# Trade-off between constitutive and inducible resistance against herbivores is only partially explained by gene expression and glucosinolate production

Sergio Rasmann[1]*, Estelle Chassin[2], Julia Bilat[2], Gaétan Glauser[3], Philippe Reymond[4]

[1] Department of Ecology and Evolutionary Biology, University of California, 92697 Irvine, CA, USA.
[2] Department of Ecology and Evolution, University of Lausanne, Biophore building, 1015 Lausanne, Switzerland.
[3] Chemical Analytical Service of the Swiss Plant Science Web, University of Neuchatel, 2000 Neuchatel, Switzerland.
[4]Department of Plant Molecular Biology, University of Lausanne, Biophore building, 1015 Lausanne, Switzerland

*Corresponding author:
e-mail: srasmann@uci.edu
phone: +1 949 824 52 84
fax: +1949 824 21 80

Estelle Chassin: estelle.chassin@wanadoo.fr
Julia Bilat: Julia.bilat@unil.ch
Gaétan Glauser: gaetan.glauser@unine.ch
Philippe Reymond: Philippe.Reymond@unil.ch

Running head: trade-off between constitutive and inducible resistance

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35 Summary statement

36 The observed partial correlation between herbivore resistance, defensive metabolites
37 accumulation, and gene expression suggests complex network of gene interactions
38 governing the postulated trade-off between constitutive defences and their
39 inducibility.

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Abstract

The hypothesis that constitutive and inducible plant resistance against herbivores should trade-off because they use the same resources and impose costs to plant fitness has been postulated for a long time. Negative correlations between modes of deployment of resistance and defences have been observed across and within species in common garden experiments. We therefore tested whether that pattern of resistance across genotypes follows a similar variation in patterns of gene expression and chemical defence production. Using the genetically tractable model *Arabidopsis thaliana* and different modes of induction, including the generalist herbivore *Spodoptera littoralis,* the specialist herbivore *Pieris brassicae*, and jasmonate application, we measured constitutive and inducibility of resistance across seven *A. thaliana* accessions that were previously selected based on constitutive levels of defence gene expression. According to theory, we found that modes of resistance traded-off among accessions, particularly against *S. littoralis,* in which, accessions investing in high constitutive resistance did not increase it substantially after attack, and vice-versa. Accordingly, the average expression of eight genes involved in glucosinolate production negatively predicted larval growth across the seven accessions. We next measured glucosinolate production and genes related to defence induction on healthy and herbivore-damaged plants. Surprisingly, we only found a partial correlation between glucosinolate production, gene expression and the herbivore resistance results. These results suggest that the defence outcome of plants against herbivores goes beyond individual molecules or genes but stands on a complex network of interactions.

Key words: glucosinolates, jasmonic acid, plant defences, plant-herbivore interaction, specificity of resistance, VSP2

## Introduction

Plants, to ward off herbivore attack, have evolved a whole array of defence traits (Schoonhoven *et al.*, 2005), which can be always present or only induced after herbivore feeding (Karban and Baldwin, 1997). The general consensus argues that inducible defences have evolved as a cost-saving strategy (Karban *et al.*, 1997), in which undamaged plants can divert resources from defence to growth and reproduction. Zangerl & Rutledge (1996) postulated that the pattern of constitutive and inducible defences, at the plant or at the organ level, depends on the probability of the attack and the value of the organ. In other words, plants or organs, which are regularly attacked by herbivores, should have high levels of constitutive defences and low levels of induced defences. By extrapolations, in populations where herbivory is low, plants should invest little in constitutive defences and more in inducibility of defence, in which inducibility is the difference between the induced state minus the constitutive state of defence in an organ of the plant. Recent examples have shown that inducibility is dependent on the spatial variation on the plant populations and herbivore pressure (e.g. Moreira *et al.*, 2014; Rasmann *et al.*, 2014), suggesting that at the landscape level there are constraints on simultaneously producing both types of defence investment within one species.

Indeed, because we know that the expression of redundant traits is costly for the plant (Koricheva *et al.*, 2004), and because we assume that constitutive and induced defences are two traits in competition for the same resources in the plant, we should expect a trade-off (or negative correlation) between them (Agrawal *et al.*, 2010). In other words, if both constitutive and inducible resistance traits are adaptive, we should observe a negative correlation between constitutive and induced resistance across populations or species of plants (Agrawal *et al.*, 2010). Several examples have shown trade-offs between constitutive and inducible resistance, both within (e.g. Gianoli, 2002; Rasmann *et al.*, 2014; Rasmann *et al.*, 2011) and across species (e.g. Kempel *et al.*, 2011; Moreira *et al.*, 2014; Rasmann and Agrawal, 2011; Zhang *et al.*, 2008). Additionally, Thaler & Karban (1997) mapped constitutive and inducible defences along the phylogeny of *Gossypium* spp., and showed independent and repeated origins and losses of both defence traits, indicating evolutionary lability and independence in the mode of defence investment. In *Acacia*, it was shown that constitutive extrafloral nectar production originated from inducible production in closely related species (Heil *et al.*, 2004). To summarize, past research indicates that

constitutive and inducibility of resistance evolve depending on the herbivore pressure and the probability of attack at a particular site. Nevertheless, constrains imposed by resource acquisition force the two mode of defence investment to negatively correlate with each other.

With this study we aimed to take a step further in the study of the interactions, and putative trade-off, between inducible and constitutive resistance and investigate the genetic bases explaining the pattern. We specifically asked whether patterns of trade-off between constitutive and inducible resistance (i.e. the effect of the plant's defensive arsenal on the performance of the herbivores, according to Karban & Baldwin (1997)) is correlated to similar patterns of defensive secondary metabolites and gene induction. To address our questions we used a highly genetically-tractable plant, the thale cress *Arabidopsis thaliana* (Brassicaceae); a small annual plant from Eurasia but naturalized across all continents expect Antarctica. Basal genome-wide expression levels have been characterized for over 750 Arabidopsis accessions. In addition, major biosynthetic pathways involved in insect resistance, including the jasmonate pathway (Howe and Jander, 2008), are well characterized (Bodenhausen and Reymond, 2007). Furthermore, Arabidopsis, like most species in the Brassicales, contains glucosinolates. When insect herbivores feed on the plant, they damage tissues and bring glucosinolates in contact with an activated enzyme, the myrosinase, which results in the production highly toxic hydrolysis breakdown products such as nitriles, isothiocyanates or thiocyanates (Halkier and Gershenzon, 2006). Moreover, several studies have already shown specificity in inducible resistance against specialists versus generalist herbivores in Arabidopsis (De Vos *et al.*, 2005; Rasmann *et al.*, 2012). Generally, it was shown that the glucosinolates have a negative impact on generalist herbivores fitness, but it has little, none, or positive effect on specialist herbivores (Mueller *et al.*, 2010; Schweizer *et al.*, 2013).

Here we hypothesize that, 1) according to classic theory, previously induced plants are more defended against subsequent herbivore attack than undamaged plants; 2) generalist herbivores are more susceptible than specialist herbivores, 3) there is a negative genetic correlation between constitutive and inducibility of resistance, and 4) both glucosinolate production, and gene expression related to defence induction correlate with patterns of induced resistance.

