.000	1.000	1.000
At5g46110	-0.394	0.035	0.259	-0.355	0.414	0.428	-0.408	0.053	0.270	-0.466	0.045	0.266	-0.313	0.079	0.286	-0.863	0.014	0.363	0.077	0.374	1.000	-0.199	0.352	0.386	0.004	0.374	0.415
At5g46295	0.509	0.051	0.276	0.662	0.256	0.396	1.061	0.033	0.253	0.000	1.000	1.000	0.000	1.000	1.000	0.464	0.502	0.435	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g46430	-0.139	0.406	0.351	-0.015	0.961	0.586	0.621	0.012	0.216	0.172	0.368	0.332	0.298	0.290	0.327	0.030	0.765	0.524	0.047	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g46580	-0.601	0.015	0.188	0.416	0.328	0.408	0.082	0.663	0.465	-0.493	0.144	0.295	-0.141	0.426	0.366	-0.265	0.459	0.418	-0.489	0.038	0.418	0.148	0.416	0.399	-0.120	0.091	0.414
At5g46800	-0.242	0.198	0.295	-0.129	0.687	0.512	-0.372	0.269	0.319	-0.383	0.103	0.295	-0.239	0.221	0.311	-0.914	0.033	0.363	-0.554	0.058	0.418	0.018	0.960	0.541	-0.097	0.184	0.414
At5g46860	0.506	0.010	0.166	0.167	0.537	0.467	0.192	0.264	0.317	0.657	0.046	0.266	0.000	1.000	1.000	0.065	0.821	0.540	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g47030	0.114	0.337	0.326	0.647	0.026	0.395	0.164	0.564	0.430	0.289	0.008	0.220	0.116	0.675	0.455	0.154	0.338	0.384	0.291	0.267	0.418	0.036	0.868	0.518	-0.039	0.483	0.458
At5g47200	0.586	0.043	0.268	0.122	0.234	0.395	0.566	0.195	0.308	0.594	0.495	0.373	0.674	0.283	0.325	0.552	0.024	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g47240	0.850	0.001	0.074	1.105	0.043	0.395	0.031	0.363	1.000	1.108	0.013	0.239	1.542	0.010	0.199	0.455	0.241	0.364	0.000	1.000	1.000	0.080	0.374	0.392	0.000	1.000	1.000
At5g48380	0.805	0.086	0.295	0.422	0.338	0.411	0.089	0.731	0.487	1.385	0.008	0.220	1.492	0.008	0.185	0.716	0.290	0.375	0.006	0.960	0.621	0.881	0.077	0.378	0.250	0.192	0.414
At5g48540	0.978	0.009	0.165	1.077	0.062	0.395	0.870	0.001	0.162	0.841	0.010	0.235	1.044	0.004	0.144	0.269	0.615	0.474	0.141	0.374	1.000	0.220	0.118	0.378	0.103	0.374	0.415
At5g48655	0.248	0.036	0.260	0.832	0.043	0.395	0.060	0.522	0.413	0.123	0.618	0.413	0.381	0.078	0.286	0.610	0.109	0.363	0.087	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g48790	-0.043	0.787	0.479	0.341	0.723	0.522	0.058	0.779	0.503	0.049	0.833	0.481	-0.239	0.091	0.289	-1.020	0.011	0.363	-0.018	0.374	1.000	-0.407	0.259	0.378	0.359	0.374	0.415
At5g48850	1.315	0.000	0.002	0.795	0.401	0.424	0.155	0.706	0.479	1.271	0.027	0.249	1.235	0.015	0.226	-0.436	0.271	0.372	0.572	0.230	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g48900	-0.187	0.110	0.295	-0.678	0.157	0.395	0.000	1.000	1.000	-0.065	0.374	1.000	-0.407	0.068	0.280	-0.700	0.019	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g48960	0.177	0.001	0.082	0.882	0.133	0.395	0.769	0.048	0.266	0.477	0.042	0.266	0.395	0.544	0.408	-0.445	0.318	0.379	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g49460	-0.331	0.022	0.212	-0.620	0.071	0.395	-0.009	0.278	0.322	-0.143	0.251	0.299	-0.311	0.053	0.256	-0.817	0.018	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g49700	0.511	0.006	0.148	0.219	0.305	0.403	0.210	0.305	0.330	0.611	0.008	0.220	0.399	0.095	0.290	0.366	0.221	0.363	0.295	0.077	0.418	0.180	0.219	0.378	0.000	1.000	1.000
At5g49890	-0.064	0.778	0.477	1.330	0.040	0.395	-0.317	0.318	0.334	-0.021	0.941	0.510	0.280	0.013	0.221	0.596	0.133	0.363	0.107	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g50160	-0.234	0.313	0.319	-0.717	0.093	0.395	0.382	0.266	0.318	-0.022	0.944	0.510	-0.603	0.047	0.249	0.044	0.352	0.386	0.003	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g50320	0.017	0.898	0.509	0.447	0.084	0.395	0.301	0.092	0.289	0.150	0.048	0.271	0.356	0.178	0.310	0.721	0.006	0.359	0.053	0.374	1.000	0.005	0.374	0.392	0.000	1.000	1.000
At5g50335	0.262	0.408	0.352	0.147	0.545	0.470	-0.009	0.986	0.561	1.130	0.028	0.249	-0.028	0.955	0.535	0.003	0.991	0.585	0.165	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g50530	0.712	0.007	0.150	1.291	0.048	0.395	0.305	0.012	0.216	0.336	0.254	0.301	0.690	0.043	0.247	0.871	0.059	0.363	0.101	0.647	0.533	0.195	0.285	0.			

