serval
serveur académique lausannois

Unil
UNIL | Université de Lausanne
Faculty of Biology and Medicine

CHUV

Current Biology

PHO1 exports phosphate from the chalazal seed coat to the embryo in developing Arabidopsis seeds

--Manuscript Draft--

Manuscript Number:	CURRENT-BIOLOGY-D-17-00537R2
Full Title:	PHO1 exports phosphate from the chalazal seed coat to the embryo in developing Arabidopsis seeds
Article Type:	Research Article
Corresponding Author:	Yves Poirier University of Lausanne Lausanne, SWITZERLAND
First Author:	Evangelia Vogiatzaki
Order of Authors:	Evangelia Vogiatzaki
	Célia Baroux
	Ji-Yul Jung
	Yves Poirier
Abstract:	Seed production requires the transfer of nutrients from the maternal seed coat to the filial endosperm and embryo. Since seed coat and filial tissues are symplasmically isolated, nutrients arriving in the seed coat via the phloem must be exported to the apoplast before reaching the embryo. Proteins implicated in the transfer of inorganic phosphate (Pi) from the seed coat to the embryo are unknown despite seed P content being an important agronomic trait. Here, we show that the Arabidopsis Pi exporter PHO1 and PHOH1 are expressed in the chalazal seed coat (CZSC) of developing seeds. PHO1 is additionally expressed in developing ovules. Phosphorus (P) content and Pi flux between seed coat and embryo was analyzed in seeds from grafts between WT roots and scions from either pho1, phoh1 or pho1 phoh1 double mutant. While P content and distribution between seed coat and embryo in fully mature dry seeds of these mutants are similar to WT, at the mature green stage of seed development the seed coat of the pho1 and pho1 phoh1 mutants, but not of the phoh1 mutant, retains approximately 2-fold more P than its WT control. Expression of PHO1 under a CZSC-specific promoter complemented the seed P distribution phenotype of the pho1 phoh1 double mutant. CZSC-specific down-expression of PHO1 also recapitulated the seed P distribution phenotype of pho1. Together, these experiments show that PHO1 expression in the CZSC is important for the transfer of P from seed coat to the embryo in developing seeds.

PHO1 exports phosphate from the chalazal seed coat to the embryo in developing Arabidopsis seeds

Evangelia Vogiatzaki[1], Célia Baroux[2], Ji-Yul Jung[1] and Yves Poirier[1, 3, *]

[1]Department of Plant Molecular Biology, Biophore Building, University of Lausanne, CH-1015 Lausanne, Switzerland

[2] Department of Plant and Microbial Biology, University of Zürich, Zollikerstrase 107, CH-8008 Zürich, Switzerland.

[3] Lead Contact

* Correspondence: yves.poirier@unil.ch

Running title: PHO1 transfers phosphate from seed coat to embryo

Summary

Seed production requires the transfer of nutrients from the maternal seed coat to the filial endosperm and embryo. Since seed coat and filial tissues are symplasmically isolated, nutrients arriving in the seed coat via the phloem must be exported to the apoplast before reaching the embryo. Proteins implicated in the transfer of inorganic phosphate (Pi) from the seed coat to the embryo are unknown despite seed P content being an important agronomic trait. Here, we show that the Arabidopsis Pi exporter PHO1 and PHOH1 are expressed in the chalazal seed coat (CZSC) of developing seeds. PHO1 is additionally expressed in developing ovules. Phosphorus (P) content and Pi flux between seed coat and embryo was analyzed in seeds from grafts between WT roots and scions from either *pho1, phoh1* or *pho1 phoh1* double mutant. While P content and distribution between seed coat and embryo in fully mature dry seeds of these mutants are similar to WT, at the mature green stage of seed development the seed coat of the *pho1* and *pho1 phoh1* mutants, but not of the *phoh1* mutant, retains approximately 2-fold more P than its WT control. Expression of *PHO1* under a CZSC-specific promoter complemented the seed P distribution phenotype of the *pho1 phoh1* double mutant. CZSC-specific down-expression of *PHO1* also recapitulated the seed P distribution phenotype of *pho1*. Together, these experiments show that *PHO1* expression in the CZSC is important for the transfer of P from seed coat to the embryo in developing seeds.

Key words: Arabidopsis, chalaza, embryo, nutrient, phosphate, seed coat

Introduction

Development and maturation of the embryo is essentially dependent on the supply of nutrients from the maternal tissues, encompassing the seed coat derived from the ovule integuments, to the filial tissues, which include the endosperm in addition to the embryo. Nutrients being transferred to the filial seed tissues include carbon and nitrogen, largely in the form of sucrose and amino acids, respectively, as well as macroelements, such as inorganic phosphate (Pi) and microelements, such as zinc and iron [1]. The bulk of nutrients reach the seed through the phloem. In the majority of monocots and dicots, the vascular strand is typically embedded throughout the ground tissue of the seed coat. In the model dicot plant *Arabidopsis thaliana* and several Brassica species (such as *Brassica napus*; rapeseed) that have anatropous ovules, the vascular bundle terminates at the end of the funiculus in the chalazal seed coat (CZSC) [1, 2]. Although the phloem and the CZSC are symplastically connected, the seed coat and filial tissues are symplasmically isolated, e.g. they are not connected via plasmodesmata [2, 3]. This symplastic isolation between maternal and filial tissues in seeds implies that nutrients arriving in the seed coat via the phloem must be exported to the apoplast in order to subsequently be acquired by the developing embryo via re-import into the symplastic space.

While several proton-coupled symporters have been identified to play important roles in the import of sucrose and amino acids into the embryo [1, 4], the identification of proteins involved in their export to the seed apoplastic space remained elusive. Recently, proteins belonging to the SWEET and UmamiT family of transporters have been implicated in the export of sucrose and amino acids, respectively, out of the seed coat for nutrient transfer to the embryo [5, 6]. Similarly, members of the HMA family of heavy metal transporting ATPase have been shown to mediate zinc export out of the seed coat [7].

Phosphorus (P) is one of the major inorganic nutrients stored in seeds. P is acquired by the seed as Pi and primarily stored in the form of phytate. Pi transport across the plasma membrane into the cytosol is mediated by members of the PHT1 family of Pi/H+ co-transporters [8]. Although several members of the *PHT1* gene family are expressed in rice (*Oryza sativa*) and wheat (*Triticum aestivum*) seed tissues [9-11], our current understanding of the dynamics and of proteins participating in seed Pi homeostasis is very fragmentary. In particular, it is currently unknown how Pi is exported from the maternal seed coat to the embryo.

The *PHO1* gene was first found to be important for the loading of Pi into the root xylem vessel, a step requiring Pi export to the xylem apoplasm [12, 13]. *A. thaliana* and rice *pho1* mutants are defective in the transfer of Pi from roots to shoots, resulting in a strong Pi deficiency in shoots [13, 14]. Specific Pi export activity of PHO1 has been demonstrated through its ectopic expression in leaves and protoplasts [15]. Furthermore, the insect and/or mammalian homologues of PHO1, named XPR1, also mediate specific Pi export when ectopically expressed in either human cultured cells [16] or tobacco leaves [17]. The *A. thaliana PHO1* gene family contains 11 members, and only the closest homologue of *PHO1*, named *PHOH1* (previously *PHO1;H1*), was shown to contribute to Pi export in the root xylem apoplasm, indicating that it can also mediate Pi export [18]. Beyond its role in Pi transfer from roots to shoots, PHO1 is expressed in guard cells and plays a role in the response of stomata to abscissic acid [19].

Considering the action of PHO1 as a Pi exporter in roots, we hypothesized that it could participate in the transfer of Pi from the seed maternal to filial tissues. In this work, we show that PHO1 and its closest homologue PHOH1 are expressed in the CZSC. Using mutants as well as CZSC-specific RNAi down-regulation and expression of PHO1, we show that PHO1 participates in the transfer of Pi from the CZSC to the embryo.

Results

Expression pattern of PHO1 and PHOH1 in Arabidopsis floral reproductive tissues and developing seeds

Publically available transcriptomic data of developing Arabidopsis seeds generated using the Affymetrix ATH1 array (http://seedgenenetwork.net/arabidopsis) [20] indicated that both *PHO1* and *PHOH1* were expressed in the CZSC, with additional *PHO1* expression in the general seed coat (Figure S1). To validate these data and expand analysis to floral reproductive tissues, transgenic *A. thaliana* lines expressing PHO1-GUS or PHOH1-GUS fusion proteins were generated using genomic DNA containing the full coding region and 2 kbp upstream of the translation start site. For both constructs, a minimum of 5 independent lines showing similar expression pattern were analyzed. PHO1-GUS expression was found in the stamen filament vasculature as well as the tissue connecting the two locules of the anthers, while in the carpel expression was in the vascular strands as well as in the developing ovules (Figure 1A). In developing seeds, PHO1-GUS expression occurred in the CZSC and micropylar seed coat in the globular stage but was essentially confined to the CZSC at later stages, including at the mature green stage (Figure 1B, C). In floral tissues, PHOH1-GUS expression was similar to PHO1-GUS, with GUS staining in stamen filament vasculature and the tissue between the locules, as well as in the carpel vascular strands, but no expression was detected in ovules (Figure 1D). In developing seeds, expression of PHOH1-GUS was confined to the CZSC from the globular stage until the mature green stage (Figure 1 E, F).

The expression pattern of PHO1-GUS in ovules was examined in more detail. PHO-GUS activity was detected as early as in the pre-meiotic stage in ovule primordia, in the meiocyte itself (ovule stage 3-I , Figure 2A) then, following meiosis, in the L1 layer of the nucellus and the functional megaspore (stages 3-II to 3-V, Figure 2B-D). Subsequently, PHO1-GUS activity was restricted to the inner integuments from stages 3-V until 4-II (Figure 2E-G).

Analysis of transgenic lines expressing either PHO1-YFP or PHOH1-GFP fusion genes under the control of their respective promoter essentially reproduced the expression pattern observed with PHO1-GUS and PHOH1-GUS constructs with the exception that a signal in the micropylar seed coat could be observed in the globular and mature green stage for PHO1-YFP and in the globular stage for PHOH1-GFP (Figure S2 and S3).