## Material and methods

### *Plant material*

Seeds of all accessions were obtained from The Nottingham Arabidopsis Stock Centre (NASC). For all the experiments (see below), all plants were grown in a growth chamber (short days, 20°C, 55% RH) with a 3:1 mix of commercial potting soil (Orbo-2, Schweizer AG, Lausanne; Switzerland) and perlite. All plants were six weeks old at the time of the experiments.

### *Microarray data*

Constitutive expression data for Arabidopsis accessions were downloaded from the ArrayExpress repository database (http://www.ebi.ac.uk/arrayexpress; experiment E-TABM-18). Data are part of the At GenExpress project (http://arabidopsis.org/portals/expression/microarray/ATGenExpress.jsp) and consist of expression values from 4-day-old seedlings from 34 accessions grown in soil in the same conditions and at the same time (Lempe *et al*, 2005).

### *Inducible resistance experiment*

To measure the specificity of trade-offs between inducible and constitutive resistance in Arabidopsis, we performed an experiment with three different induction treatments: a control treatment (no induction), a jasmonic acid (JA) application, and an herbivore induction. Jasmonic acid has been shown to be the master regulator of plant inducible resistance against chewing herbivores in many plants, including Arabidopsis (Howe, 2004; Howe and Jander, 2008). For the herbivore treatment, we chose the highly generalist herbivore *Spodoptera littoralis* (Lepidoptera, Noctuidae), and the cabbage family specialist herbivore *Pieris brassicae* (Lepidoptera, Pieridae). Eggs of *S. littoralis* were provided by Syngenta (Stein Switzerland) and first-instar larvae were obtained by placing eggs at 30°C during three days. First-instar larvae of *P. brassicae* were obtained from rearing insects on cabbage (*Brassica oleracea*) in controlled greenhouse conditions at the University of Lausanne.

For all treatments, plants were enclosed in hermetic Plexiglas boxes (N = 7 genotypes x 3 treatments x 2 herbivores x 3 plants = 63 plants). Treatments were performed as follow: 1) the control-treated plants were left without further treatment for three days; 2) the JA treatment included plants that were induced by putting three cotton buds in the box, each one spiked with 5 µL of Methyl Jasmonate (MeJA)

(Sigma-Aldrich CAS Nb 39924-52-2). JA treatment lasted 24h after which lids were opened allowing the evaporation of the JA left in the box. Finally, 3) plants were induced by placing 8-10 first-instar *S. littoralis* larvae per pot. Larvae were allowed to feed for three days prior to removal. We used *S. littoralis* for the induction treatment as this herbivore was used to measure the induction of defence genes in selected accessions (see below).

After the induction, plants were individually surrounded with 330 ml volume deli plastic cups with the bottom cut off, and 10 *S. littoralis* or 10 *P. brassicae* larvae were added to each plant (N = 30 larvae per herbivore, per genotype and per treatment). Cups were covered with fine-meshed nylon nets to prevent larvae from escaping, and larvae were allowed to feed for 7 days, after which, all surviving larvae were flash frozen in liquid nitrogen, oven-dried for 4 days at 50 °C and weighed.

*Glucosinolate and gene expression analyses*

For glucosinolate and gene analyses we planted 12 plants per genotype and after six weeks, half of the plants were induced with 10 *S. littoralis* caterpillars for three days as described above. At the end the induction treatment, 200 mg of fresh tissue per plant was ground with a homogenizer in 2 ml ice cold MeoH:water (70:30, v/v) with 25 µl of sinalbin 1.56 mmol as the internal standard. Samples were then incubated for 15 min at 80 °C in a block heater (Techne dri-block, Staffordshire, UK), centrifuged at 3500 x g for 10 min, and the supernatant was transferred to an appropriate vial for analysis. Glucosinolate identification and quantification was performed using an Acquity UPLC from Waters (Milford, MA, USA) interfaced to a Synapt G2 QTOF from Waters with electrospray ionization, using the separation and identification method as described in Glauser *et al.* (2012).

For gene expression analyses, two leaves were sampled from half of the control and treated plants (n = 3), added together in one Eppendorf tube and flash frozen in liquid nitrogen. We selected three genes known to be induced after caterpillar attack in Col-0 (Reymond *et al.*, 2000), including: 1) *ALLENE OXIDE CYCLYSE2* (*AOC2*), a gene that catalyses an essential step in jasmonic acid biosynthesis*; 2) VEGETATIVE STORAGE PROTEIN2* (*VSP2*), a highly inducible gene after herbivory or JA treatment; and 3) *CYTOCHROME P450 79B3* (*CYP79B3*), a gene involved in indole-glucosinolate biosynthesis. RNA extraction and qPCR analyses were done following standard protocols using the reference gene

*At2g28390* (Arabidopsis SAND family protein) as described in Hilfiker *et al.* (2014). Primer efficiencies (E) were assessed by a five-step dilution regression. The expression level of a target gene (TG) was normalized to the reference gene (RG) and calculated as Normalized Relative Quantity (NRQ) as follows: $NRQ=E^{CtRG}/E^{CtTG}$.

*Statistical analyses*

We analysed the effect of the genotypes, the induction treatment, and the two herbivore species using a full-factorial three-way ANOVA. Secondly, to test for trade-offs between constitutive and inducibility of resistance, we regressed the inducibility (i.e. the difference in mean larval mass values for each genotype between control and induced plants) against the genotype mean of that trait in the control treatment (i.e. the constitutive level). As we regressed a variable against a difference that includes the same variable (i.e. inducibility of resistance = induced plants – control plants), the errors in the two axes are not independent, and thus there is a possibility of obtaining spurious correlations from these analyses (Morris *et al.*, 2006). Therefore, to evaluate the significance of these correlations, we employed the Monte Carlo simulation procedure proposed by Morris et al. (2006) using MATLAB (Version 7.5.0.342 – R2007b, MathWorks Inc., USA).

Glucosinolate data were analysed with a three-way permutation ANOVA using the package LmPerm in R (Wheeler, 2010), because we could not reach normality of the errors, and included genotype, herbivore treatment, and compound identity as main effects.

## Results

*Selection of Arabidopsis accessions with contrasting constitutive defences*

To investigate genotypic variation in constitutive versus inducible resistance we selected seven accessions of Arabidopsis, based on the expression of 16 genes known to be related to defence against chewing herbivores (Reymond *et al.*, 2004); Supplementary Material Table S1). For each individual gene, 34 accessions for which whole-genome expression data were available (see methods) were ranked based on the constitutive expression of defence genes. The computation of the average constitutive expression across all genes provided a list of seven accessions

(Table S2), including HR-5, Kindalville-0 (Kin-0), Niederzenz-1 (Nd-1), Columbia-0 (Col-0), Moscow-0 (Ms-0), C-24, and Shahdara (Sha).