At5g52390	0.000	1.000	1.000	1.012	0.112	0.395	0.082	0.746	0.492	0.175	0.266	0.304	0.000	1.000	1.000	1.320	0.007	0.361	-0.010	0.974	0.624	0.000	1.000	1.000	0.000	1.000	1.000
At5g52780	-0.394	0.005	0.126	-0.468	0.104	0.395	-0.161	0.256	0.314	-0.613	0.028	0.249	-1.013	0.001	0.083	-0.626	0.006	0.359	-0.092	0.150	0.418	-0.170	0.142	0.378	0.085	0.374	0.415
At5g52810	0.118	0.251	0.303	0.321	0.219	0.395	0.127	0.068	0.283	0.197	0.046	0.268	0.614	0.021	0.226	0.197	0.515	0.439	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g53280	0.174	0.386	0.344	-0.753	0.001	0.385	0.198	0.418	0.371	-0.372	0.013	0.239	0.537	0.103	0.292	-0.106	0.720	0.508	0.135	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g53450	0.004	0.981	0.530	0.179	0.343	0.412	0.117	0.576	0.434	-0.250	0.004	0.166	0.419	0.270	0.321	0.941	0.015	0.363	0.093	0.829	0.592	0.470	0.067	0.378	-0.094	0.374	0.415
At5g53490	-0.334	0.053	0.277	-0.176	0.763	0.533	-0.173	0.453	0.386	-0.443	0.157	0.295	-0.383	0.039	0.243	-0.728	0.010	0.363	0.046	0.465	0.462	0.077	0.625	0.452	0.000	1.000	1.000
At5g54080	-0.046	0.706	0.454	0.495	0.176	0.395	-0.399	0.112	0.297	-0.106	0.655	0.428	0.480	0.118	0.300	0.764	0.040	0.363	0.192	0.178	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g54160	-0.157	0.328	0.322	-0.357	0.608	0.488	0.124	0.339	0.341	0.145	0.179	0.295	0.667	0.025	0.226	0.190	0.549	0.452	0.000	1.000	1.000	0.019	0.374	0.392	0.000	1.000	1.000
At5g54170	0.435	0.018	0.200	0.600	0.034	0.395	0.492	0.011	0.216	1.534	0.004	0.171	1.206	0.017	0.226	0.265	0.219	0.363	-0.132	0.583	0.506	0.105	0.374	0.392	0.000	1.000	1.000
At5g54490	0.743	0.055	0.277	1.151	0.045	0.395	0.137	0.755	0.495	0.520	0.179	0.295	0.802	0.026	0.226	-0.318	0.579	0.461	0.000	1.000	1.000	0.015	0.374	0.392	0.000	1.000	1.000
At5g54770	-0.928	0.009	0.161	-0.245	0.317	0.405	-0.078	0.818	0.516	-0.942	0.032	0.250	0.106	0.634	0.442	-0.013	0.949	0.575	0.427	0.352	0.423	0.280	0.324	0.383	-0.034	0.892	0.591
At5g54810	0.316	0.053	0.276	0.323	0.394	0.421	0.236	0.248	0.313	0.529	0.243	0.297	0.795	0.009	0.189	0.060	0.884	0.560	0.000	1.000	1.000	0.048	0.295	0.378	0.000	1.000	1.000
At5g54930	0.642	0.010	0.168	-0.062	0.900	0.571	0.303	0.208	0.309	0.518	0.113	0.295	0.885	0.012	0.219	0.591	0.030	0.363	-0.106	0.797	0.581	-0.022	0.866	0.517	0.000	1.000	1.000
At5g55530	-0.333	0.033	0.253	-0.428	0.203	0.395	-0.095	0.331	0.337	-0.636	0.029	0.249	-0.484	0.002	0.127	-0.001	0.996	0.586	-0.063	0.374	1.000	-0.111	0.158	0.378	0.000	1.000	1.000
At5g55750	0.222	0.146	0.295	0.595	0.013	0.395	0.185	0.029	0.248	0.152	0.048	0.271	0.447	0.210	0.311	0.220	0.090	0.363	0.283	0.181	0.418	-0.033	0.204	0.378	0.000	1.000	1.000
At5g56010	0.142	0.631	0.431	0.136	0.638	0.498	0.253	0.214	0.309	1.249	0.018	0.247	0.914	0.079	0.286	-0.093	0.665	0.491	0.018	0.812	0.587	0.952	0.023	0.378	0.414	0.129	0.414
At5g56090	0.118	0.644	0.434	0.180	0.586	0.481	0.184	0.392	0.362	0.650	0.306	0.317	0.605	0.050	0.254	0.323	0.249	0.365	-0.075	0.374	1.000	-0.021	0.374	0.392	0.000	1.000	1.000
At5g56150	0.092	0.348	0.329	-0.213	0.458	0.443	-0.001	0.995	0.563	0.038	0.838	0.482	0.639	0.017	0.226	0.009	0.974	0.582	-0.375	0.223	0.418	-0.146	0.378	0.394	0.000	1.000	1.000
At5g56760	0.743	0.040	0.265	0.079	0.237	0.395	0.439	0.277	0.321	0.316	0.428	0.350	0.328	0.480	0.385	-0.034	0.785	0.530	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g57035	-0.006	0.351	1.000	0.100	0.494	0.453	0.000	1.000	1.000	0.000	1.000	1.000	0.757	0.374	1.000	0.599	0.027	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g57110	-0.059	0.807	0.484	-0.172	0.461	0.444	-0.121	0.268	0.319	0.973	0.001	0.091	0.836	0.006	0.168	-0.068	0.658	0.488	0.000	1.000	1.000	0.019	0.180	0.378	0.000	1.000	1.000
At5g57150	0.299	0.064	0.285	0.258	0.047	0.395	0.205	0.210	0.309	1.456	0.013	0.239	0.672	0.001	0.083	0.206	0.075	0.363	-0.006	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g57170	-0.293	0.227	0.299	-0.253	0.532	0.466	0.086	0.484	0.398	-0.707	0.046	0.267	-0.527	0.152	0.310	-0.053	0.774	0.526	-0.030	0.798	0.582	0.076	0.553	0.432	0.000	1.000	1.000
At5g57300	0.077	0.782	0.478	-1.255	0.029	0.395	0.095	0.741	0.491	-0.236	0.397	0.339	-0.114	0.710	0.465	-0.507	0.306	0.376	-0.591	0.183	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g57560	1.562	0.001	0.082	2.558	0.016	0.395	-0.539	0.260	0.315	1.095	0.017	0.246	1.911	0.034	0.235	0.549	0.591	0.466	-0.072	0.805	0.584	0.277	0.093	0.378	0.260	0.264	0.414
At5g57630	0.862	0.098	0.295	1.037	0.311	0.404	0.565	0.250	0.313	1.251	0.039	0.260	1.460	0.037	0.238	1.360	0.034	0.363	0.166	0.208	0.418	0.391	0.284	0.378	0.000	1.000	1.000