Analysis of size, weight and P content in seeds of *pho1 phoh1* double mutants

The implication of both PHO1 and PHOH1 in the export of Pi [15, 21] as well as their overlapping expression profile indicated that they were likely to have redundant function in developing seeds. While *pho1* mutants are viable and fertile, all above ground tissues experience continuous Pi deficiency stress, leading to reduced growth and seeds with low P content [13]. The double mutant *pho1 phoh1* shows a more severe phenotype and does not complete its life cycle under normal growth conditions [18]. In order to bypass the negative effects of the constant shoot Pi-deficiency stress of both *pho1* single mutants and *pho1 phoh1* double mutants, and allow an analysis of the contribution of these genes to seed development and Pi homeostasis, analysis was restricted to grafted plants between a mutant scion and a WT root. Such grafted plants have previously been shown to have normal shoot Pi (because WT roots supply adequate amount of Pi to the shoot) and show WT-like shoot and inflorescence growth as well as normal seed yield, even though the shoots and developing seeds are genetically mutant [18] (Figure S4).

To generate a seed stock of the *pho1 phoh1* double mutant, segregating seed population obtained from *pho1/PHO1 phoh1/phoh1* or *pho1/pho1 phoh1/PHOH1* plants was germinated in an agar-based medium and then shoots were grafted onto WT roots. The resulting grafted plants were grown in soil and subsequently genotyped to identify the grafts with shoots derived from homozygous *pho1 phoh1* plants.

Analysis of seed size distribution, average seed weight as well as total P and Pi content in fully mature dry seeds did not reveal a significant and reproducible difference between WT/WT self-grafted controls (for all grafts the numerator represents the shoot and the denominator represents the root) and grafts of two independent double mutants made using distinct *pho1* and *phoh1* alleles, namely the *pho1-1 phoh1-1* double mutant (named thereafter DM1.1) and the *pho1-2 phoh1-4* double mutant (named thereafter DM2.4) (Figure 3). Analysis by light microscopy of seed development in *pho1-1*/WT and DM1.1/WT grafted plants did not detect any significant changes compared to WT/WT self-grafted controls (Figure S5).

Flux of Pi from seed coat to embryos

Expression of PHO1 and PHOH1 in the CZSC suggested that transfer of Pi from the maternal seed coat to the filial embryo could be compromised in the corresponding mutants. To examine this, a seed Pi flux assay was developed whereby ^{33}Pi was supplied to siliques with embryos at the mature green stage via the immersion of the cut end of the inflorescence in a radioactive solution for 12-18 hours, followed by manual dissection of the seed coat from the embryo and determination of ^{33}P content in both fractions. While the distribution of ^{33}P was approximately equal between seed coat and embryo for seeds of WT/WT and either *phoh1-1*/WT or *phoh1-4*/WT, a higher proportion of ^{33}P was found in the seed coat of seeds from both *pho1-1*/WT and *pho1-2*/WT as well as for the double mutant combinations DM1.1/WT and DM2.4/WT (Figure 4A, B). No enhancement in seed coat ^{33}P retention was observed when seeds from the DM/WT plants were compared to the *pho1*/WT plants. Since PHO1 is known to mediate Pi export but not sulfate (SO$_4$) export [15], ^{35}SO$_4$ distribution between seed coat and embryo was used as a control. None of the *pho1*/WT, *phoh1*/WT or DM/WT grafts tested showed significant differences to WT/WT grafts in the distribution of radioactive sulfur (^{35}S) between embryo and seed coat. Together, these data indicate that *PHO1* but not *PHOH1* had a significant role to play in the transfer of Pi from seed coat to embryo at mature green stage of development.

CZSC-specific expression and down-regulation of PHO1

To further examine the implication of the expression of PHO1 in the CZSC for seed Pi homeostasis, the gene was cloned as a GFP fusion (*PHO1-GFP*) under the control of the CZSC-specific promoter from the *pARR22* gene [22]. The construct was first transformed into DM1.1/WT grafted plants, the resulting seeds were selected on agar-based medium for hygromycin resistance and then immediately grafted onto a WT root. Successful grafts from independent transgenic plants were grown in soil to generate a stock of transgenic seeds named DM1.1-CP. Expression of PHO1-GFP specifically to the CZSC was confirmed in the DM1.1-CP lines by confocal microscopy (Figure 5A).

Using the ^{33}Pi seed flux assay, it was observed that the CZSC-specific expression of PHO1-GFP in the DM1.1 genetic background line resulted in a decrease in the proportion of ^{33}P that is found in the seed coat, with values being similar to WT/WT plants (Figure 5B). No

significant change in the distribution of ^{35}S could be observed when ^{35}SO$_4$ was used instead of ^{33}Pi (Figure 5C).

An artificial microRNA aimed at down-regulating the expression of the *PHO1* gene (amiRNA*PHO1*) was constructed and shown to efficiently reproduce the phenotype of the *pho1* mutant when expressed under the *PHO1* promoter (Figure S6). The same amiRNA*PHO1* construct was then placed under the control of the *pARR22* promoter and transformed into ungrafted WT plants to generate the independent transgenic lines amiRNA1.2 and amiRNA2.3. The ^{33}Pi/^{35}SO$_4$ seed flux assay revealed an increase in the proportion of ^{33}P that is retained in the seed coat of these two transgenic lines compared to the WT control, while the flux of ^{35}SO$_4$ was unchanged (Figure 6). Altogether, these experiments showed that expression of *PHO1* specifically in the CZSC was sufficient to modify the flux of Pi from the seed coat to the embryo at the mature green stage of seed development.

Impact of PHO1 on the P content in seeds

The impact of PHO1 expression on the distribution of total P between embryo and seed coat was first measured at the mature green stage of development (Figure 7A). The proportion of total P in the seed coat was significantly higher in DM/WT and *pho1*/WT compared to WT/WT but not in *phoh1*/WT seeds. Furthermore, CZSC-specific expression of *PHO1* in transformed DM plants DM1.1-CP.1 and DM1.1-CP.2 decreased the proportion of P in the seed coat to levels similar to WT/WT plants. However, analysis of the distribution of total P in fully mature dry seeds did not show a significant difference between the DM/WT plants and the WT/WT control (Figure 7B). Similarly, distribution of total P in fully mature dry seeds did not show a significant difference between the Pi-deficient plants DM/*pho1.2* and the WT/*pho1.2* control (Figure 7C).

8

Discussion

The seed coat plays numerous important roles in seed physiology, including the protection of the developing embryo and the control of both dormancy and germination [23]. The CZSC is, however, distinctive from the rest of the seed coat in several aspects. Being the site where the vascular strand ends in many seeds, including Arabidopsis, the CZSC represents an area of nutrient unloading and an important interface connecting the maternal and filial tissues [2]. The Arabidopsis CZSC epidermis is morphologically distinct from the more distal outer seed coat, with no secondary wall thickening made of cellulose or mucilage, and fewer and smaller starch granules [24]. Transcriptomic analysis of the CZSC and distal seed coat showed that while these tissues share extensive gene expression patterns early in development, they have distinct transcriptional programs later in the maturation phases. For example, numerous genes involved in either water, sugar and amino acid transport are more expressed in the maturing CZSC than the distal seed coat [24].

Analysis of plants expressing GFP from the companion cell-specific *SUC2* promoter showed that the symplastic post-phloem movement of GFP in developing seeds was restricted to the CZSC, or unloading domain, and that no GFP could move to the more distant seed coat layers or towards the endosperm [2]. If the symplastic path to Pi is the same as GFP, this would imply that transfer of Pi beyond the CZSC would require an export to the apoplasm. The current work shows that at the mature green stage of seed development, PHO1 expression in the CZSC accounted for nearly half of the Pi flux from seed coat to embryo (Figure 4, 5). These results reveal that Pi export to the CZSC apoplast is an important step for the subsequent acquisition of Pi by the developing embryo. However, the observation that in the absence of PHO1 the embryo still receives enough Pi to sustain normal seed development implies that other genes and/or pathways also contribute to the transfer of Pi from the maternal to the filial tissues. The expression pattern of PHO1 in the CZSC is similar to the amino acid facilitator UmamiT11 and 14, involved in amino acid export from the seed coat, while UmamiT28 was expressed in the endothelium and cellularized endosperm, and UmamiT29 in the seed coat inner and outer integuments. Interestingly, single mutants in any of these four UmamiT genes resulted in an increase in amino acid content in seeds, indicating that efflux of amino acids in all of these tissues contribute to the seed nitrogen content [6]. Similar involvement of several SWEET sucrose exporters in distinct seed tissues, including the micropyle, endosperm, CZSC and seed coat integuments, are thought to sequentially

9

contribute to sucrose export to the embryo [5]. Transcriptomic data of developing Arabidopsis seeds provide clues to other genes that could contribute to Pi flux from maternal to filial tissues [20]. For example, the *PHO1* homologues *PHO1;H3*, *PHO1;H5* and *PHO1;H8* are also expressed in various seed tissues, including endosperm, CZSC and distal seed coat, and could thus potentially act redundantly or complementarily with PHO1 in seed Pi homeostasis (Figure S7).

While DM/WT grafts showed an increased accumulation of P in the seed coat at the mature green stage of seed development compared to the WT/WT control, fully mature dry seeds of DM/WT grafts show no difference in P distribution compared to WT/WT control (Figure 7A, B). There was also no significant difference in P distribution in fully mature seeds between DM and WT when plants were subjected to Pi deficiency via the use of a *pho1* rootstock in the grafts (DM/*pho1-2* versus WT/*pho1-2*) (Figure 7 C). These data indicate that between the mature green stage of seed development and the fully mature dry seeds, P that accumulated in the seed coat was redistributed to the embryo. During the early stage of seed development, the Arabidopsis seed coat is only 5-cell thick on most of the seed surface and all layers die during the late stage of seed maturation [25, 26]. Release of Pi into the seed apoplast from the dying cells of the seed coat and its subsequent acquisition by the embryo may explain the late P redistribution between seed coat and embryo. P is known to be highly mobile element in plants as compared to microelements, such as Zn, which have limited mobility [27]. Such difference in element mobility may explain why mutation in the seed coat Zn exporter HMA led to zinc accumulation in the mature seed coat while no such accumulation is evident for P in the *pho1* mutant [7].