*Induction experiment*

In accordance with classic predictions, we found an overall effect of previous induction on resistance (Figure 1, Table 1). Particularly, larvae of both species grew 22% and 14% less (for *S. littoralis* and *P. brassicae*, respectively) on plants that were previously induced by *S. littoralis* (Figure 1, Table 1), and to a lesser extent on plants that were induced with JA (17% and 10%, respectively, see no effect of treatment by species interaction in Table 1). Overall, we found strong variation in resistance across accessions (Table 1) and strong specificity in resistance across accessions (see significant genotype by species interaction in Table 1).

Across seven accessions of Arabidopsis we found a negative genetic correlation between the constitutive resistance and the inducibility of resistance, particularly for the generalist herbivore *S. littoralis* (Figure 2, for *S. littoralis*, larval induction, $r = -0.94$, $p = 0.02$; and JA induction, $r = -0.94$, $p = 0.01$; and for *P. brassicae*, larval induction, $r = -0.82$, $p = 0.09$; and JA induction, $r = -0.08$, $p = 0.74$). For *S. littoralis*, the ranking of inducibility from high-induced susceptibility to high-induced resistance for both the larval and jasmonate induction was: C-24, HR-5, Sha, Col-0, Kin-0, Ms-0, and Nd-1. In other words, Nd-1 showed the largest inducibility of resistance, whereas C-24 had the smallest. Interestingly, we observed in some instances that larvae were larger on induced plants than on uninduced ones (Table S3). This was the case for *S. littoralis* feeding on HR-5 and C-24 after treatment with JA, and for *P. brassicae* feeding on Sha and C-24, after herbivory.

We next assessed whether natural variation in gene expression could directly influence resistance. We therefore regressed the average expression values of 8 genes related to glucosinolate production, and 8 genes including JA marker genes and JA biosynthesis in Arabidopsis (Table S2) against the larval weight of the generalist *S. littoralis* on each genotype (Table S3). We only used *S. littoralis* data for this analysis since only generalist herbivores should be affected by glucosinolates in plants. Additionally, we only used the control treatment as gene expression was measured on undamaged plants. We found that the constitutive expression of glucosinolate biosynthesis-related genes negatively predicted larval weight gain (Figure 3, $n = 7$, $r = 0.80$, $p = 0.03$). This was not true when regressing the average

expression of genes related to JA signalling and production (n = 7, r = 0.07, p = 0.87). To test whether or not results for the glucosinolate genes were spurious due to random gene sampling, we performed a permutation analysis using the 10'000 averages of 10 randomly selected genes from the whole pool of 22'759 genes present in Arabidopsis. As shown in Figure S1, our data indicate that the glucosinolate result is well below the 0.1, and the 0.05 probabilities when compared to correlations with random genes, indicating that the *S. littoralis* result cannot be obtained from random gene sampling of defence genes.

*Glucosinolate and gene expression analyses*

Because we observed a negative relationship between constitutive and inducible resistance (particularly against *S. littoralis*), we next sought defence mechanisms behind the observed trade-off and measured glucosinolates and gene expression of Col-0, HR-0, Ms-0, Nd-1. Our initial results from the resistance experiment indicated that Col-0 and HR-5 showed little or none induced resistance, Ms-0 showed intermediate levels of induced resistance, and ND-1 showed the highest levels of induced resistance (Figure 2A). We therefore predicted that glucosinolate and gene expression profiles would mimic the larval resistance results, and Nd-1 would show the highest induction of defensive metabolites and genes related to defence induction, and Col-0 and HR-5 the lowest (Figure 4A).

Glucosinolate analyses yielded 14 individual glucosinolate compounds, all showing different overall levels (Table S4, see compound effect in Table 2) and different inducibilities after herbivore attack (see treatment by compound effect in Table 2), overall, with herbivore treatment increasing average glucosinolate levels by 27% compared to control plants (see treatment effect in Table 2). Accessions showed little variation in total amount of glucosinolates, and only Nd-1 and Col-0 showed variation in glucosinolate induction after herbivore attack (Figure 4B, Table S4, and see treatment by genotype interaction in Table 2). Strikingly, some glucosinolates were almost exclusively found in a single accession (Table S4).

Expression analyses of selected insect-inducible genes showed strong induction after *S. littoralis* treatment (Figure 4C-E, and Table 3). *VSP2* had the highest inducibility, with 14-fold induction overall (Figure 4E), compared to 2.6-fold and 1.55-fold for *AOC2* and *CYP79B3* (Figures 4C, and 4D, respectively). We also found strong genotype effect, and genotype by treatment effect for inducibility of

genes (Table 3). For *VSP2*, Col-0 and Nd-1 showed the strongest induction, MS-0 showed average induction and HR-5 the lowest induction after herbivore attack. However, *AOC2* was strongly induced in Col-0, moderately in both HR-5 and Nd-1, but not in Ms-0. Finally, *CYP79B3* was only induced in Col-0 (Figure 4D). Since this enzyme is involved in the synthesis of indole-glucosinolates (Halkier and Gershenzon, 2006), and its expression correlates with accumulation of glucosinolates in Col-0 (Schweizer *et al.*, 2013), it was interesting to see that levels of the main indole-glucosinolates I3M, and to a lesser extent 1MOI3M, increased in Col-0 after herbivory (Table S4). Additionally, both compounds were also induced in Nd-1 and I3M was higher in Ms-0 without the respective changes in *CYP79B3* expression. Thus, our data show that there is not a consistent correlation between inducibility of resistance, accumulation of glucosinolates and defence gene induction between accessions, as it was predicted by the model in Figure 4A.

## Discussion

We found that overall inducible resistance against herbivores in Arabidopsis is underlined by strong genotypic variation, in which accessions that have high constitutive resistance are weak inducer, whereas accessions that have low constitutive resistance are strong inducers. This pattern generates the predicted trade-off between constitutive and inducible resistance in plants. Interestingly, despite the fact that basal expression of genes related to glucosinolate biosynthesis also predicts the observed resistance to herbivory, we found that constitutive and induced glucosinolate levels and defence gene induction only partially relate to the observed resistance. This suggests that plant defence allocation strategies goes beyond the individual molecules or genes but stands on a complex network of interactions. Below we discuss the possible causes and consequences of the observed results.

### *Specificity of induction of defences and herbivore responses*

The seminal book on plant defence induction by Karban and Baldwin (1997) has paved the way to the general wisdom that plants, under herbivore attack, are able to increase their basal levels of defences to a higher level. Whereas the ability to increase resistance only after attack has undoubtedly clear benefits in term of costs (Karban *et al.*, 1997), several drawbacks still impair a full grasp on the phenomenon,

including high specificity on the induction/response, and strong genotypic variation in induction.

First, as we show here, there is high level of specificity on both sides, in which either the induction agent (an insect or a phytohormone in our case) can result in different inducibilities, and the response of the herbivore is species specific. Indeed, plant induction of defences is driven by the complex chemistry of plant-herbivore interaction (Halitschke *et al.*, 2003; Walling, 2000), which takes into account the counter-response of the herbivore (Felton and Eichenseer, 2000; Karban and Agrawal, 2002), and surely goes beyond simple application of jasmonic acid to the plant (but see e.g. Rasmann *et al.*, 2012). Therefore, only by studying the effect of several inducing agents can we generalize on the existing patterns. Next, we show that specialist herbivores such as *P. brassicae* are less affected by previous plant induction than the generalist herbivore *S. littoralis*, and this seems to be a general rule in plant-insect interaction studies (Ali and Agrawal, 2012). Whether variation in induced resistance and subsequent formation of trade-offs is mainly generated by generalist herbivores is an enticing questions, and to our view merits further studies.