At5g57710	0.428	0.150	0.295	0.163	0.490	0.452	0.211	0.402	0.365	1.403	0.025	0.249	0.604	0.261	0.320	0.554	0.051	0.363	0.449	0.279	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g57760	0.007	0.979	0.529	-0.797	0.025	0.395	-0.287	0.232	0.311	-0.405	0.098	0.292	-0.681	0.024	0.226	-0.011	0.981	0.583	0.043	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g58250	-0.524	0.049	0.274	-0.383	0.601	0.485	-0.046	0.838	0.523	-0.668	0.027	0.249	-0.143	0.635	0.442	-0.474	0.182	0.363	0.065	0.231	0.418	-0.202	0.338	0.386	0.073	0.374	0.415
At5g58260	-0.256	0.131	0.295	0.295	0.113	0.395	-0.224	0.178	0.308	-0.859	0.217	0.295	-1.167	0.038	0.242	-0.178	0.542	0.450	-0.011	0.374	1.000	-0.115	0.374	0.392	0.000	1.000	1.000
At5g58300	0.601	0.064	0.285	1.006	0.105	0.395	0.670	0.056	0.276	0.625	0.030	0.250	0.958	0.023	0.226	-0.381	0.090	0.363	0.017	0.374	1.000	0.434	0.162	0.378	0.068	0.193	0.414
At5g58310	0.609	0.016	0.192	1.029	0.081	0.395	0.162	0.359	0.350	0.740	0.004	0.171	1.146	0.002	0.111	-0.038	0.786	0.530	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g58680	0.407	0.021	0.210	-0.036	0.770	0.535	-0.035	0.776	0.501	0.802	0.005	0.185	0.892	0.019	0.226	0.069	0.870	0.555	-0.218	0.267	0.418	0.023	0.809	0.507	0.000	1.000	1.000
At5g59080	0.467	0.088	0.295	0.174	0.760	0.533	-0.895	0.031	0.249	-0.481	0.420	0.348	0.216	0.179	0.310	-0.106	0.737	0.515	0.005	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g59330	0.090	0.785	0.479	-1.280	0.031	0.395	-0.228	0.557	0.427	-0.161	0.736	0.454	-0.037	0.945	0.533	-1.078	0.274	0.372	-0.142	0.374	1.000	-0.242	0.702	0.477	-0.174	0.419	0.429
At5g59500	-0.156	0.260	0.304	0.781	0.031	0.395	0.095	0.627	0.454	-0.052	0.821	0.478	0.105	0.360	0.346	-0.005	0.987	0.584	-0.060	0.374	1.000	0.000	1.000	1.000	0.032	0.374	0.415
At5g59820	1.581	0.001	0.074	1.176	0.204	0.395	1.020	0.016	0.234	1.131	0.032	0.252	1.457	0.031	0.233	0.802	0.411	0.404	0.263	0.374	1.000	0.008	0.374	0.392	0.000	1.000	1.000
At5g59960	0.214	0.265	0.305	-0.577	0.213	0.395	-0.661	0.016	0.234	0.026	0.928	0.505	0.076	0.698	0.462	0.299	0.157	0.363	-0.078	0.374	1.000	-0.002	0.374	0.392	0.000	1.000	1.000
At5g60680	0.636	0.032	0.247	-0.133	0.607	0.487	-0.020	0.961	0.556	-0.305	0.395	0.339	-0.098	0.495	0.390	0.431	0.571	0.458	-0.023	0.868	0.600	0.000	1.000	1.000	0.000	1.000	1.000
At5g60750	-0.129	0.426	0.359	0.384	0.175	0.395	-0.264	0.027	0.246	-0.644	0.114	0.295	-0.778	0.038	0.242	-0.274	0.412	0.404	-0.215	0.514	0.478	0.154	0.501	0.420	-0.118	0.797	0.561
At5g60960	-0.095	0.446	0.367	0.572	0.269	0.396	0.100	0.303	0.330	0.443	0.108	0.295	0.577	0.011	0.210	0.725	0.024	0.363	-0.118	0.338	0.423	0.194	0.426	0.400	0.115	0.475	0.458
At5g62280	0.500	0.319	0.321	0.214	0.779	0.537	-1.689	0.012	0.216	-0.499	0.164	0.295	-1.085	0.047	0.249	-0.096	0.902	0.564	-0.419	0.295	0.419	-0.239	0.021	0.378	-0.222	0.374	0.415
At5g62350	0.074	0.223	0.298	0.095	0.692	0.514	0.000	1.000	1.000	0.740	0.013	0.239	0.049	0.761	0.481	-0.167	0.429	0.410	0.000	1.000	1.000	0.360	0.144	0.			

At5g63880	0.031	0.933	0.517	-0.044	0.956	0.585	0.710	0.039	0.256	0.264	0.572	0.398	-0.031	0.938	0.531	-0.178	0.432	0.410	-0.076	0.843	0.594	0.320	0.151	0.378	0.314	0.179	0.414
At5g63980	0.441	0.029	0.240	1.007	0.003	0.395	0.172	0.155	0.308	0.345	0.081	0.291	0.597	0.058	0.266	0.267	0.418	0.406	-0.142	0.185	0.418	0.091	0.275	0.378	0.000	1.000	1.000
At5g64040	-0.190	0.250	0.303	0.155	0.759	0.532	-0.488	0.063	0.278	-0.259	0.271	0.305	-0.107	0.550	0.410	-0.860	0.026	0.363	-0.298	0.297	0.419	0.020	0.918	0.529	-0.086	0.844	0.580
At5g64260	1.473	0.002	0.087	1.482	0.028	0.395	0.395	0.109	0.297	2.479	0.000	0.075	1.883	0.009	0.192	1.325	0.178	0.363	0.183	0.456	0.458	1.422	0.036	0.378	0.447	0.276	0.414
At5g64660	0.744	0.008	0.158	0.658	0.029	0.395	1.034	0.014	0.228	0.251	0.152	0.295	0.834	0.022	0.226	0.939	0.093	0.363	0.006	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g65250	0.288	0.169	0.295	0.207	0.241	0.395	-0.080	0.802	0.510	0.880	0.016	0.246	0.551	0.205	0.311	0.264	0.247	0.364	0.270	0.249	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g65280	1.070	0.000	0.015	0.765	0.231	0.395	0.771	0.018	0.237	1.401	0.030	0.250	1.870	0.001	0.069	-0.374	0.090	0.363	0.000	1.000	1.000	0.022	0.374	0.392	0.000	1.000	1.000
At5g65720	0.475	0.098	0.295	1.033	0.033	0.395	0.524	0.015	0.232	0.596	0.069	0.291	0.645	0.088	0.289	0.982	0.065	0.363	0.135	0.374	1.000	0.270	0.266	0.378	-0.044	0.374	0.415
At5g65810	0.279	0.143	0.295	-0.172	0.539	0.468	0.181	0.451	0.386	0.714	0.014	0.239	0.527	0.062	0.272	-0.101	0.692	0.500	0.107	0.474	0.465	0.240	0.090	0.378	0.081	0.374	0.415