PHOH1 has previously been shown to complement the *pho1* mutant when expressed under the control of the *PHO1* promoter [18]. Furthermore, the *pho1 phoh1* double mutant has lower root-to-shoot Pi transfer capacity compared to the single *pho1* mutant [18]. These data indicated that like PHO1, PHOH1 also mediate Pi export to the root xylem apoplast. However, in developing seeds, expression of *PHOH1* did not play a significant role in the transfer of Pi from the seed coat to the embryo. This conclusion is supported by the absence of statistically significant difference in Pi distribution or Pi flux from seed coat to embryo in mature green seeds between either WT and *phoh1*, or *pho1* and *pho1 phoh1* (Figure 4, 7). The lack of evidence for a role of PHOH1 in seed Pi homeostasis was surprising considering the overlap in the expression domains of PHO1-GUS and PHOH1-GUS in developing seeds. It is

possible that distinct post-translational regulation exists between these proteins in developing seeds influencing their Pi export activity.

The expression of PHO1 in the megaspore mother cell, functional megaspore and inner integuments is intriguing. Although ovule development has not been examined in detail in the *pho1* mutant, *pho1*/WT or DM/WT grafted plants show no evidence of reduced fertility compared to WT/WT control, indicating that the ovules of the *pho1* mutant are competent for fertilization. It is possible that functional redundancy exists between *PHO1* and other members of the *PHO1* gene family expressed during ovule development, thus masking any phenotypes in the single *pho1* mutant. While the need for Pi export in the developing ovule is unclear, PHO1 may not only be important in Pi transfer between tissues (e.g. root to shoot, and seed coat to embryo) but may also play more subtle roles in regulating ion fluxes associated with signaling pathways in specific cells, such as the role of PHO1 in the response of guard cells to abscisic acid [19]. It is thus perhaps in that context that PHO1 participates in ovule development.

P content in seeds is an important agricultural trait. On one hand, high seed P content can promote early seedling establishment and vigor, which can positively impact yield [28]. On the other hand, harvest of grains with high P content is an important factor in the global P cycle, driving soil P depletion that must be replenished via the application of fertilizers, a practice associated with risks of eutrophication [29]. Furthermore, phytate, the main storage form of P in seeds, is an efficient chelator of essential microelements, such as zinc, and high phytate content in cereals is associated with microelement deficiencies [30]. There is thus a need to optimize P accumulation in seeds to maintain seedling vigor while limiting its negative impact on global P cycling and animal nutrition [31]. In this context, this work on the role of PHO1 in seeds represents an important first step in the quest to gain fundamental insights into the molecular players participating in seed P homeostasis in model plants, such as Arabidopsis, and crops.

Author contributions

E.V. and Y.P. designed experiments and wrote the paper. E.V., C.B. and Y.P interpreted the data. E.V. performed most of the experiments. Y.P. elaborated of the assay to measure total P in seeds and the seed Pi flux, J.-Y.J. created and tested the amiRNA*PHO1* construct, and C.B. performed the imaging of PHO-YFP in the developing ovules and seeds. Y.P. supervised the whole research project.

References

1. Zhang, W.H., Zhou, Y.C., Dibley, K.E., Tyerman, S.D., Furbank, R.T., and Patrick, J.W. (2007). Nutrient loading of developing seeds. Funct. Plant Biol. *34*, 314-331.

2. Stadler, R., Lauterbach, C., and Sauer, N. (2005). Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplastic domains in Arabidopsis seeds and embryos. Plant Physiol. *139*, 701-712.

3. Patrick, J.W., and Offler, C.E. (2001). Compartmentation of transport and transfer events in developing seeds. J. Exp. Bot. *52*, 551-564.

4. Tegeder, M., and Rentsch, D. (2010). Uptake and partitioning of amino acids and peptides. Mol Plant *3*, 997-1011.

5. Chen, L.Q., Lin, I.W.N., Qu, X.Q., Sosso, D., McFarlane, H.E., Londono, A., Samuels, A.L., and Frommer, W.B. (2015). A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. Plant Cell *27*, 607-619.

6. Muller, B., Fastner, A., Karmann, J., Mansch, V., Hoffmann, T., Schwab, W., Suter-Grotemeyer, M., Rentsch, D., Truernit, E., Ladwig, F., et al. (2015). Amino acid export in developing Arabidopsis seeds depends on UmamiT facilitators. Curr. Biol. *25*, 3126-3131.

7. Olsen, L.I., Hansen, T.H., Larue, C., Osterberg, J.T., Hoffmann, R.D., Liesche, J., Kramer, U., Surble, S., Cadarsi, S., Samson, V.A., et al. (2016). Mother-plant-mediated pumping of zinc into the developing seed. Nature Plants *2*, 16036.

8. Nussaume, L., Kanno, S., Javot, H., Marin, E., Pochon, N., Ayadi, A., Nakanishi, T.M., and Thibaud, M.C. (2011). Phosphate import in plants: focus on the PHT1 transporters. Front Plant Sci *2*, 83.

9. Li, Y.T., Zhang, J., Zhang, X., Fan, H.M., Gu, M., Qu, H.Y., and Xu, G.H. (2015). Phosphate transporter OsPht1;8 in rice plays an important role in phosphorus redistribution from source to sink organs and allocation between embryo and endosperm of seeds. Plant Sci. *230*, 23-32.

10. Shukla, V., Kaur, M., Aggarwal, S., Bhati, K.K., Kaur, J., Mantri, S., and Pandey, A.K. (2016). Tissue specific transcript profiling of wheat phosphate transporter genes and its association with phosphate allocation in grains. Sci Rep *6*, 39293.

11. Zhang, F., Sun, Y.F., Pei, W.X., Jain, A., Sun, R., Cao, Y., Wu, X.N., Jiang, T.T., Zhang, L., Fan, X.R., et al. (2015). Involvement of OsPht1;4 in phosphate acquisition and mobilization facilitates embryo development in rice. Plant J. *82*, 556-569.

12. Hamburger, D., Rezzonico, E., MacDonald-Comber Petétot, J., Somerville, C., and Poirier, Y. (2002). Identification and characterization of the *Arabidopsis PHO1* gene involved in phosphate loading to the xylem. Plant Cell *14*, 889-902.

13. Poirier, Y., Thoma, S., Somerville, C., and Schiefelbein, J. (1991). A mutant of *Arabidopsis* deficient in xylem loading of phosphate. Plant Physiol. *97*, 1087-1093.

14. Secco, D., Baumann, A., and Poirier, Y. (2010). Charcaterization of the rice *PHO1* gene family reveals a key role for *OsPHO1;2* in phosphate homeostasis and the evolution of a distinct clade in dicotyledons. Plant Physiol. *152*, 1693-1704.

15. Arpat, A.B., Magliano, P., Wege, S., Rouached, H., Stefanovic, A., and Poirier, Y. (2012). Functional expression of PHO1 to the Golgi and *trans*-Golgi network and its role in export of inorganic phosphate. Plant J. *71*, 479-491.

16. Giovannini, D., Touhami, J., Charnet, P., Sitbon, M., and Battini, J.-L. (2013). Inorganic phosphate export by the retrovirus receptor XPR1 in metazoans. Cell Rep *3*, 1866-1873.

17. Wege, S., and Poirier, Y. (2014). Expression of the mammalian XENOTROPIC POLYTROPIC VIRUS RECEPTOR 1 (XPR1) in tobacco leaves leads to phosphate export. FEBS Lett. *588*, 482-489.

18. Stefanovic, A., Ribot, C., Rouached, H., Wang, Y., Chong, J., Belbahri, L., Delessert, S., and Poirier, Y. (2007). Members of the *PHO1* gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. Plant J. *50*, 982-994.

19. Zimmerli, C., Ribot, C., Vavasseur, A., Bauer, H., Hedrich, R., and Poirier, Y. (2012). PHO1 expression in guard cells mediates the stomatal response to abscisic acid in Arabidopsis. Plant J. *72*, 199-211.

20. Belmonte, M.F., Kirkbride, R.C., Stone, S.L., Pelletier, J.M., Bui, A.Q., Yeung, E.C., Hashimoto, M., Fei, J., Harada, M., Munoz, M.D., et al. (2013). Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. Proc. Natl. Acad. Sci. USA *110*, E435-E444.

21. Stefanovic, A., Arpat, A.B., Bligny, R., Gout, E., Vidoudez, C., Bensimon, M., and Poirier, Y. (2011). Overexpression of PHO1 in Arabidopsis leaves reveals its role in mediating phosphate efflux. Plant J. *66*, 689-699.

22. Horak, J., Grefen, C., Berendzen, K.W., Hahn, A., Stierhof, Y.D., Stadelhofer, B., Stahl, M., Koncz, C., and Harter, K. (2008). The *Arabidopsis thaliana* response regulator ARR22 is a putative AHP phospho-histidine phosphatase expressed in the chalaza of developing seeds. BMC Plant Biol. *8*.

23. Radchuk, V., and Borisjuk, L. (2014). Physical, metabolic and developmental functions of the seed coat. Front Plant Sci *5*, 510.

24. Khan, D., Millar, J.L., Girard, I.J., and Belmonte, M.F. (2014). Transcriptional circuitry underlying seed coat development in Arabidopsis. Plant Sci. *219*, 51-60.

25. Beeckman, T., De Rycke, R., Viane, R., and Inze, D. (2000). Histological study of seed coat development in *Arabidopsis thaliana*. J. Plant Res. *113*, 139-148.

26. Haughn, G., and Chaudhury, A. (2005). Genetic analysis of seed coat development in Arabidopsis. Trends Plant Sci. *10*, 472-477.

27. Marschner, H. (1995). Mineral nutrition of higher plants, (London: Academic Press).

28. White, P.J., and Veneklaas, E.J. (2012). Nature and nurture: the importance of seed phosphorus content. Plant Soil *357*, 1-8.

29. MacDonald, G.K., Bennett, E.M., Potter, P.A., and Ramankutty, N. (2011). Agronomic phosphorus imbalances across the world's croplands. Proc. Natl. Acad. Sci. USA *108*, 3086-3091.