Second, this is not the first example of genotypes becoming more susceptible to herbivores after induction. Indeed, induced susceptibility is more common than we might expect (Karban and Baldwin, 1997), and it has been suggested that defence suppression could even benefit the plant rather than the herbivore (Kahl *et al.*, 2000). Although there is generally still little evidence for it, other studies show that plants decrease their defences (Bede *et al.*, 2006; Kahl *et al.*, 2000; Lawrence *et al.*, 2008), and become more susceptible to attacks by herbivores after previous attacks by other species of herbivores (Poelman *et al.*, 2008; Sarmento *et al.*, 2011; Sauge *et al.*, 2006). Mechanisms behind induced susceptibility might include trade-offs between defence types against different herbivore species (via so-called antagonistic cross-talk between signalling pathways involved in plant defence (Thaler, 1999), even within the same species (Bruessow *et al.*, 2010). It is therefore possible that the physiological (and evolutionary) constraints generating the trade-offs between constitutive and inducibility of resistance might also be behind patterns of induced susceptibility, and future work with Arabidopsis in this regard might answer this question.

*Genetic correlations among resistance strategies*

By measuring caterpillar growth on undamaged and previously damaged plants, we found a negative genetic correlation between constitutive resistance and inducibility of resistance. Thus, Arabidopsis accessions appear to have a maximal potential for resistance, and this is either allocated constitutively (i.e. always present), following herbivore attack, or in equal balance between the two. Such trade-offs between constitutive and induced responses suggests that the expression of resistance traits in plants is costly or otherwise constrained, or that there is simply no benefit in to additional resistance beyond a particular threshold level (Agrawal *et al.*, 2010). Similar patterns in deployment strategies of defence were previously observed within genotypes (Rasmann *et al.*, 2011), or across species of plants (Kempel *et al.*, 2011; Moreira *et al.*, 2014). Nevertheless, others have failed to observe trade-offs between constitutive defences and inducibility, at least across species (Rasmann and Agrawal, 2011). Such discrepancies in the experimental observations are difficult to explain as long as we lack a mechanistic understanding of how trade-offs arise, particularly at the gene level (Agrawal *et al.*, 2010). As mentioned above, variable production of defences can be triggered by insect-derived elicitors (Halitschke *et al.*, 2003), plant hormones (Harfouche *et al.*, 2006), herbivore-induced volatile organic compounds (Ton *et al.*, 2007), or indeed, differential constitutive levels of gene expression (Ahmad *et al.*, 2011)

Additionally, differential investment in plant defence deployment could arise from different herbivore pressures across the effective niche distribution of the species. For instance, we have recently shown that *Vicia sepium* plants at high elevation have lower basal levels of volatile organic compounds production but are more inducible than their conspecifics at lower elevation. This pattern of defence deployment goes hand-in-hand with lower herbivore pressure and lower abundance of predatory ants at high elevation (Rasmann *et al.*, 2014). We thus suggest that the observed pattern in Arabidopsis accessions is generated both by the physiological constrains of the plant (i.e. some genotypes are simply at the maximum level of resistance and thus could not be induced even more as was shown in Córdova-Campos *et al.* (2012)), and the different selection pressures at different locations where the accessions originated.

*Genotype – phenotype correlations*

Contrary to our expectations, we did not observe a consistent correlation between the phenotypic response (i.e. herbivore growth), glucosinolate production and defence gene induction. For instance, although the increasing induction of VSP2 between HR-5, Ms-0 and Nd-1 was correlated with the inducibility of resistance results (as predicted in Figure 4A), Col-0 displayed the strongest induction of defence genes and it displayed a high constitutive defence. Similarly, accumulation of glucosinolates after *S. littoralis* feeding was not higher in Nd-1 than Col-0, despite their different inducibility of resistance. In addition, the constitutive expression level of glucosinolate biosynthesis genes was negatively correlated with larval weight, although this was not true for glucosinolate levels, implying another level of complexity. In a related study with Arabidopsis, Ahmad *et al.* (2011) showed that a high induction of the defence gene *PR1* was correlated with a reduced bacterial infection in different accessions.

Clearly, more work is needed to better understand these discrepancies. For example, the apparent absence of correlation between total glucosinolates levels and inducibility of resistance might be explained by the fact that different accessions contain specific glucosinolates. These molecules may have different deterrent properties, and a careful examination of the contribution of each glucosinolate compound to defence will be needed. Furthermore, we restricted our investigation to genes of the jasmonate pathway and to glucosinolates, which are established components of defence against herbivory. Nevertheless, additional factors may contribute to the inducibility of resistance, such as priming (Ahmad *et al.*, 2011; van Hulten *et al.*, 2006), epigenetic modifications (Rasmann *et al.*, 2012), or post-transcriptional effects (Gfeller *et al.*, 2011; Savchenko *et al.*, 2013). A study with a larger number of accessions and defence traits might be needed to explain the mechanistic aspects of the trade-off between constitutive and induced defences.

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Tables and Figures

Table 1. Three-way ANOVA for assessing the effect of the seven Arabidopsis accessions, the induction treatment (with *S. littoralis* or with methyl jasmonate), on the growth the two herbivore species (*S. littoralis* and *P. brassicae*).

| Factor | df | F ratio | P value |
|---|---|---|---|
| Genotypes (G) | 6 | 5.646 | <.0001 |
| Treatments (T) | 2 | 3.999 | 0.022 |
| G*T | 12 | 1.400 | 0.183 |
| Species (S) | 1 | 261.774 | <.0001 |
| G*S | 6 | 3.354 | 0.005 |
| T*S | 2 | 0.214 | 0.807 |
| G*T*S | 12 | 1.327 | 0.220 |
| Residuals | 82 | | |

Table 2. Three-way permutation ANOVA table for individual glucosinolate levels across four Arabidopsis accessions. Plants were either left undamaged or induced with *S. littoralis* caterpillars for three days (i.e. treatment effect).

| Factor | Df | Iter | P value |
|---|---|---|---|
| Genotype (G) | 3 | 51 | 1 |
| Treatment (T) | 1 | 3985 | 0.024 |
| G*T | 3 | 3026 | 0.032 |
| Compound (C) | 13 | 5000 | < 0.0001 |
| G*C | 39 | 5000 | <0.0001 |
| T*C | 13 | 5000 | 0.025 |
| G*T*C | 39 | 5000 | 0.004 |
| Residuals | 560 | | |

Table 3. Three-way permutation ANOVA table for individual gene expression levels across four Arabidopsis accessions. Plants were either left undamaged or induced with *S. littoralis* caterpillars for three days (i.e. treatment effect).

| Factor | Df | Iter | P value |
|---|---|---|---|
| Genotype (G) | 3 | 5000 | < 0.0001 |
| Treatment (T) | 1 | 5000 | < 0.0001 |
| G*T | 3 | 5000 | < 0.0001 |
| Genes (Gn) | 2 | 5000 | < 0.0001 |
| G*Gn | 6 | 5000 | < 0.0001 |
| T*Gn | 2 | 5000 | < 0.0001 |
| G*T*Gn | 6 | 5000 | < 0.0001 |
| Residuals | 48 | | |

Figure legends

Figure 1. Induced resistance against chewing herbivores. Shown are means (± SE) of *P. brassicae* (open bars) and *S. littoralis* (shaded bars) larval mass on Arabidopsis plants that were either left untouched (control), previously induced with *S. littoralis* caterpillar or previously induced with methyl jasmonate (JA). Shown is the average of resistance across seven Arabidopsis accessions. Different letters above bars means difference after post-hoc Tukey test, $p < 0.05$.