At5g65870	0.246	0.384	0.343	0.340	0.268	0.396	0.093	0.363	1.000	0.827	0.108	0.295	0.810	0.025	0.226	-0.414	0.239	0.364	0.000	1.000	1.000	0.182	0.120	0.378	0.000	1.000	1.000
At5g66210	1.075	0.026	0.228	0.489	0.342	0.412	-0.032	0.940	0.553	1.144	0.086	0.291	1.116	0.065	0.273	0.359	0.645	0.484	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g66570	-0.411	0.028	0.240	-0.946	0.100	0.395	-0.569	0.015	0.232	-0.512	0.103	0.295	-0.685	0.031	0.233	-1.284	0.001	0.216	-0.441	0.006	0.418	0.076	0.762	0.495	-0.161	0.294	0.414
At5g66580	-0.108	0.656	0.438	0.187	0.168	0.395	0.405	0.178	0.308	1.447	0.037	0.259	0.083	0.848	0.506	0.668	0.125	0.363	0.013	0.374	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g66760	0.083	0.482	0.379	0.449	0.022	0.395	0.445	0.026	0.246	0.587	0.002	0.123	0.490	0.023	0.226	0.422	0.016	0.363	0.239	0.398	0.436	0.081	0.575	0.440	0.018	0.374	0.415
At5g66815	0.174	0.098	0.295	0.138	0.640	0.498	0.247	0.180	0.308	0.496	0.104	0.295	-0.185	0.342	0.340	0.724	0.032	0.363	-0.053	0.667	0.538	-0.088	0.727	0.485	-0.022	0.374	0.415
At5g67220	0.020	0.828	0.489	0.243	0.452	0.442	-0.081	0.396	0.363	0.061	0.691	0.439	0.129	0.284	0.325	0.620	0.031	0.363	0.000	1.000	1.000	0.000	1.000	1.000	0.000	1.000	1.000
At5g67300	1.045	0.000	0.025	1.354	0.008	0.395	-0.032	0.922	0.547	1.383	0.023	0.249	0.832	0.070	0.282	0.292	0.533	0.447	-0.107	0.156	0.418	0.000	1.000	1.000	0.000	1.000	1.000
At5g67470	0.060	0.426	0.360	-0.189	0.382	0.418	0.222	0.023	0.242	0.780	0.001	0.091	0.371	0.069	0.281	-0.024	0.851	0.549	0.036	0.374	1.000	0.099	0.231	0.378	0.000	1.000	1.000

## ANNEX 2

<b>AGI code</b>	<b>P FL</b>	<b>P-value</b>	<b>q-value</b>	<b>OP</b>	<b>P-value</b>	<b>q-value</b>	<b>O</b>	<b>P-value</b>	<b>q-value</b>
At1g02405	-0.214	0.716	0.374	1.327	0.111	0.325	0.809	0.019	0.247
At1g03090	0.514	0.099	0.294	0.006	0.977	0.522	0.880	0.042	0.278
At1g03600	-0.700	0.005	0.294	-0.945	0.048	0.325	-0.203	0.437	0.389
At1g05135	0.746	0.000	0.141	0.627	0.033	0.325	0.690	0.006	0.192
At1g05940	0.189	0.124	0.294	0.136	0.163	0.325	0.615	0.006	0.192
At1g06680	-0.226	0.222	0.294	-0.086	0.276	0.327	-0.625	0.030	0.269
At1g06830	-0.480	0.175	0.294	-0.142	0.393	0.343	-0.795	0.023	0.254
At1g07040	0.039	0.649	0.356	0.025	0.747	0.458	0.685	0.003	0.165
At1g07110	-0.129	0.205	0.294	-0.038	0.374	1.000	-1.253	0.025	0.257
At1g07230	-0.744	0.055	0.294	-1.335	0.016	0.325	0.475	0.141	0.314
At1g07600	0.727	0.019	0.294	0.706	0.014	0.325	0.630	0.027	0.260
At1g08380	-0.747	0.050	0.294	-0.616	0.218	0.325	-0.879	0.094	0.296
At1g08550	-0.640	0.096	0.294	-0.152	0.156	0.325	0.659	0.010	0.206
At1g08670	-0.480	0.018	0.294	-0.778	0.029	0.325	-0.504	0.031	0.270
At1g09070	0.205	0.383	0.294	0.090	0.549	0.397	1.050	0.019	0.247
At1g09330	0.482	0.339	0.294	0.715	0.039	0.325	0.519	0.181	0.314
At1g09340	-0.577	0.024	0.294	-0.589	0.009	0.325	-0.334	0.210	0.314
At1g11840	1.304	0.017	0.294	0.867	0.057	0.325	-0.247	0.519	0.423
At1g11860	-0.763	0.008	0.294	-0.195	0.021	0.325	-0.600	0.050	0.283
At1g11910	2.559	0.001	0.294	-0.202	0.766	0.464	0.733	0.316	0.349
At1g12500	0.547	0.120	0.294	0.814	0.178	0.325	0.995	0.006	0.191
At1g15130	-0.223	0.101	0.294	-0.631	0.005	0.325	-0.159	0.179	0.314
At1g15580	-0.187	0.194	0.294	-0.181	0.111	0.325	-0.633	0.031	0.270
At1g15810	-1.082	0.006	0.294	-0.860	0.152	0.325	-0.851	0.082	0.294
At1g15820	-0.681	0.021	0.294	-0.589	0.015	0.325	-0.682	0.021	0.250
At1g17290	0.372	0.346	0.294	0.000	1.000	1.000	0.944	0.048	0.283
At1g17420	2.140	0.019	0.294	1.982	0.039	0.325	-0.178	0.151	0.314
At1g18730	-0.255	0.127	0.294	-0.132	0.156	0.325	-0.682	0.028	0.265
At1g19190	-0.006	0.852	0.411	0.068	0.374	1.000	0.596	0.006	0.192
At1g19400	0.306	0.161	0.294	0.067	0.455	0.363	1.019	0.002	0.149
At1g20020	-0.845	0.006	0.294	-0.251	0.732	0.453	0.994	0.075	0.291
At1g20770	0.075	0.433	0.298	-0.376	0.231	0.325	0.748	0.017	0.242
At1g21130	0.712	0.038	0.294	0.767	0.076	0.325	0.160	0.374	1.000
At1g21500	-0.403	0.107	0.294	-0.111	0.472	0.370	-0.612	0.006	0.192
At1g22030	0.270	0.183	0.294	0.098	0.415	0.351	0.913	0.002	0.149
At1g22530	-0.749	0.012	0.294	-1.436	0.013	0.325	-0.162	0.696	0.483
At1g23740	0.015	0.783	0.393	0.000	1.000	1.000	-0.854	0.014	0.238
At1g24909	0.809	0.011	0.294	0.469	0.058	0.325	-0.349	0.023	0.254
At1g26630	-0.249	0.381	0.294	0.227	0.186	0.325	0.665	0.031	0.270
At1g29070	-0.749	0.043	0.294	-0.319	0.439	0.359	-0.182	0.642	0.465
At1g29395	0.073	0.642	0.354	-0.116	0.184	0.325	-1.018	0.007	0.192
At1g29910	-0.038	0.356	1.000	0.000	1.000	1.000	-0.654	0.017	0.242
At1g30380	-0.871	0.036	0.294	-0.436	0.098	0.325	-0.604	0.193	0.314