30. Raboy, V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. Plant Sci. *177*, 281-296.

31. Wang, F.M., Rose, T., Jeong, K., Kretzschmar, T., and Wissuwa, M. (2016). The knowns and unknowns of phosphorus loading into grains, and implications for phosphorus efficiency in cropping systems. J. Exp. Bot. *67*, 1221-1229.

32. Liu, T.Y., Huang, T.K., Tseng, C.Y., Lai, Y.S., Lin, S.I., Lin, W.Y., Chen, J.W., and Chiou, T.J. (2012). PHO2-dependent degradation of PHO1 modulates phosphate homeostasis in *Arabidopsis*. Plant Cell *24*, 2168-2183.

33. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods *9*, 676-682.

34. Turnbull, C.G.N., Booker, J.P., and Leyser, H.M.O. (2002). Micrografting techniques for testing long-distance signalling in Arabidopsis. Plant J. *32*, 255-262.

35. Marsch-Martínez, N., Franken, J., Gonzalez-Aguilera, K., de Folter, S., Angenent, G., and Alvarez-Buylla, E. (2013). An efficient flat-surface collar-free grafting method for *Arabidopsis thaliana* seedlings. Plant Methods *9*, 14.

36. Ames, B.N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol. *8*, 115-118.

37. Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods *9*, 671-675.

38. Curtis, M.D., and Grossniklaus, U. (2003). A gateway cloning vectors set for high-throughput functional analysis of genes in planta. Plant Physiol. *133*, 462-469.

39. Gattolin, S., Alandete-Saez, M., Elliott, K., Gonzalez-Carranza, Z., Naomab, E., Powell, C., and Roberts, J.A. (2006). Spatial and temporal expression of the response regulators ARR22 and ARR24 in *Arabidopsis thaliana*. J. Exp. Bot. *57*, 4225-4233.

40. Schwab, R., Ossowski, S., Riester, M., Warthmann, N., and Weigel, D. (2006). Highly specific gene silencing by artificial microRNAs in Arabidopsis. Plant Cell *18*, 1121-1133.

41. Koncz, C., and Schell, J. (1986). The promoter of Tl-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of *Agrobacterium* binary vector. Mol. Gen. Genet. *204*, 383-396.

42. Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. *6*, 735-743.

43. Logemann, E., Birkenbihl, R.P., Ulker, B., and Somssich, I.E. (2006). An improved method for preparing Agrobacterium cells that simplifies the Arabidopsis transformation protocol. Plant Methods *2*.

44. Neff, M.N., Neff, J.D., Chory, J., and Pepper, A.E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. Plant J. *14*, 387-392.

Figure legends

Figure 1. Expression pattern of the PHO1 and PHOH1 in reproductive tissues and developing seeds. PHO1-GUS (A-C) and PHOH1-GUS (D-E) fusion protein expressed in anthers and pistils before pollen release (A, D),during the globular (B, E) and mature green stage (C, F) of seed development. MSC; mycropile seed coat, CZSC; chalazal seed coat. See also Figure S1, S2, S3 and S7.

Figure 2. Expression pattern of PHO1-GUS during ovule development. PHO1-GUS was expressed in the megaspore mother cell (MMC) of ovules at the 3-I stage (A), the L1 layer and the functional megaspore (FM) of the ovules between stage 3-II (early and late 2-nucleate) and stage 3-V (B, C and D), and the inner integuments (I.I.) of the stage 3-V, 3-VI and 4-II stages (E, F and G). Bars = 50 μm. See also Figure S2.

Figure 3. Size, Pi and P content of seeds produced from WT/WT, DM1.1/WT and DM2.4/WT plants. (A) Distribution of size (x-axis represents seed area measured as pixels using ImageJ) of fully mature dry seeds. (B) Average weight of 100 mature dry seeds. Error bars denote standard deviations with n=7. P (C) and Pi (D) content of fully mature dry seeds. Error bars denote standard deviations with n=4. For B, C and D, no statistical difference (p>0.05) to WT/WT control according to Student t-test. See also Figure S4 and S5.

Figure 4. ^{33}P and ^{35}S distribution between seed coat and embryo in mutant seeds at the mature green stage of development. For all panels, black and grey represent the % value of radioactivity distribution between seed coat and embryo, respectively, after 18h of supply of either $^{33}PO_4$ (A, B) or $^{35}SO_4$ (C,D) to the inflorescence. Error bars denote standard deviations with n=8-10. Asterisks denote statistical significance (p < 0.05) compared to WT/WT control according to Student t-test.

Figure 5. Specific expression of PHO1-GFP in the CZSC complements the P distribution phenotype at the mature green stage of development. (A) Localization of PHO1-GFP fusion protein expressed using the chalazal seed coat-specific promoter pARR22 (right panel) compared to WT control (left panel). CZSC; chalazal seed coat. For panels B and C, black and grey represent the % value of radioactivity distribution between seed coat and embryo, respectively, after 18h of supply of either $^{33}PO_4$ (B) or $^{35}SO_4$ (C) to the inflorescence. Error

bars denote standard deviations with n=8-10. Asterisks denote statistical significance (p < 0.05) compared to WT/WT control according to Student t-test.

Figure 6. ^{33}P and ^{35}S distribution between seed coat and embryo in seeds at the mature green stage of development in plants expressing a CZSC-specific amiRNA targeting *PHO1*. Seeds from two independent transgenic lines (amiRNA1.2 and amiRNA2.3) were compared to WT. For all panels, black and grey represent the % value of radioactivity distribution between seed coat and embryo, respectively, after 18h of supply of either $^{33}PO_4$ (A) or $^{35}SO_4$ (B) to the inflorescence. Error bars denote standard deviations with n=8-10. Asterisks denote statistical significance (p < 0.05) compared to WT control according to Student t-test. See also Figure S6.

Figure 7. Total P distribution between seed coat and embryo in seeds. For all panels, black and grey represent the % value of P between seed coat and embryo, respectively. Analysis of P distribution from seeds (A) at the mature green stage of development, or (B, C) in fully mature dry seeds. Error bars denote standard deviations with n=8-10. Asterisks denote statistical significance (p < 0.05) compared to WT/WT control according to Student t-test.

STAR METHODS

KEY RESOURCES TABLE

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yves Poirier (yves.poirier@unil.ch).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All *A. thaliana* plants were of the Columbia-0 (Col-0) ecotype. The *pho1-1* and *pho1-2* mutants were previously described [12], as well as the *phoh1-1* mutant [18]. The *phoh1-4* allele was derived from the GABI line 462F04. Transgenic plants expressing the PHO1-YFP fusion under the *PHO1* promoter were kindly provided by Tzyy-Jen Chiou (National Chung-Hsing University and Academia Sinica, Taiwan) and were previously described [32]. For *in vitro* experiments, plants were first surface-sterilized with 15% NaClO for 4 minutes followed by two washes with sterile water before sowing on half-strength Murashige and Skoog (MS) salts (2.2g/L) containing 1% (w/v) sucrose and 0.8% (w/v) agar. Plants were typically grown in soil at 22°C, 60% humidity, 100 μmol m^{-2} sec^{-1} of white light and a photoperiod of 16h light/8h dark.

METHOD DETAILS

Microscopy

Localization of both PHO1-GUS and PHOH1-GUS proteins in reproductive tissues and seeds was performed using either a Leica DM5000 or DM5500 microscope with transmitted light and differential interference contrast (DIC). Ovules and seeds were dissected and cleared with chloral hydrate solution (2.7g/ml in 30% glycerol) for several minutes before imaging. Imaging of PHO1-GFP and PHOH1-GFP signals was performed using confocal laser scanning microscopy with one of the following instruments: Zeiss LSM 700 or LSM 710 with Apochromat 63× NA1.2 water immersion or multi-immersion objective, or Leica SP5R with a HC PL APO CS2 63x NA1.3 glycerol immersion objective. A 488nm excitation line was used and fluorescence was collected within a 500-540nm range. Whole-mount ovule primordia, mature ovules and seeds were carefully dissected from *Arabidopsis* transgenic plants just before imaging and freshly mounted in sterile, cold water, or counterstained with 20 μg/ml FM4-64 for 15 min (young ovules and seeds) and 1 h (mature seeds), on ice, before

imaging. Projection from image series was done using Imaris 8.3.1 (Bitplane, Switzerland) and single-plane channel overlay was done in Leica Advanced SuiteX-Light or Fiji [33].

Hypocotyl micro-grafting.

Micro-grafting was performed essentially as previously described [34] except that the cotyledons of the seedlings were removed [35]. Seedlings for grafting were first grown vertically on plate containing half-strength MS salts with 0.5% (w/v) sucrose and 0.8% (w/v) agar for 5 d in a growth cabinet under 100 μmol m^{-2} sec^{-1} white light and short-day conditions (8 h light and 16 h dark) at 26°C. Coyteldons were removed and the hypocotyls were sectioned transversely using a sharp razor blade. The scions and rootstocks were inserted in a short (2 mm) sterile medical-grade silicon tubing (0.3 mm diameter). Plants were then returned to the growth cabinet and grown for a further 7 d on the same media and growth conditions. Graft unions were examined for the absence of adventitious roots, and successful grafts were transferred to soil.

Glucuronidase staining

Seedlings, ovules and developing seeds were dissected and stained for GUS activity by infiltrating, under mild vacuum, a solution containing 1mM (seedlings, developing seeds) or 2mM (ovules) 5-bromo-4-chloro-3-indolyl-β-glucoronic acid (X-Gluc), 10mM EDTA, 2mM potassium ferricyanide, 2mM potassium ferrocyanide, 0.1 % Triton X100 and 100mM sodium phosphate, pH7. The enzymatic reaction was performed overnight at 37°C in the dark. Stained tissues were washed with a graded ethanol series (10% - 80%) and cleared in chloral hydrate solution (2.7g/ml in 30% glycerol).

$^{33}PO_4$ and $^{35}SO_4$ flux measurement in seeds.

Inflorescences with maturing siliques were cut at the base with a razor blade and placed for 16 h in an Eppendorf tube that contained 1 ml of 10 μCi/ml $^{33}PO_4$ or $^{35}SO_4$ in water. Seed coat and embryo from seeds (5 seeds per sample, 8-10 samples) were manually dissected under a microscope and placed in vials filled with 5 ml of Ultima Gold scintillation liquid. Radioactivity was measured in the Perkin Elmer tri-carb 2800TF Scintillation counter.