Figure 2. Trade-off between constitutive and inducibility of resistance. Shown are means of A) *S. littoralis* and B) *P. brassicae* larval mass when feeding on seven Arabidopsis accessions. Plants were either left undamaged (constitutive) or previously induced by herbivores (open circles, dotted lines), or induced with methyl jasmonate (black dots, solid lines). Inducibility is the average difference of larval weight between induced and constitutive conditions, therefore a negative value means induced resistance, and the lowest values indicate the highest induction of resistance. Lines indicate significant correlation, $p < 0.05$. Legend besides open circles or inside black circles indicate accessions' names: N = Nd-1, M = Ms-0, K = Kin-0, S = Sha, Co = Col-0, H = HR-5, and C = C-24.

Figure 3. Relationship between constitutive gene expression and resistance against chewing herbivores. Shown is the genotypic relationship across seven Arabidopsis accessions of resistance against *S. littoralis* larvae and average gene expression of 8 genes related to glucosinolate production ($p < 0.05$).

Figure 4. Defence induction across accessions. A) shows the predicted defence induction of four Arabidopsis accessions based on the resistance bioassay in Figure 2A, in which Nd-1 should have the highest inducibility, HR-5 and Col-0 should have the lowest inducibility, and Ms-0 should have intermediate levels of inducibility. B) show the mean (± SE) levels of constitutive (open bars) and induced (black bars) production of glucosinolates, and C) – E) show the relative expression of *AOC2*, *CYP79B3*, and *VSP2*, respectively. Induction was performed with *S. littoralis* caterpillars. Values (± SE) are the average of three technical replicates.

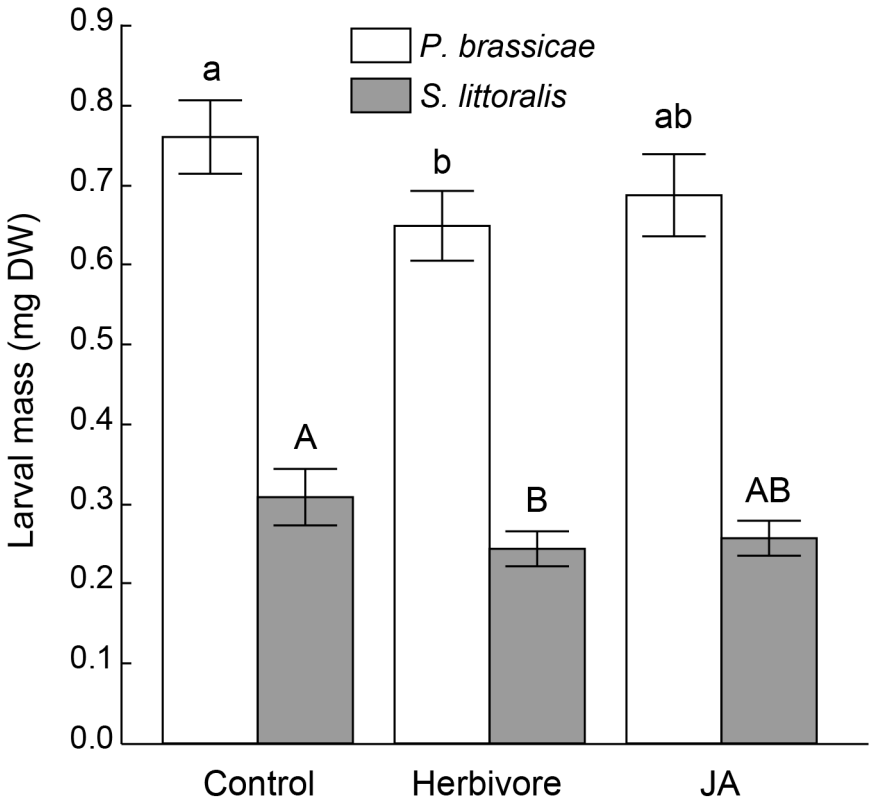
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Larval mass (mg DW)
P. brassicae
S. littoralis
a
A
b
B
ab
AB
Control
Herbivore
JA

Figure 1.

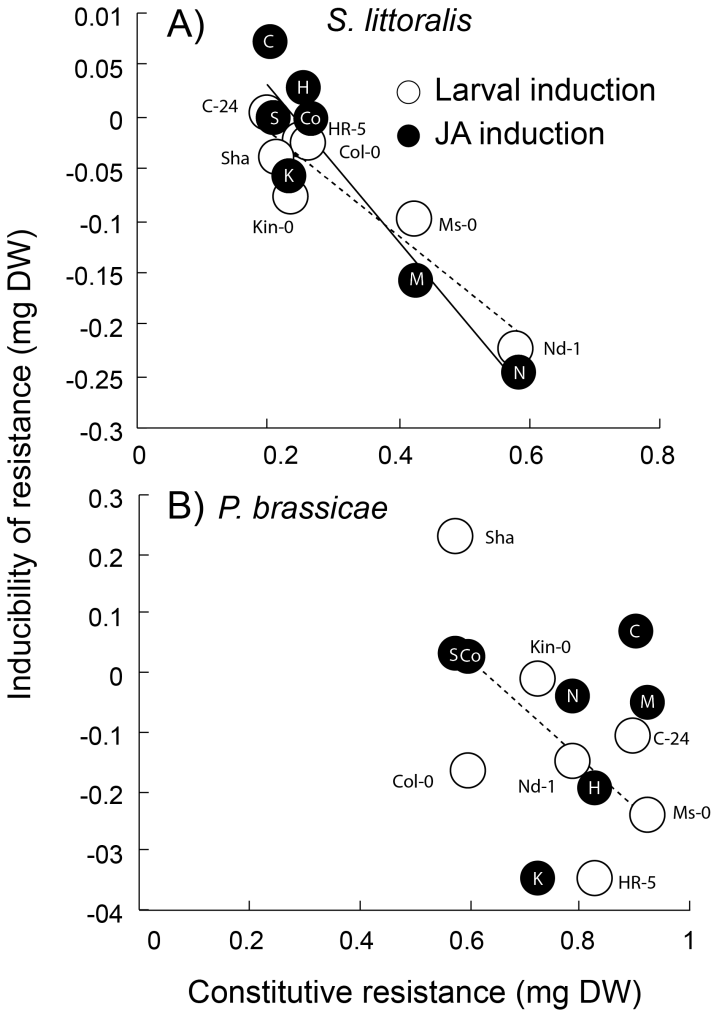
A)
S. littoralis
Larval induction
JA induction
C
H
C-24
S
Co
HR-5
Col-0
Sha
K
Kin-0
Ms-0
M
Nd-1
N
B) P. brassicae
Sha
C
SCo
Kin-0
N
M
C-24
Col-0
Nd-1
H
Ms-0
K
HR-5
Inducibility of resistance (mg DW)
Constitutive resistance (mg DW)

Figure 2.