At1g32060	-0.729	0.030	0.294	-0.037	0.741	0.456	-0.768	0.084	0.294
At1g32640	0.589	0.020	0.294	0.527	0.043	0.325	-0.335	0.040	0.272
At1g32920	0.951	0.007	0.294	1.148	0.121	0.325	0.000	1.000	1.000
At1g33360	0.912	0.008	0.294	0.734	0.000	0.018	0.547	0.048	0.283
At1g33970	-0.356	0.086	0.294	0.040	0.910	0.503	0.932	0.015	0.238
At1g34400	0.703	0.023	0.294	0.387	0.372	0.340	0.770	0.100	0.299
At1g44020	0.163	0.529	0.321	0.627	0.028	0.325	-0.138	0.410	0.381
At1g44040	-0.475	0.080	0.294	-0.551	0.000	0.061	-0.595	0.038	0.270
At1g44575	-0.343	0.083	0.294	-0.272	0.180	0.325	-1.192	0.008	0.202
At1g48920	0.113	0.237	0.294	0.087	0.374	1.000	1.104	0.027	0.260
At1g50970	0.004	0.911	0.425	-0.004	0.374	1.000	0.783	0.041	0.277



At1g51340	-0.198	0.070	0.294	-0.095	0.245	0.325	0.670	0.021	0.250
At1g52400	2.148	0.002	0.294	1.638	0.066	0.325	-0.091	0.913	0.545
At1g54130	-0.050	0.834	0.405	0.083	0.757	0.461	-0.587	0.035	0.270
At1g54650	0.325	0.063	0.294	0.239	0.103	0.325	0.588	0.012	0.226
At1g55090	-0.695	0.010	0.294	-0.885	0.005	0.325	-0.943	0.004	0.174
At1g60950	-0.658	0.050	0.294	-0.329	0.031	0.325	-1.195	0.008	0.202
At1g64710	1.170	0.029	0.294	0.108	0.725	0.451	0.232	0.614	0.456
At1g65930	0.185	0.298	0.294	0.210	0.327	0.332	0.842	0.002	0.149
At1g67590	0.544	0.026	0.294	0.259	0.114	0.325	0.791	0.004	0.174
At1g67870	-0.190	0.012	0.294	-0.587	0.038	0.325	-0.555	0.074	0.291
At1g70450	0.036	0.294	0.294	0.000	1.000	1.000	1.527	0.043	0.278
At1g70810	0.091	0.279	0.294	0.214	0.260	0.325	0.930	0.018	0.244
At1g71360	0.366	0.044	0.294	0.011	0.778	0.469	0.634	0.032	0.270
At1g72290	1.500	0.011	0.294	1.483	0.046	0.325	-0.958	0.338	0.355
At1g72610	-0.789	0.111	0.294	-0.419	0.133	0.325	-1.395	0.001	0.144
At1g73630	0.819	0.044	0.294	0.352	0.194	0.325	0.312	0.111	0.306
At1g74360	0.149	0.399	0.294	0.000	1.000	1.000	0.912	0.034	0.270
At1g74470	-0.712	0.034	0.294	-0.229	0.148	0.325	-0.604	0.045	0.279
At1g76090	-0.718	0.041	0.294	-0.309	0.184	0.325	-0.204	0.323	0.350
At1g79040	-0.756	0.009	0.294	-0.077	0.313	0.330	-1.084	0.034	0.270
At1g80840	1.332	0.010	0.294	1.357	0.087	0.325	0.000	1.000	1.000
At2g01890	0.126	0.362	0.294	0.035	0.741	0.456	0.805	0.024	0.255
At2g02220	-1.506	0.013	0.294	-1.611	0.000	0.061	-1.491	0.007	0.192
At2g02990	0.416	0.166	0.294	0.283	0.374	1.000	1.952	0.003	0.165
At2g03570	0.378	0.021	0.294	0.295	0.090	0.325	0.672	0.001	0.138
At2g04220	0.640	0.004	0.294	0.527	0.043	0.325	1.005	0.001	0.149
At2g04350	0.127	0.611	0.344	0.080	0.723	0.450	0.612	0.035	0.270
At2g05580	0.835	0.111	0.294	0.889	0.185	0.325	1.462	0.011	0.225
At2g07696	-0.953	0.014	0.294	-0.783	0.019	0.325	-1.054	0.009	0.202
At2g10270	-0.836	0.050	0.294	-1.009	0.005	0.325	-1.351	0.003	0.165
At2g10940	-0.012	0.356	1.000	0.102	0.374	1.000	1.375	0.019	0.247
At2g12190	0.660	0.045	0.294	0.024	0.374	1.000	-0.034	0.919	0.546
At2g15490	0.751	0.017	0.294	0.772	0.099	0.325	0.459	0.043	0.278
At2g15500	0.004	0.968	0.439	0.228	0.213	0.325	-0.585	0.040	0.273
At2g15730	0.338	0.025	0.294	0.265	0.117	0.325	1.783	0.032	0.270
At2g17190	0.163	0.484	0.309	0.126	0.643	0.425	0.668	0.037	0.270
At2g17660	0.188	0.202	0.294	0.029	0.737	0.454	0.789	0.018	0.246
At2g18680	1.185	0.015	0.294	0.694	0.080	0.325	0.236	0.166	0.314
At2g19560	0.281	0.105	0.294	-0.034	0.882	0.496	0.602	0.003	0.165
At2g20770	0.172	0.352	0.294	0.000	1.000	1.000	0.788	0.039	0.270
At2g21330	-0.360	0.233	0.294	0.181	0.196	0.325	-0.656	0.024	0.254
At2g22425	0.725	0.042	0.294	0.216	0.318	0.330	-0.466	0.097	0.298
At2g23760	0.208	0.100	0.294	0.426	0.170	0.325	1.040	0.017	0.242
At2g25540	0.828	0.039	0.294	0.839	0.043	0.325	0.379	0.387	0.371
At2g28840	-0.724	0.008	0.294	-1.053	0.027	0.325	0.070	0.762	0.503
At2g29100	-0.019	0.735	0.380	-0.065	0.374	1.000	1.196	0.009	0.202
At2g29110	-0.002	0.356	1.000	-0.058	0.374	1.000	1.096	0.029	0.268
At2g29340	0.078	0.667	0.361	-0.088	0.616	0.418	1.028	0.045	0.279
At2g29490	0.833	0.004	0.294	0.457	0.047	0.325	0.059	0.334	0.353
At2g29630	-1.051	0.004	0.294	-0.875	0.008	0.325	-0.826	0.032	0.270
At2g30360	1.103	0.015	0.294	0.454	0.204	0.325	0.477	0.095	0.297
At2g30570	0.608	0.359	0.294	1.182	0.075	0.325	1.918	0.006	0.192
At2g30790	-0.535	0.046	0.294	-0.278	0.652	0.427	-1.210	0.014	0.238
At2g31890	0.710	0.040	0.294	0.300	0.348	0.338	0.591	0.014	0.238
At2g32990	-0.034	0.356	1.000	0.000	1.000	1.000	-0.654	0.004	0.174
At2g33380	0.936	0.088	0.294	0.994	0.021	0.325	-0.117	0.427	0.386
At2g33450	-0.651	0.018	0.294	-0.344	0.136	0.325	-0.567	0.076	0.291
At2g34420	-1.407	0.022	0.294	-0.484	0.139	0.325	-1.841	0.005	0.187

At2g34620	-0.327	0.169	0.294	-0.294	0.087	0.325	-1.012	0.037	0.270