Inorganic phosphate and total phosphorus measurements

For the determination of Pi content of leaves, the Pi content was first released from plant tissues by repeated freeze-thaw cycles into distilled water. For seeds, 4 mg per sample were

homogenized in 0.4N HCl and shaken for 3.5 hours at room temperature. The Pi concentration in the solution was then quantified by the molybdate colorimetric assay [36]. Briefly, a master mix of 6 volumes of 0.42% (w/v) ammonium molybdate in 1N H_2SO_4 and 1 volume of 10% ascorbic acid in water was prepared, distributed in 96-well plates and was mixed with the sample in a 4:1 proportion, respectively. The plates were incubated at 37°C for 30 minutes and the absorbance read at 820 nm. Phosphoric acid was used to make a standard curve.

Total P measurement of dissected seed coats and embryos (5 per sample) at the mature green stage of development or of fully mature seeds (6 per sample) was done using a wet ashing procedure, converting P into Pi. Samples were heated for 16 h at 80°C in borosilicate tubes containing 150 µl of 90% HNO_3 in a well-ventilated hood. The tubes were next heated at 145°C to completely evaporate the HNO_3. After cooling at room temperature (RT), 60 µl of 10% (w/v) $Mg(NO_3)_2$ in ethanol was added and the tubes were first heated at 100°C to slowly evaporate the ethanol, and then heated at 300°C for 2h. After cooling down at RT, 300 µl of 1N H_2SO_4 was added, the tubes were closed with a Teflon-lined screw cap and incubated at 100°C for 1h. After cooling down at RT, concentration of Pi was determined by the molybdate colorimetric assay as described above. Phytic acid was used to make a standard curve.

Seed size measurements.

Dried seeds were photographed using a Leica MZ16 FA fluorescent stereomicroscope. The size (area) of seeds were analyzed with the ImageJ software [37].

Cloning. Genomic sequences from the start to the penultimate codon of *PHO1* (5483bp) and of *PHOH1* (3866bp) were first cloned into pENTR2B vectors using the In-Fusion HD Cloning System (Clontech) (see Table S1 for list of oligonucleotides). The insert was then transferred, using the Gateway Cloning System (InVitrogen) into the binary plant expression vectors pMDC107 (for GFP fusions) [38] and pMDC163 (for GUS fusions) [38] that were modified by replacing the CaMV35S promoter with either 2 kb sequence upstream of the translation start of *PHO1* or *PHOH1* gene [18], or the 1150 bp fragment containing the promoter of the pARR22 (At3g04280) gene using the oligos ARR22-R and ARR22-F [39]. An amiRNA aimed at targeting the *PHO1* gene was constructed by modifying the endogenous amiR-319 [40] using the oligos miRNA-PHO1-1-F and miRNA-PHO1-1-R (Table S1). The resulting amiR-PHO1 was introduced, using the Gateway Cloning System, into a modified pMDC32 [38] where the CaMV35S promoter was replaced by the pARR22 promoter.

All the constructs were introduced into *Agrobacterium tumefaciens* pGV3101(pMP90) [41] and used for transformation of *A. thaliana* by the flower dip method [42, 43]. Bacteria were first grown at 30°C on plates containing Luria Broth, 2% agar, 50 µg/ml kanamycin and 50 µg/ml spectinomycin. Bacteria were scraped from the plates and resuspended in 120 ml of 5% (w/v) sucrose solution with 0.03% Silwet L-77 to reach an OD_{600} of 0.8. Flowers of plants were subsequently dipped into the bacterial solution for 10 seconds. The procedure was repeated once more after 1 week to ensure a high transformation rate. Transformed plants were kept in a long day chamber and seeds were harvested upon full maturation.

Genotyping

Genotyping the *pho1-1* and *pho1-2* alleles was done by digesting PCR fragments of the *PHO1* gene with restriction enzymes generating bands that are distinct for the various genotypes, according to the CAPS/dCAPS technique [44]. Oligonucleotides and restriction enzymes used to genotype the different *pho1* and *phoh1* alleles are shown in Table S2.

QUANTIFICATION AND STATISTICAL ANALYSIS

Values are presented as mean ± SD. Student's t test was used for statistical analyses, with $p < 0.05$ considered significant. The number of replicates and sample sizes for all experiments are indicated in the figure legends.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Agrobacterium tumefaciens pGV3101(pMP90)	Koncz and Schell, [41]	N/A
Chemicals, Peptides, and Recombinant Proteins		
Murashige and Skoog medium	Duchefa Biochemie	M0221
sucrose	AppliChem	131621
agar	AppliChem	A2111
chloral hydrate	Acros Organics	232475000
glycerol	AppliChem	A2926
FM4-64	Invitrogen	T13320
silicon tubing	Freudenberg Medical	www.freudenbergmedical.com
^{33}P phosphoric acid	Hartmann Analytic	FF-1
^{35}S sulfuric acid	Hartmann Analytic	S-RA-1
Ultima Gold scintillation liquid	Perkin Elmer	6013329
Hydrochloric acid	Sigma-Aldrich	30721
Nitric acid	Fischer Scientific	9598-34
Ammonium molybdate	Sigma-Aldrich	09878
Ascorbic acid	Sigma-Aldrich	A0278
$Mg(NO_3)_2$	Sigma-Aldrich	M5296
Sulfuric acid	Merck Millipore	1.00731
Ethanol	Fischer Scientific	E/0650DF/17
Phytic acid	Sigma-Aldrich	P5681
BsrI (BseNI) restriction enzyme	ThermoFisher	FD0884
MfeI (MnuI) restriction enzyme	ThermoFisher	ER0752
5-bromo-4-chloro-3-indolyl-β-glucoronic acid	Duchefa Biochemie	X1405
potassium ferricyanide	Sigma-Aldrich	60299
potassium ferrocyanide	Sigma-Aldrich	60279

Triton X100	AppliChem	A4975
Phosphoric acid	Sigma-Aldrich	79617
Kanamycin sulfate	Sigma-Aldrich	60615
Spectinomycin sulfate	Alfa Aesar	J61820
Luria Broth	Difco	244620
Silwet L-77	Lehle Seeds	VIS-30
Experimental Models: Organisms/Strains		
pho1-1	Hamburger et al., [12]	N/A
pho1-2	Hamburger et al., [12]	N/A
phoh1-1	Stefanovic et al., [18]	N/A
phoh1-4	GABI collection (www.gabi-kat.de)	line 462F04
pho1-1 phoh1-1	This study	N/A
pho1-2 phoh1-4	This study	N/A
Col-0	ABRC (arabidopsis.org)	CS60000
PHO1-YFP in Col-0	Liu et al., [32]	N/A
Oligonucleotides		
Oligonucleotides used for plasmid construction and genotyping are listed in Table S1 and S2.	This study	N/A
Recombinant DNA		
pENTR2B	Invitrogen	A10463
pMDC107	Curtsi and Grossniklaus [38]	N/A
pMDC163	Curtsi and Grossniklaus [38]	N/A
amiR-319	Schwab et al., [39]	N/A
pMDC32	Curtsi and Grossniklaus [38]	N/A
Software and Algorithms		
Imaris	Bitplane	www.bitplane.com

LASX-light	Leica	www.leica-microsystems.com
Fiji	Schindelin et al., [33]	https://fiji.sc/
ImageJ	Schneider et al., [37]	imagej.net
Others		
In-Fusion HD Cloning System	Clontech	638909
Gateway LR Clonase	Invitrogen	12538120

Figure 1

Figure 2

3-I

A

MMC

3-II early

B

L1 layer

FM

3-II late

C

L1 layer

L1 layer

FM

3-V

D

L1 layer

FM

E

I.I.

3-V

F

I.I.

I.I.

3-VI

G

I.I.