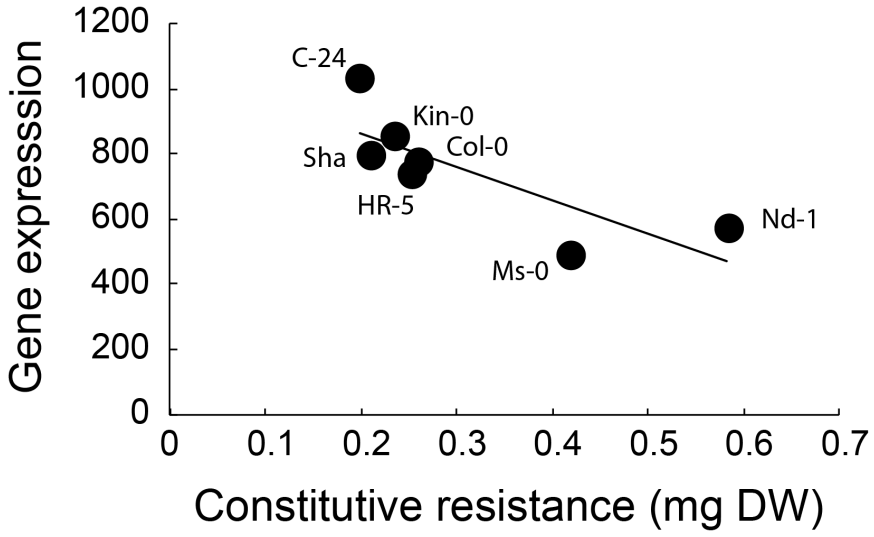
Gene expresssion
1200
1000
800
600
400
200
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Constitutive resistance (mg DW)
C-24
Kin-0
Col-0
Sha
HR-5
Nd-1
Ms-0

Figure 3.

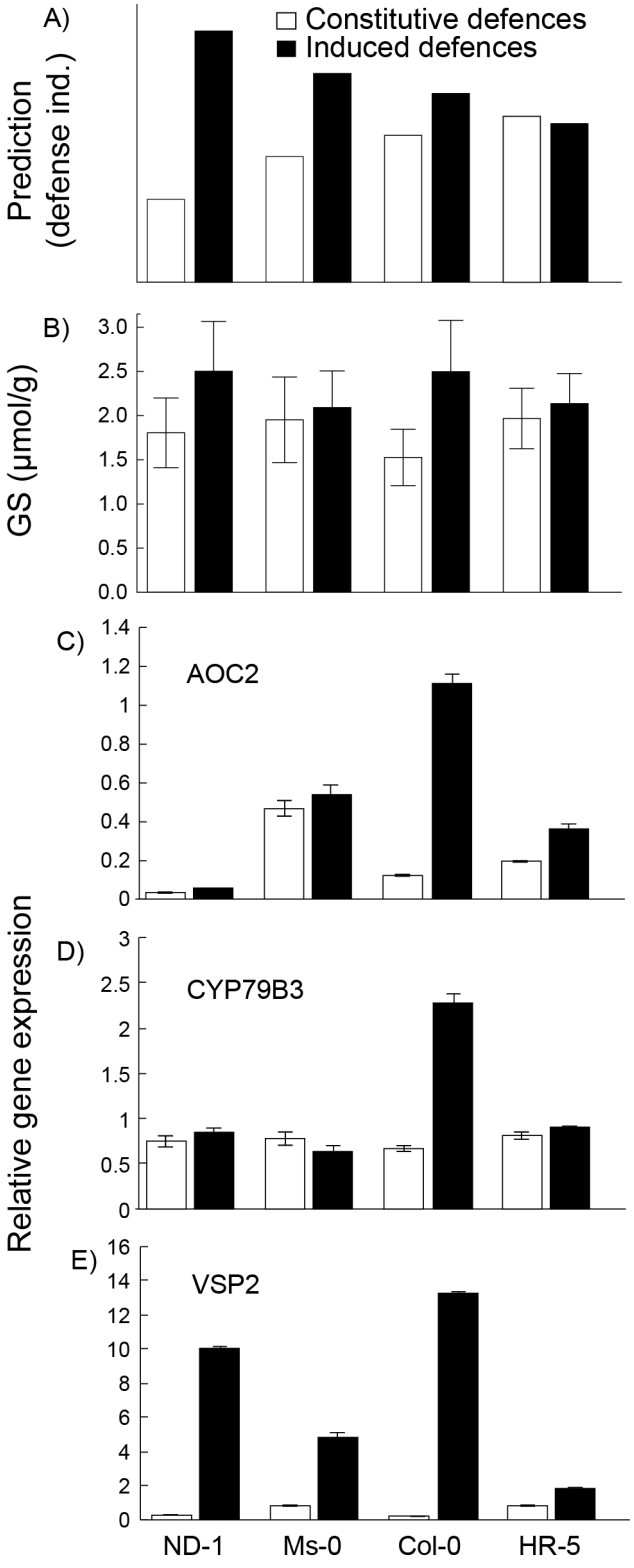
A)
Constitutive defences
Induced defences
Prediction (defense ind.)
B)
GS (µmol/g)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C)
AOC2
1.4
1.2
1
0.8
0.6
0.4
0.2
0
D)
CYP79B3
3
2.5
2
1.5
1
0.5
0
E)
VSP2
16
14
12
10
8
6
4
2
0
Relative gene expression
ND-1
Ms-0
Col-0
HR-5

Figure 4.

Supplementary information

Table S1. Genes known to be inducible after chewing herbivore attack in Arabidopsis. Those genes were used for the classification of 34 Arabidopsis accessions based on constitutive gene expression levels, and the selection of the seven accessions used for the experiments.

| Function | AGI | Short Description (TAIR10) |
|---|---|---|
| Glucosinolates | At4g39950 | CYP79B2 (cytochrome P450) |
| | At1g74100 | SOT16 (sulfotransferase) |
| | At5g60890 | MYB34 (transcription factor) |
| | At3g16390 | NSP3 (nitrile specifier protein 3) |
| | At1g52030 | MBP2 (myrosinase-binding protein) |
| | At2g39330 | Jacalin lectin family protein, myrosinase-associated protein |
| | At3g16470 | Jacalin lectin family protein, myrosinase-associated protein |
| | At1g54000 | GDSL-like lipase, myrosinase-associated protein |
| JA synthesis and signalling | At1g17420 | LOX3 (lipoxygenase) |
| | At1g72520 | LOX4 (lipoxygenase) |
| | At5g07010 | ST2A (hydroxyjasmonate sulfotransferase) |
| | At5g13220 | JAZ10 (jasmonate-ZIM-domain protein) |
| JA marker | At5g44420 | PDF1.2 (low-molecular-weight cysteine-rich 77) |
| | At1g12240 | Vacuolar invertase betaFruct4 |
| | At2g24850 | TAT3 (tyrosine aminotransferase 3); transaminase |
| | At5g24420 | Glucosamine/galactosamine-6-phosphate isomerase-related |