At2g36630	0.247	0.240	0.294	0.238	0.299	0.330	-0.650	0.037	0.270
At2g37270	0.128	0.118	0.294	0.228	0.374	1.000	0.586	0.026	0.259
At2g37710	0.094	0.686	0.366	-0.020	0.916	0.505	0.846	0.023	0.254
At2g38170	-0.178	0.316	0.294	0.118	0.791	0.473	1.605	0.001	0.138
At2g38240	1.643	0.007	0.294	1.272	0.089	0.325	0.000	1.000	1.000
At2g38780	-0.154	0.494	0.311	0.082	0.732	0.453	0.610	0.004	0.174
At2g38790	1.594	0.004	0.294	1.452	0.011	0.325	0.072	0.374	1.000
At2g38940	0.022	0.866	0.414	0.129	0.183	0.325	1.103	0.007	0.192
At2g39720	0.450	0.152	0.294	0.768	0.101	0.325	1.384	0.041	0.276
At2g40100	-0.625	0.026	0.294	-0.253	0.140	0.325	-0.593	0.062	0.291
At2g40205	0.418	0.000	0.294	0.455	0.104	0.325	0.976	0.003	0.167
At2g40475	-0.006	0.990	0.444	-0.260	0.398	0.345	0.792	0.001	0.138
At2g40840	-0.071	0.675	0.364	-0.284	0.392	0.343	-0.719	0.022	0.254
At2g41000	0.201	0.088	0.294	0.000	1.000	1.000	0.826	0.049	0.283
At2g41380	0.359	0.062	0.294	0.580	0.061	0.325	1.071	0.015	0.238
At2g43590	0.096	0.535	0.323	-0.254	0.453	0.363	-1.014	0.029	0.268
At2g44060	-0.020	0.921	0.428	-0.030	0.761	0.462	0.744	0.025	0.257
At2g44220	-0.141	0.243	0.294	-0.176	0.301	0.330	1.764	0.030	0.269
At2g44280	0.348	0.082	0.294	-0.336	0.250	0.325	0.779	0.036	0.270
At2g45000	0.302	0.079	0.294	-0.117	0.181	0.325	0.894	0.005	0.191
At2g45180	-0.709	0.151	0.294	0.595	0.035	0.325	0.747	0.049	0.283
At2g46300	0.353	0.197	0.294	0.462	0.247	0.325	1.237	0.019	0.247
At2g46400	1.128	0.036	0.294	0.856	0.171	0.325	0.285	0.099	0.299
At2g46600	0.620	0.034	0.294	0.437	0.556	0.399	2.405	0.001	0.149
At2g46710	0.093	0.563	0.330	0.234	0.182	0.325	0.959	0.003	0.167
At2g46920	0.092	0.424	0.296	0.091	0.426	0.355	0.740	0.003	0.168
At2g47400	-0.150	0.013	0.294	-0.351	0.171	0.325	-0.907	0.016	0.241
At3g01120	-0.177	0.261	0.294	-0.280	0.193	0.325	-0.979	0.046	0.280
At3g01500	0.013	0.937	0.432	0.195	0.263	0.325	-1.030	0.028	0.266
At3g01680	-0.214	0.241	0.294	-0.473	0.171	0.325	-0.635	0.030	0.270
At3g03290	0.042	0.234	0.294	0.000	1.000	1.000	1.192	0.022	0.252
At3g03770	-0.020	0.248	0.294	0.000	1.000	1.000	-0.788	0.044	0.278
At3g04070	0.075	0.356	1.000	0.000	1.000	1.000	0.833	0.034	0.270
At3g05900	-0.007	0.954	0.436	-0.459	0.156	0.325	0.950	0.050	0.283
At3g06070	-0.986	0.002	0.294	-0.856	0.017	0.325	0.198	0.067	0.291
At3g07390	0.707	0.035	0.294	0.761	0.129	0.325	0.165	0.374	1.000
At3g09360	0.129	0.271	0.294	0.129	0.374	1.000	1.725	0.003	0.167
At3g10920	0.341	0.264	0.294	0.168	0.360	0.339	1.407	0.001	0.144
At3g11410	0.589	0.007	0.294	0.332	0.214	0.325	-0.352	0.005	0.191
At3g14420	-0.859	0.010	0.294	-1.235	0.007	0.325	-0.142	0.517	0.422
At3g14440	1.489	0.014	0.294	1.285	0.070	0.325	-0.087	0.787	0.508
At3g14990	0.632	0.025	0.294	0.221	0.163	0.325	0.474	0.126	0.310
At3g15260	-0.174	0.234	0.294	-0.045	0.089	0.325	-0.603	0.008	0.202
At3g15530	0.480	0.013	0.294	0.512	0.042	0.325	0.681	0.010	0.207
At3g15540	0.920	0.012	0.294	0.393	0.256	0.325	-0.104	0.342	0.356
At3g16140	0.000	0.998	0.447	-0.356	0.167	0.325	-0.886	0.009	0.202
At3g16590	0.091	0.315	0.294	0.052	0.374	1.000	0.587	0.031	0.270
At3g20770	0.354	0.074	0.294	0.113	0.562	0.400	0.925	0.010	0.207
At3g22800	0.391	0.103	0.294	0.700	0.126	0.325	0.604	0.006	0.192
At3g23920	0.803	0.131	0.294	0.631	0.050	0.325	0.667	0.019	0.247
At3g24100	0.084	0.512	0.316	0.025	0.707	0.446	0.702	0.004	0.174
At3g24480	0.839	0.001	0.294	0.493	0.223	0.325	1.202	0.003	0.168
At3g24860	0.390	0.038	0.294	0.226	0.261	0.325	0.650	0.006	0.191
At3g25220	-0.007	0.964	0.438	0.232	0.316	0.330	1.040	0.044	0.278
At3g27090	0.525	0.036	0.294	0.711	0.026	0.325	-0.015	0.911	0.544
At3g27140	1.334	0.003	0.294	0.981	0.050	0.325	0.049	0.374	1.000
At3g27510	0.668	0.038	0.294	0.253	0.574	0.404	-1.813	0.036	0.270

At3g28450	0.645	0.007	0.294	0.166	0.700	0.444	0.762	0.001	0.149
At3g28540	-1.774	0.002	0.294	-1.922	0.000	0.088	-2.137	0.001	0.138
At3g32990	-0.026	0.946	0.434	-0.035	0.775	0.467	-1.342	0.045	0.278
At3g33002	-0.768	0.031	0.294	-0.577	0.099	0.325	-0.803	0.167	0.314
At3g45140	3.126	0.001	0.294	2.745	0.005	0.325	-0.717	0.377	0.368
At3g45640	1.005	0.006	0.294	1.353	0.021	0.325	-0.183	0.548	0.434
At3g46220	0.092	0.591	0.338	0.216	0.188	0.325	1.034	0.030	0.269
At3g46830	-0.506	0.369	0.294	0.535	0.294	0.330	0.850	0.033	0.270
At3g47070	-0.568	0.031	0.294	-0.642	0.017	0.325	-1.249	0.044	0.278
At3g47960	0.787	0.030	0.294	0.576	0.053	0.325	-0.002	0.987	0.563
At3g48070	0.048	0.496	0.312	0.340	0.183	0.325	1.005	0.015	0.238
At3g48180	1.176	0.029	0.294	0.606	0.141	0.325	1.092	0.068	0.291
At3g48700	0.104	0.383	0.294	0.482	0.222	0.325	1.273	0.004	0.174