4-II
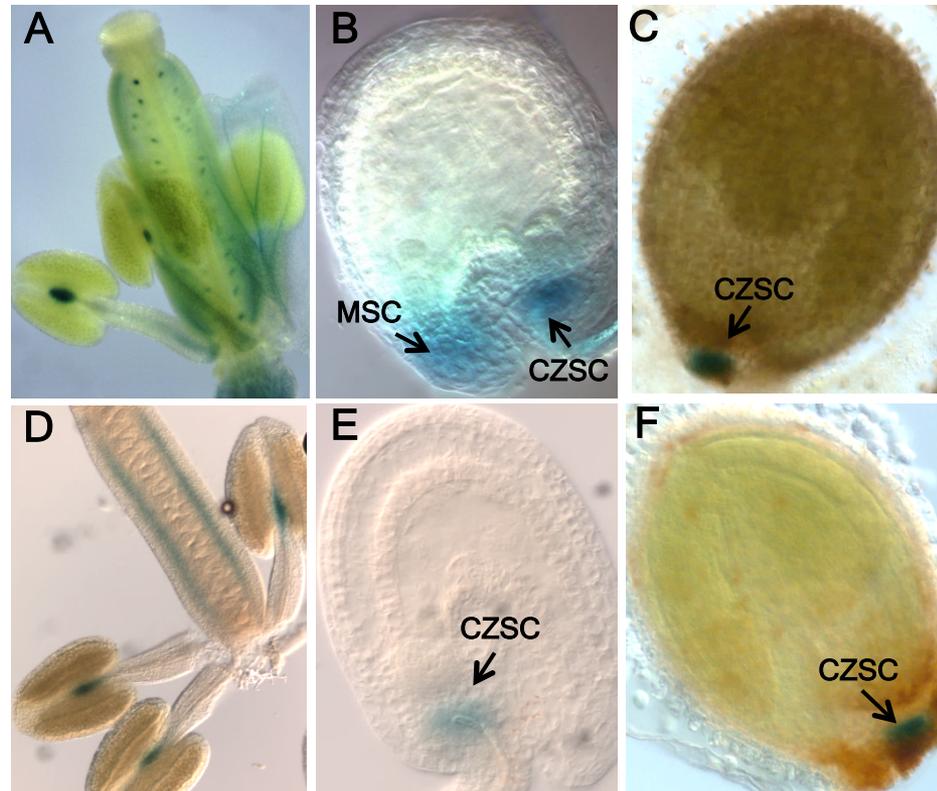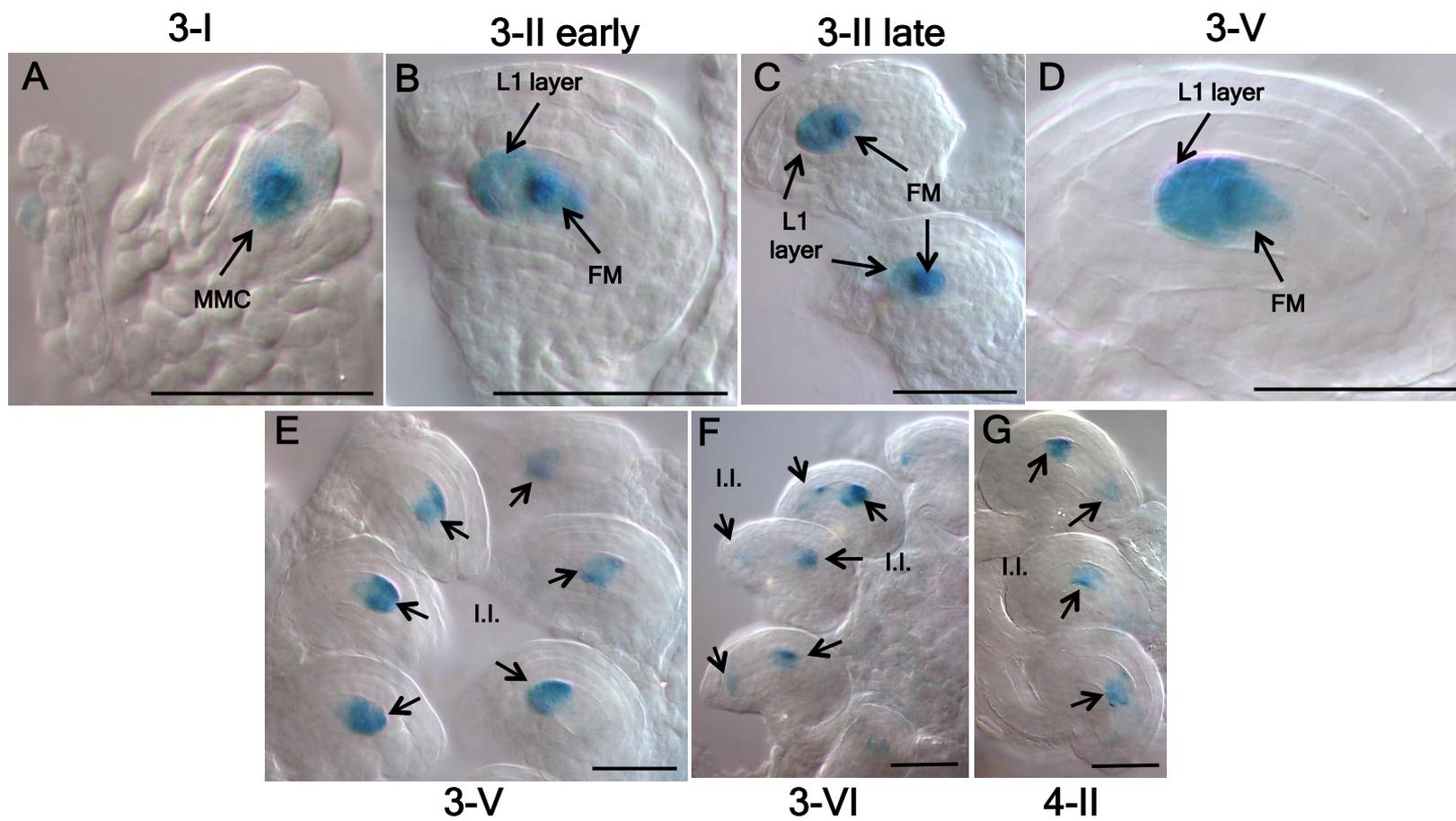
Figure 3

Figure 4

Figure 5

A

Control   DM1-CP.1/WT

CZSC

B

^{33}P distribution (%)

WT/WT   DM1.1/WT   DM1.1-CP.1/WT   DM1.1-CP.2/WT

C

^{35}S distribution (%)