Table S2. Constitutive expression of genes involved in glucosinolate biosynthesis and regulation, and in jasmonate biosynthesis, signaling, and response, for seven Arabidopsis accessions.

| Type | AGI ID | C-24 | Kin-0 | SH | HR-5 | Col-0 | ND-1 | Ms-0 |
|---|---|---|---|---|---|---|---|---|
| Glucosinolates | At4g39950 | 254.77 | 635.1 | 524.33 | 371.7 | 285.2 | 242.6 | 201.5 |
| | At1g74100 | 704.87 | 706.67 | 1017.97 | 632.4 | 450.37 | 681.07 | 492 |
| | At5g60890 | 212.67 | 169.9 | 203.87 | 264.6 | 226.37 | 143.7 | 120.7 |
| | At3g16390 | 2087.93 | 1667.5 | 1149.27 | 747.6 | 1601.1 | 1325.47 | 970.5 |
| | At1g52030 | 12.33 | 28.83 | 309.37 | 24.3 | 28.63 | 40.3 | 49.6 |
| | At2g39330 | 15.23 | 20.57 | 15.37 | 7.2 | 16.43 | 10.83 | 20.9 |
| | At3g16470 | 808.47 | 582.3 | 819.9 | 1048.4 | 632.03 | 725.13 | 358.3 |
| | At1g54000 | 2079.9 | 1536.83 | 1370.87 | 1780.7 | 1459.83 | 1096.13 | 860.1 |
| JA synthesis | At1g17420 | 55.43 | 19.1 | 36.73 | 23.9 | 42.43 | 45.87 | 29.5 |
| | At1g72520 | 10.73 | 8.97 | 29.23 | 13.3 | 14.03 | 13.43 | 6.2 |
| | At5g07010 | 12.3 | 12.5 | 12.83 | 11.1 | 6.07 | 9.93 | 22 |
| | At5g13220 | 69.63 | 86.2 | 58.57 | 91.1 | 54.2 | 64 | 50.6 |
| JA marker | At5g44420 | 9.07 | 3.17 | 15.17 | 28.5 | 15 | 8.23 | 2 |
| | At1g12240 | 659.97 | 944.67 | 659.53 | 931.9 | 811.13 | 684.23 | 444.3 |
| | At2g24850 | 28.4 | 20.43 | 18.57 | 27.8 | 24.6 | 20.43 | 25.6 |
| | At5g24420 | 94.47 | 104.1 | 222.87 | 153.4 | 141.03 | 323.43 | 196.1 |
| Average | | 541 | 483 | 447 | 406 | 435 | 397 | 283 |

Table S3. Constitutive (control treatment) and induced (*S. littoralis* and MeJA treatment) resistance of seven *A. thaliana* accessions against the specialist caterpillar *P. brassicae*, and the generalist caterpillar *S. littoralis*. Data represent averages (± SE) caterpillar dry weight.

| Induction treatment | Accession | *P. brassicae* (mg) | *S. littoralis* (mg) |
|---|---|---|---|
| Control | C-24 | 0.899 +/- 0.024 | 0.199 +/- 0.026 |
| | Col-0 | 0.594 +/- 0.029 | 0.26 +/- 0.013 |
| | HR-5 | 0.826 +/- 0.086 | 0.254 +/- 0.01 |
| | Kin-0 | 0.725 +/- 0.03 | 0.235 +/- 0.01 |
| | Moscow-0 | 0.922 +/- 0.026 | 0.42 +/- 0.017 |
| | ND-1 | 0.784 +/- 0.024 | 0.582 +/- 0.057 |
| | SH | 0.573 +/- 0.065 | 0.211 +/- 0.006 |
| *S. littoralis* | C-24 | 0.794 +/- 0.045 | 0.201 +/- 0.023 |
| | Col-0 | 0.427 +/- 0.034 | 0.234 +/- 0.011 |
| | HR-5 | 0.482 +/- 0.053 | 0.233 +/- 0.005 |
| | Kin-0 | 0.714 +/- 0.05 | 0.157 +/- 0.014 |
| | Moscow-0 | 0.681 +/- 0.034 | 0.322 +/- 0.028 |
| | ND-1 | 0.636 +/- 0.022 | 0.359 +/- 0.014 |
| | SH | 0.808 +/- 0.037 | 0.175 +/- 0.015 |
| MeJA | C-24 | 0.971 +/- 0.016 | 0.272 +/- 0.03 |
| | Col-0 | 0.619 +/- 0.066 | 0.257 +/- 0.013 |
| | HR-5 | 0.628 +/- 0.021 | 0.284 +/- 0.041 |
| | Kin-0 | 0.376 +/- 0.061 | 0.178 +/- 0.003 |
| | Moscow-0 | 0.869 +/- 0.043 | 0.265 +/- 0.023 |
| | ND-1 | 0.744 +/- 0.034 | 0.335 +/- 0.03 |
| | SH | 0.605 +/- 0.01 | 0.209 +/- 0.006 |