At3g50080	0.275	0.070	0.294	0.169	0.355	0.338	1.175	0.004	0.174
At3g50770	0.409	0.133	0.294	0.050	0.721	0.449	2.891	0.000	0.115
At3g50830	0.002	0.356	1.000	-0.012	0.202	0.325	0.685	0.008	0.200
At3g50950	0.889	0.020	0.294	0.531	0.115	0.325	0.378	0.431	0.387
At3g51000	0.083	0.330	0.294	0.011	0.374	1.000	0.661	0.006	0.192
At3g51440	-0.106	0.580	0.335	-0.012	0.816	0.480	0.678	0.047	0.282
At3g51660	0.554	0.151	0.294	-0.264	0.577	0.404	1.031	0.027	0.265
At3g51920	0.501	0.139	0.294	0.394	0.168	0.325	0.969	0.014	0.236
At3g53490	-3.065	0.000	0.004	-3.387	0.006	0.325	-2.995	0.000	0.115
At3g54140	-0.007	0.356	1.000	-0.264	0.374	1.000	1.323	0.019	0.247
At3g54680	0.068	0.217	0.294	0.074	0.374	1.000	0.841	0.031	0.270
At3g55800	-0.561	0.016	0.294	-0.286	0.180	0.325	-0.943	0.018	0.244
At3g56150	0.633	0.022	0.294	0.102	0.230	0.325	0.737	0.039	0.270
At3g56940	-0.099	0.715	0.374	-0.755	0.260	0.325	-1.015	0.042	0.278
At3g57450	1.426	0.002	0.294	0.875	0.065	0.325	0.057	0.338	0.355
At3g57550	0.607	0.042	0.294	0.411	0.153	0.325	0.229	0.286	0.338
At3g57930	1.226	0.022	0.294	0.790	0.076	0.325	0.107	0.502	0.417
At3g58020	0.659	0.013	0.294	0.272	0.370	0.340	0.784	0.030	0.269
At3g59970	0.107	0.648	0.356	0.350	0.251	0.325	-1.063	0.043	0.278
At3g60520	0.347	0.219	0.294	0.165	0.206	0.325	0.672	0.033	0.270
At3g61470	-0.928	0.003	0.294	-0.399	0.254	0.325	-1.322	0.013	0.235
At3g62510	0.440	0.257	0.294	0.760	0.038	0.325	0.331	0.196	0.314
At3g63140	-0.179	0.455	0.301	-0.154	0.565	0.401	-0.996	0.028	0.265
At4g00300	0.347	0.046	0.294	0.349	0.122	0.325	0.806	0.016	0.238
At4g01290	0.294	0.403	0.294	-0.244	0.313	0.330	0.655	0.045	0.278
At4g02770	-0.643	0.069	0.294	-1.151	0.016	0.325	-0.479	0.001	0.149
At4g02840	0.128	0.234	0.294	0.231	0.125	0.325	0.695	0.009	0.202
At4g03000	-0.602	0.019	0.294	-0.117	0.370	0.340	-0.278	0.134	0.313
At4g03210	-0.684	0.012	0.294	-0.708	0.058	0.325	-0.167	0.171	0.314
At4g05230	0.629	0.019	0.294	0.500	0.105	0.325	-0.228	0.193	0.314
At4g08980	0.288	0.164	0.294	0.072	0.432	0.357	0.701	0.013	0.233
At4g10340	-0.387	0.063	0.294	-0.195	0.261	0.325	-1.498	0.012	0.230
At4g14540	0.577	0.005	0.294	0.585	0.028	0.325	0.519	0.016	0.238
At4g16410	-0.869	0.009	0.294	-0.539	0.248	0.325	-0.304	0.220	0.316
At4g16500	-0.284	0.412	0.295	0.275	0.474	0.371	0.726	0.034	0.270
At4g16590	0.019	0.356	1.000	0.000	1.000	1.000	-1.123	0.025	0.257
At4g16720	-0.220	0.669	0.362	0.654	0.132	0.325	1.121	0.017	0.242
At4g17840	0.213	0.038	0.294	-0.031	0.575	0.404	0.642	0.050	0.285
At4g19200	0.270	0.514	0.317	0.426	0.016	0.325	1.278	0.025	0.257
At4g20390	0.557	0.010	0.294	0.367	0.090	0.325	1.239	0.004	0.174
At4g20760	-0.874	0.013	0.294	-1.665	0.005	0.325	-1.795	0.000	0.115
At4g20890	-0.361	0.041	0.294	-0.645	0.038	0.325	-0.579	0.002	0.149
At4g21120	0.138	0.466	0.304	0.065	0.317	0.330	0.711	0.040	0.273
At4g22710	1.113	0.000	0.294	0.713	0.036	0.325	0.714	0.030	0.270
At4g23100	0.696	0.005	0.294	0.743	0.079	0.325	0.024	0.920	0.546

At4g23880	1.058	0.014	0.294	0.658	0.048	0.325	1.166	0.066	0.291
At4g23890	-0.703	0.000	0.083	-0.325	0.148	0.325	-0.618	0.107	0.305
At4g24380	0.668	0.038	0.294	0.510	0.134	0.325	-0.107	0.489	0.411
At4g24690	-0.004	0.989	0.444	-0.169	0.738	0.455	1.218	0.002	0.149
At4g26240	-0.022	0.775	0.390	-0.304	0.179	0.325	0.894	0.017	0.242
At4g27280	0.367	0.088	0.294	0.366	0.184	0.325	0.939	0.012	0.230
At4g27740	-0.142	0.124	0.294	-0.248	0.112	0.325	1.290	0.006	0.191
At4g28290	-0.118	0.249	0.294	0.000	1.000	1.000	0.745	0.030	0.269
At4g29010	0.205	0.363	0.294	0.253	0.260	0.325	0.682	0.046	0.280
At4g29780	1.184	0.011	0.294	0.769	0.257	0.325	0.000	1.000	1.000
At4g29900	0.144	0.137	0.294	0.159	0.154	0.325	0.699	0.000	0.115
At4g31800	1.441	0.012	0.294	1.129	0.045	0.325	0.420	0.148	0.314
At4g32560	0.096	0.644	0.354	0.000	1.000	1.000	0.652	0.035	0.270
At4g33050	0.820	0.014	0.294	0.605	0.015	0.325	0.063	0.723	0.490
At4g33300	0.198	0.293	0.294	0.217	0.186	0.325	1.016	0.000	0.115
At4g39260	-0.404	0.381	0.294	0.060	0.668	0.433	1.716	0.012	0.233
At4g39660	0.057	0.714	0.373	0.409	0.194	0.325	0.942	0.035	0.270
At5g01290	0.185	0.071	0.294	0.639	0.003	0.325	0.329	0.038	0.270
At5g01740	-0.648	0.046	0.294	-0.375	0.087	0.325	-0.019	0.930	0.548
At5g03210	0.835	0.075	0.294	0.946	0.004	0.325	-0.504	0.189	0.314
At5g03710	0.641	0.053	0.294	0.723	0.016	0.325	0.781	0.018	0.242
At5g04440	-0.087	0.272	0.294	-0.052	0.360	0.339	-1.096	0.013	0.234
At5g06300	0.140	0.301	0.294	0.190	0.261	0.325	0.645	0.001	0.138
At5g07020	-0.625	0.036	0.294	-0.259	0.409	0.349	-0.356	0.118	0.307
At5g07280	0.208	0.098	0.294	0.000	1.000	1.000	0.924	0.014	0.236