WT/WT   DM1.1/WT   DM1.1-CP.1/WT   DM1.1-CP.2/WT
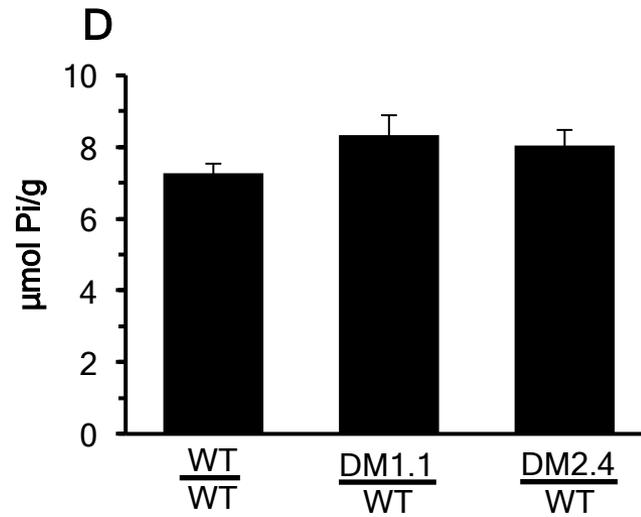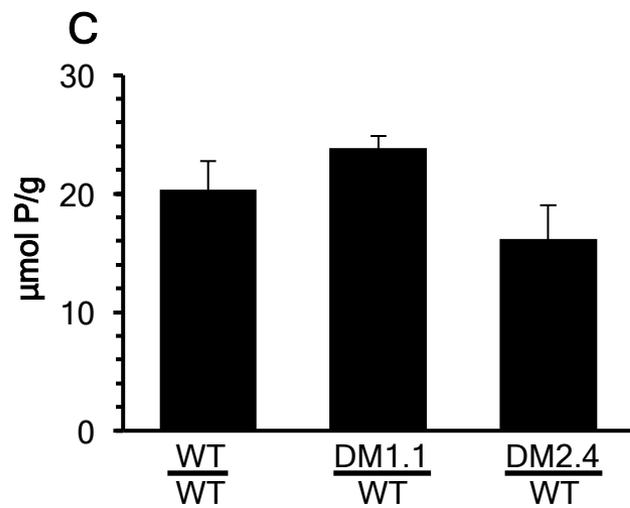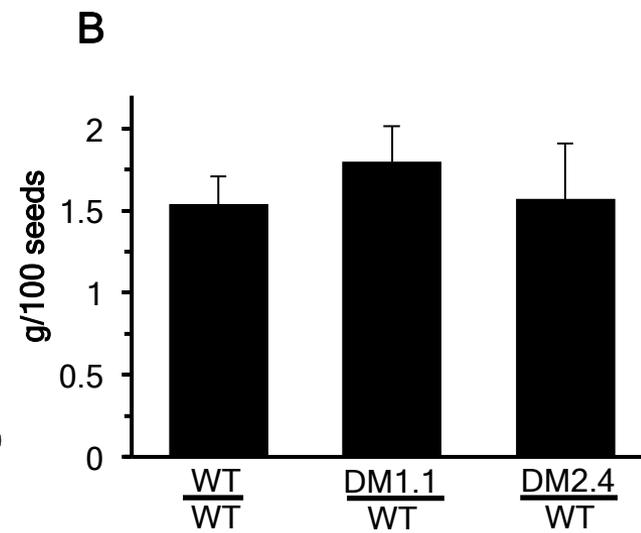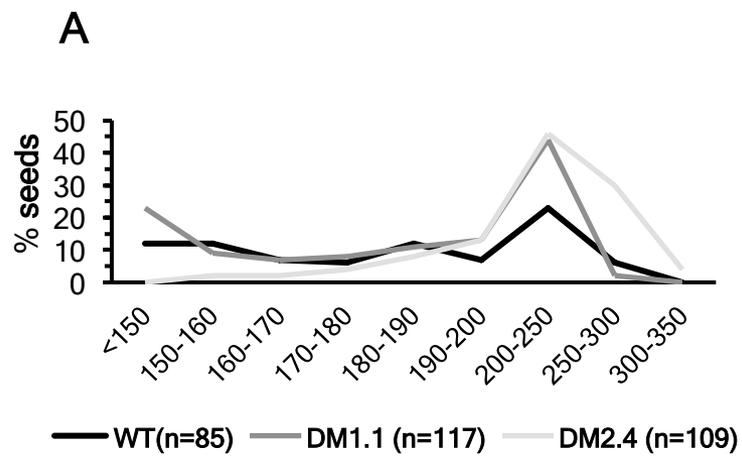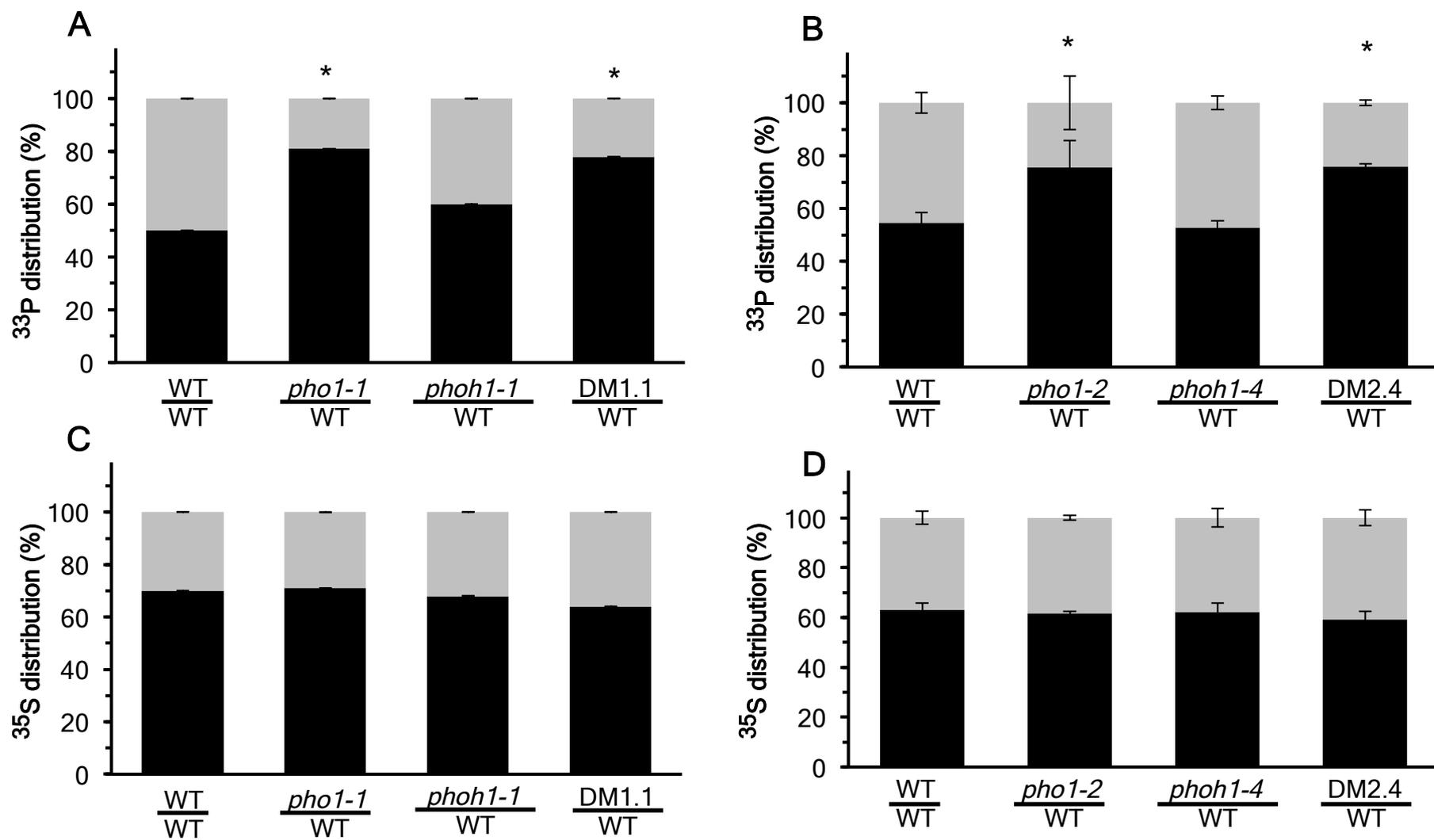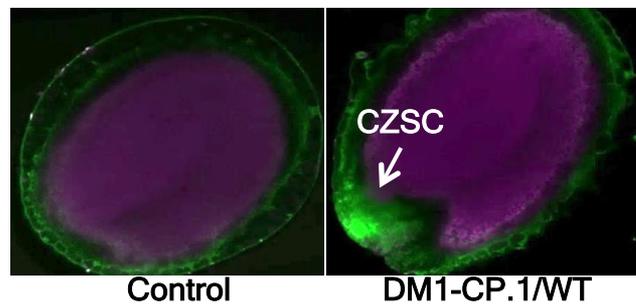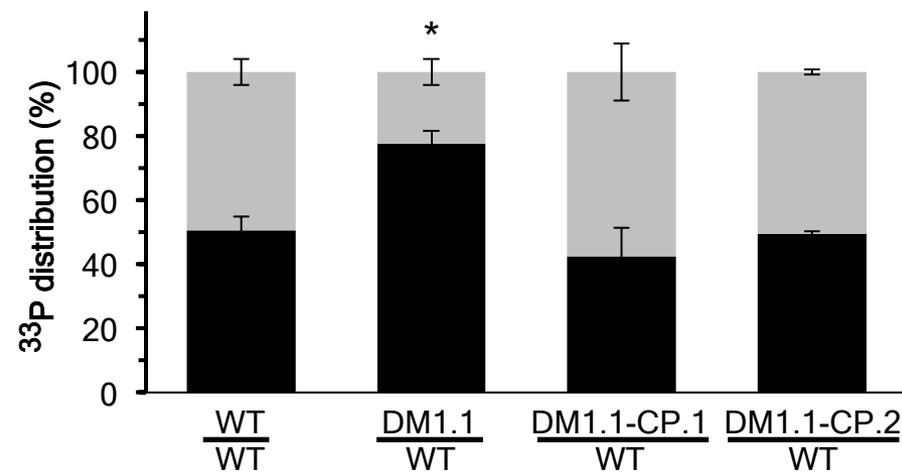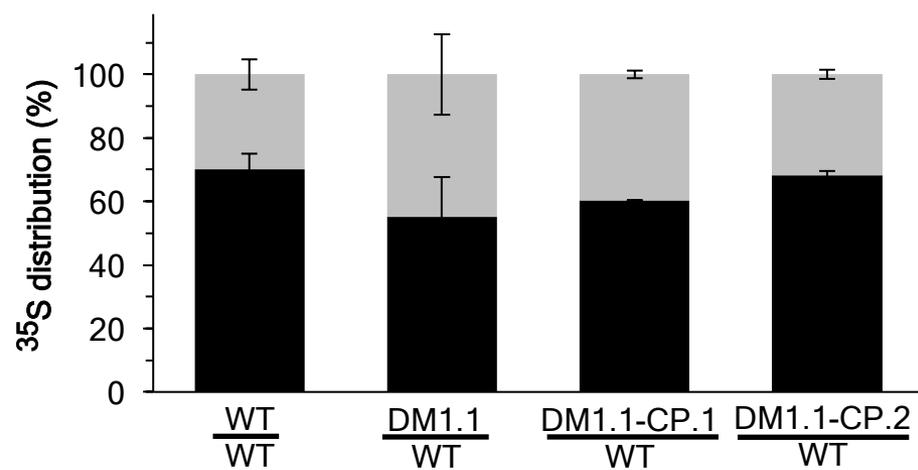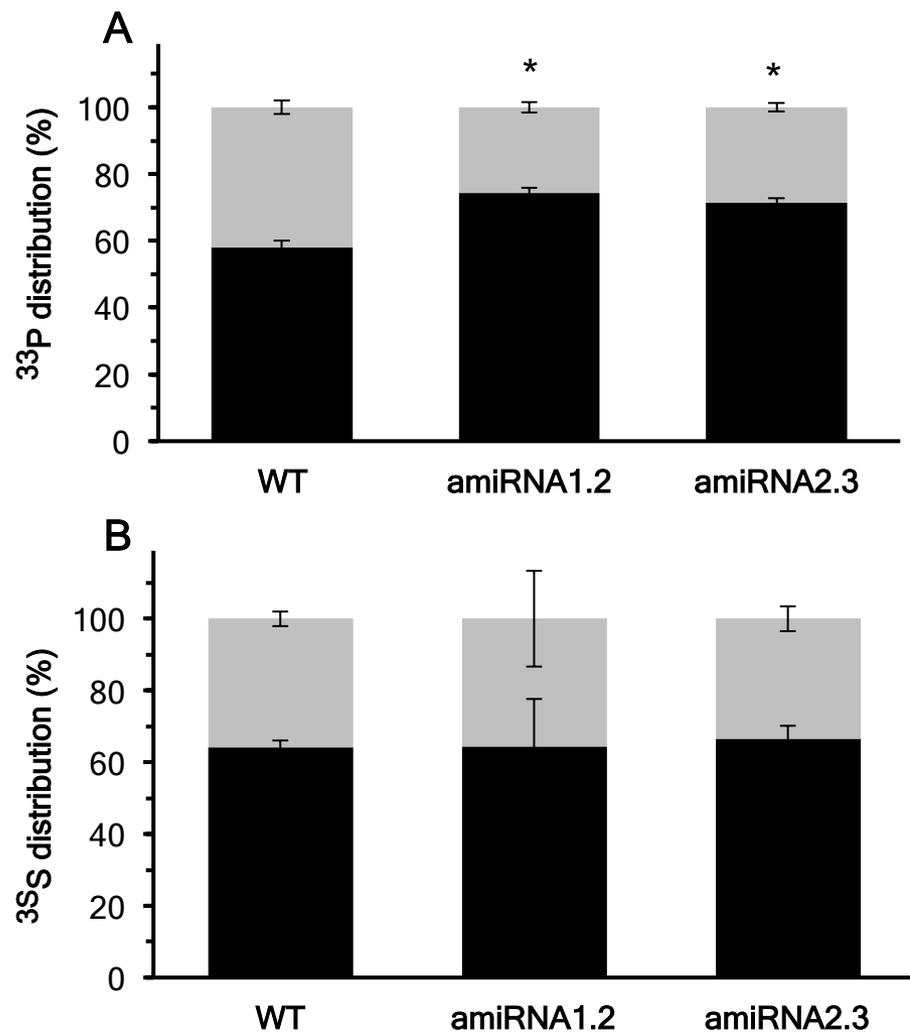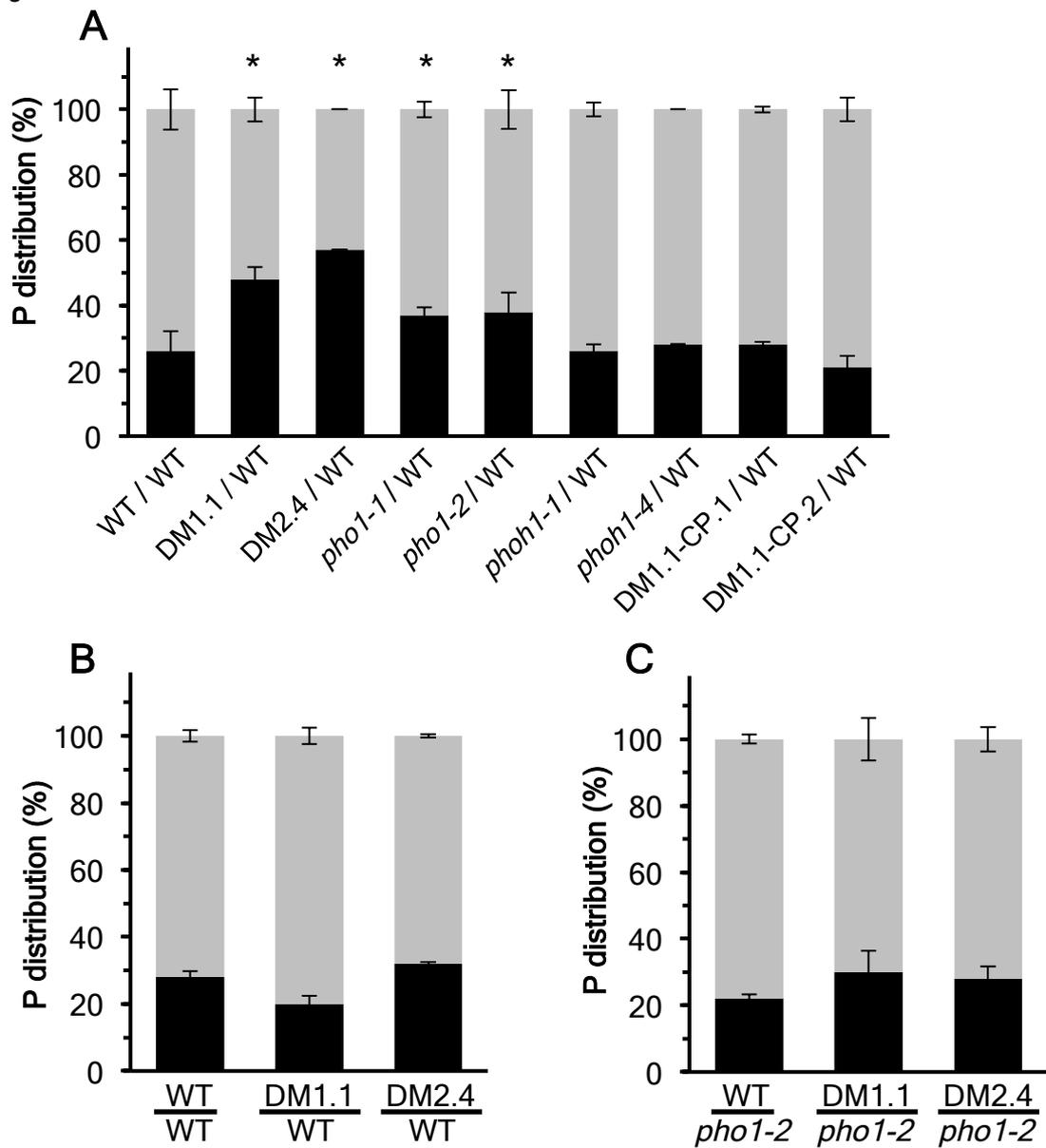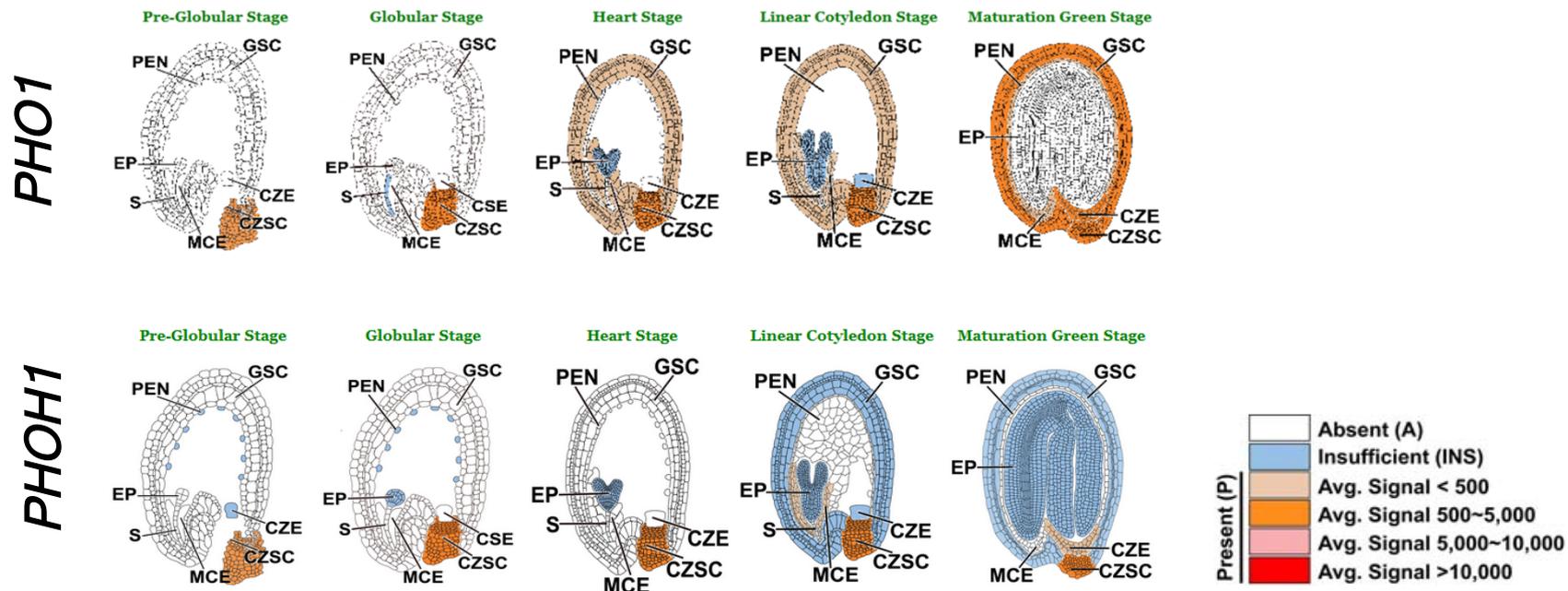
Figure 6