Table S4. Glucosinolate levels in four Arabidopsis accessions

| | Col-0 | | HR-5 | | Ms-0 | | Nd-1 | |
|---|---|---|---|---|---|---|---|---|
| **Glucosinolate** | **Control** | **Induced** | **Control** | **Induced** | **Control** | **Induced** | **Control** | **Induced** |
| 2-propenyl | 0 +/- 0 | 0 +/- 0 | 0.017 +/- 0.006 | 0.024 +/- 0.004 | 0.839 +/- 0.244 | 0.743 +/- 0.140 | 0.005 +/- 0.001 | 0.005 +/- 0.003 |
| 3-hydroxypropyl | 0.001 +/- 0.001 | 0 +/- 0 | 0.275 +/- 0.172 | 0.088 +/- 0.083 | 0 +/- 0 | 0.001 +/- 0.001 | 0.871 +/- 0.113 | 1.191 +/- 0.209 |
| 7-methylthioheptyl (7MTH) | 0.026 +/- 0.002 | 0.027 +/- 0.001 | 0.050 +/- 0.011 | 0.059 +/- 0.012 | 0.025 +/- 0.003 | 0.033 +/- 0.010 | 0.020 +/- 0.002 | 0.023 +/- 0.002 |
| 8-methylthiooctyl (8MTO) | 0.064 +/- 0.005 | 0.051 +/- 0.004 | 0.177 +/- 0.017 | 0.188 +/- 0.037 | 0.216 +/- 0.042 | 0.194 +/- 0.019 | 0.130 +/- 0.016 | 0.150 +/- 0.018 |
| glucobrassicanapin | 0 +/- 0 | 0 +/- 0 | 0.033 +/- 0.011 | 0.041 +/- 0.008 | 0 +/- 0 | 0.006 +/- 0.006 | 0 +/- 0 | 0 +/- 0 |
| glucobrassicin (I3M) | 0.181 +/- 0.027 | 0.586 +/- 0.163 | 0.154 +/- 0.017 | 0.203 +/- 0.015 | 0.117 +/- 0.025 | 0.226 +/- 0.030 | 0.228 +/- 0.027 | 0.424 +/- 0.080 |
| glucoerucin (4MTB) | 0.190 +/- 0.026 | 0.132 +/- 0.019 | 0.003 +/- 0.002 | 0.004 +/- 0.002 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 |
| glucohirsutin (8MSOO) | 0.116 +/- 0.017 | 0.106 +/- 0.010 | 0.334 +/- 0.040 | 0.344 +/- 0.055 | 0.534 +/- 0.128 | 0.397 +/- 0.041 | 0.272 +/- 0.058 | 0.311 +/- 0.070 |
| glucoiberin (3MSOP) | 0.103 +/- 0.014 | 0.161 +/- 0.022 | 0.023 +/- 0.014 | 0.028 +/- 0.028 | 0.034 +/- 0.008 | 0.032 +/- 0.006 | 0.122 +/- 0.017 | 0.187 +/- 0.032 |
| gluconapin | 0.001 +/- 0.001 | 0 +/- 0 | 0.344 +/- 0.117 | 0.449 +/- 0.099 | 0.029 +/- 0.006 | 0.094 +/- 0.068 | 0 +/- 0 | 0 +/- 0 |
| glucoraphanin (4MSOB) | 0.715 +/- 0.1 | 1.200 +/- 0.172 | 0.008 +/- 0.004 | 0.008 +/- 0.002 | 0 +/- 0 | 0.001 +/- 0.001 | 0.020 +/- 0.002 | 0.025 +/- 0.004 |
| glucotropeolin | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 | 0 +/- 0 |
| methoxyglucobrassicin (4MOI3M) | 0.019 +/- 0.001 | 0.025 +/- 0.003 | 0.015 +/- 0.001 | 0.016 +/- 0.001 | 0.020 +/- 0.002 | 0.020 +/- 0.001 | 0.012 +/- 0 | 0.015 +/- 0.001 |
| neoglucobrassicin (1MOI3M) | 0.003 +/- 0.002 | 0.039 +/- 0.010 | 0 +/- 0 | 0 +/- 0 | 0.004 +/- 0.003 | 0.011 +/- 0.002 | 0 +/- 0 | 0 +/- 0 |
| progoitrin isomer | 0 +/- 0 | 0 +/- 0 | 0.097 +/- 0.032 | 0.130 +/- 0.028 | 0 +/- 0 | 0.045 +/- 0.045 | 0 +/- 0 | 0 +/- 0 |
| progoitrin | 0.001 +/- 0.001 | 0 +/- 0 | 0.302 +/- 0.101 | 0.405 +/- 0.088 | 0 +/- 0 | 0.143 +/- 0.143 | 0 +/- 0 | 0 +/- 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TOTAL | 1.420 +/- 0.197 | 2.327 +/- 0.404 | 1.832 +/- 0.545 | 1.987 +/- 0.462 | 1.818 +/- 0.461 | 1.946 +/- 0.513 | 1.680 +/- 0.236 | 2.331 +/- 0.419 |

Values (μmol/g FW) are the mean (±SE) of 6 measurements

Table S5. List of primers used in this study

| | | |
|---|---|---|
| AOC2 (At3g25770) | Fwd | 5'-CACGTCCCAGAGAAGAAAGG-3' |
| | Rev | 3'-CGAGGAACGAATCCTCGTAA-3' |
| | | |
| CYP79B3 (At2g22330) | Fwd | 5'-CTTTGCTTACCGCTGATGAA-3' |
| | Rev | 5'-GCGTTTGA TGGGTTGTCTG-3' |
| | | |
| VSP2 (At5g24770) | Fwd | 5'-GGTGCCCGCAAATTGCAAAGACTA-3' |
| | Rev | 5'-GGTTGATGCTCCGGTCCCTAACCA-3' |
| | | |
| SAND (At2g28390) | Fwd | 5'-AACTCTATGCAGCATTTGATCCACT-3' |
| | Rev | 5'-TGATTGCATATCTTTATCGCCATC-3' |

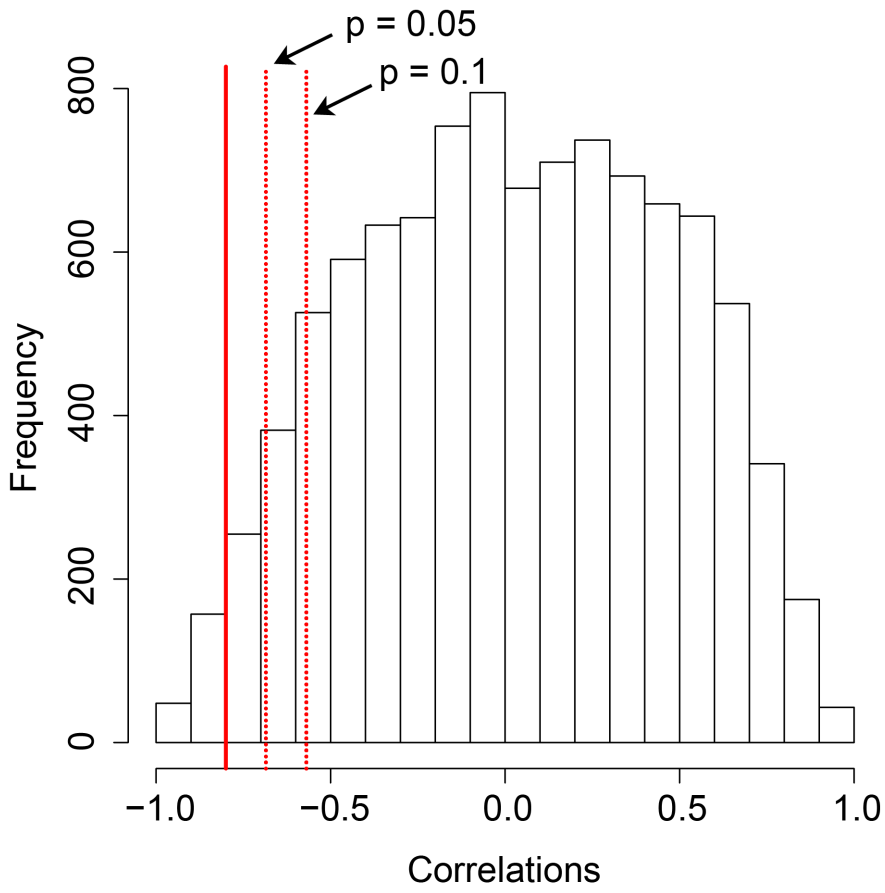


Figure S1. Selection of random genes. Shown is the histogram of correlations between the average of 10 randomly selected genes and the constitutive resis agaisnt *S. littoralis* across seven Arabidopsis accessions. Solid line indicate t correlation coefficient for the 8 genes related to glucosinolate producion. Do represent the 10% and 5% quantile for the 10000 correlations using random g selection.