At5g07650	0.268	0.186	0.294	0.405	0.091	0.325	0.753	0.004	0.174
At5g09220	-1.042	0.001	0.294	-0.870	0.017	0.325	-0.115	0.397	0.376
At5g11650	0.228	0.480	0.308	0.042	0.255	0.325	0.626	0.013	0.234
At5g11670	0.740	0.096	0.294	0.973	0.038	0.325	-0.240	0.086	0.295
At5g15490	1.314	0.006	0.294	0.914	0.108	0.325	-0.273	0.038	0.270
At5g15820	-0.275	0.335	0.294	-0.240	0.374	1.000	0.915	0.031	0.270
At5g17000	0.275	0.167	0.294	0.312	0.282	0.327	0.872	0.002	0.149
At5g17230	-0.782	0.006	0.294	-0.331	0.145	0.325	-0.656	0.043	0.278
At5g17290	-0.091	0.668	0.362	0.000	1.000	1.000	0.624	0.049	0.283
At5g17670	-0.041	0.587	0.337	-0.112	0.178	0.325	-0.724	0.007	0.198
At5g19080	0.485	0.010	0.294	0.769	0.097	0.325	0.834	0.003	0.165
At5g19090	0.592	0.026	0.294	0.293	0.169	0.325	0.724	0.001	0.149
At5g19190	-0.737	0.027	0.294	-0.872	0.015	0.325	0.747	0.013	0.233
At5g19220	-0.601	0.019	0.294	-0.408	0.013	0.325	-0.453	0.050	0.285
At5g19440	0.618	0.026	0.294	0.427	0.089	0.325	0.000	0.999	0.566
At5g19590	-0.721	0.019	0.294	-0.372	0.037	0.325	0.631	0.080	0.294
At5g20960	0.003	0.960	0.437	-0.071	0.186	0.325	0.646	0.045	0.278
At5g23950	0.276	0.130	0.294	-0.005	0.871	0.493	0.854	0.002	0.159
At5g24313	0.569	0.173	0.294	0.883	0.016	0.325	-0.359	0.476	0.404
At5g24810	-0.010	0.831	0.405	0.000	1.000	1.000	0.634	0.037	0.270
At5g25320	0.041	0.356	1.000	0.000	1.000	1.000	1.100	0.049	0.283
At5g27380	0.083	0.866	0.414	0.808	0.038	0.325	-0.320	0.264	0.330
At5g28610	-0.554	0.091	0.294	-0.808	0.036	0.325	0.042	0.801	0.512
At5g35170	0.270	0.052	0.294	0.328	0.106	0.325	0.652	0.022	0.252
At5g35732	-0.683	0.018	0.294	-0.428	0.165	0.325	0.000	1.000	1.000
At5g37600	0.013	0.943	0.433	-0.184	0.178	0.325	1.595	0.004	0.173
At5g38870	0.626	0.093	0.294	0.627	0.182	0.325	1.028	0.001	0.149
At5g39040	-0.118	0.344	0.294	-0.074	0.184	0.325	0.641	0.002	0.149
At5g39590	0.079	0.669	0.362	0.216	0.139	0.325	0.656	0.048	0.283
At5g41340	-0.006	0.947	0.434	0.138	0.374	1.000	0.691	0.002	0.149
At5g42020	0.638	0.034	0.294	0.441	0.176	0.325	-0.144	0.704	0.487
At5g42050	0.879	0.036	0.294	0.590	0.165	0.325	0.347	0.034	0.270
At5g42635	0.257	0.124	0.294	0.497	0.105	0.325	0.941	0.008	0.202

At5g44005	1.022	0.047	0.294	0.752	0.090	0.325	0.492	0.073	0.291
At5g44130	0.160	0.294	0.294	0.298	0.178	0.325	0.650	0.019	0.247
At5g44420	-1.010	0.006	0.294	-0.848	0.129	0.325	-0.522	0.614	0.456
At5g45340	0.914	0.002	0.294	0.920	0.140	0.325	0.000	1.000	1.000
At5g45410	0.656	0.026	0.294	0.386	0.181	0.325	0.371	0.123	0.310
At5g45810	-0.049	0.845	0.408	0.549	0.199	0.325	0.833	0.001	0.138
At5g46295	0.850	0.018	0.294	0.563	0.229	0.325	0.000	1.000	1.000
At5g46800	-0.699	0.036	0.294	-0.331	0.206	0.325	-0.958	0.011	0.212
At5g47040	0.015	0.939	0.432	0.109	0.210	0.325	0.653	0.009	0.202
At5g47120	0.559	0.011	0.294	0.557	0.034	0.325	0.668	0.046	0.280
At5g47130	-0.114	0.592	0.338	0.246	0.302	0.330	0.624	0.010	0.211
At5g48380	0.466	0.149	0.294	0.449	0.174	0.325	0.773	0.016	0.238
At5g48540	0.677	0.050	0.294	0.433	0.048	0.325	0.042	0.851	0.527
At5g49700	0.677	0.007	0.294	0.325	0.158	0.325	0.252	0.364	0.363
At5g50730	-0.049	0.721	0.376	0.309	0.290	0.330	0.828	0.007	0.192
At5g51990	0.331	0.184	0.294	0.720	0.093	0.325	1.620	0.003	0.165
At5g52650	0.142	0.601	0.341	0.219	0.289	0.329	1.713	0.005	0.191
At5g54170	0.690	0.031	0.294	0.597	0.019	0.325	0.080	0.516	0.422
At5g54770	-0.559	0.094	0.294	0.319	0.510	0.382	-1.206	0.019	0.247
At5g57560	1.098	0.044	0.294	1.233	0.178	0.325	0.000	1.000	1.000
At5g57630	0.428	0.042	0.294	0.238	0.603	0.413	1.516	0.021	0.252
At5g58250	-0.818	0.001	0.294	-0.488	0.182	0.325	-0.355	0.172	0.314
At5g58270	0.036	0.227	0.294	0.031	0.374	1.000	1.320	0.029	0.268
At5g58300	0.079	0.120	0.294	0.191	0.147	0.325	-0.856	0.004	0.174
At5g59330	0.544	0.062	0.294	-0.008	0.981	0.523	-0.616	0.007	0.192
At5g59820	1.949	0.000	0.167	1.667	0.032	0.325	0.272	0.036	0.270
At5g60750	-0.184	0.481	0.308	-0.185	0.478	0.372	-0.611	0.015	0.238
At5g63400	0.599	0.039	0.294	0.547	0.183	0.325	0.128	0.685	0.481
At5g63470	0.056	0.149	0.294	0.498	0.228	0.325	0.949	0.020	0.247
At5g64260	1.071	0.006	0.294	0.977	0.047	0.325	0.744	0.016	0.241
At5g64660	0.685	0.033	0.294	1.184	0.006	0.325	0.001	0.374	1.000
At5g64920	0.544	0.069	0.294	-0.045	0.929	0.508	0.638	0.036	0.270
At5g65280	0.900	0.044	0.294	0.680	0.184	0.325	-0.946	0.078	0.293
At5g66210	1.324	0.032	0.294	0.898	0.199	0.325	0.251	0.173	0.314
At5g66570	-0.838	0.015	0.294	-0.547	0.041	0.325	-0.581	0.087	0.295
At5g66760	-0.058	0.501	0.314	0.425	0.045	0.325	0.589	0.021	0.252
At5g67300	-0.041	0.570	0.332	0.103	0.289	0.329	0.896	0.034	0.270