Figure 7

Figure S1. Expression pattern of *PHO1* and *PHOH1* in developing seeds, related to Figure 1.
The expression pattern shown is according to [S1]. Figure adapted from data obtained on http://seedgenenetwork.net/arabidopsis.
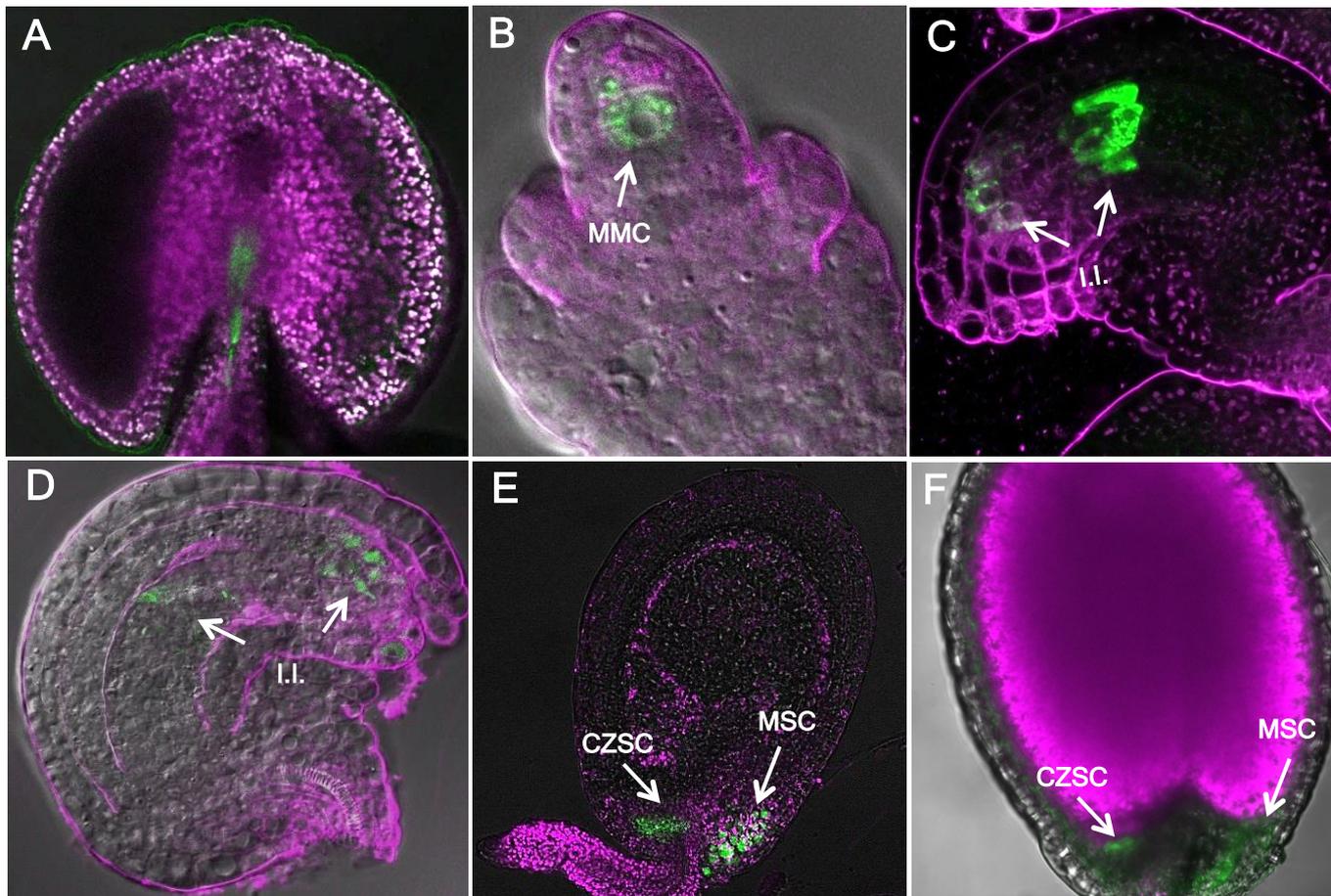
Figure S2. Expression pattern of the PHO1-YFP fusion protein in reproductive tissues and developing seed, related to Figure 1.

PHO1-YFP is expressed in the connecting tissue and vascular strand of the anthers (**A**), the megaspore mother cell (MMC) of the ovule primordia (**B**), the inner integuments (I.I.) of stage 3-V (**C**) and stage 4-II (**D**) of the developing ovules, and in the micropylar seed coat (MSC) and the chalazal seed coat (CZSC) of the globular (**E**) and mature green stages (**F**) of seed development.
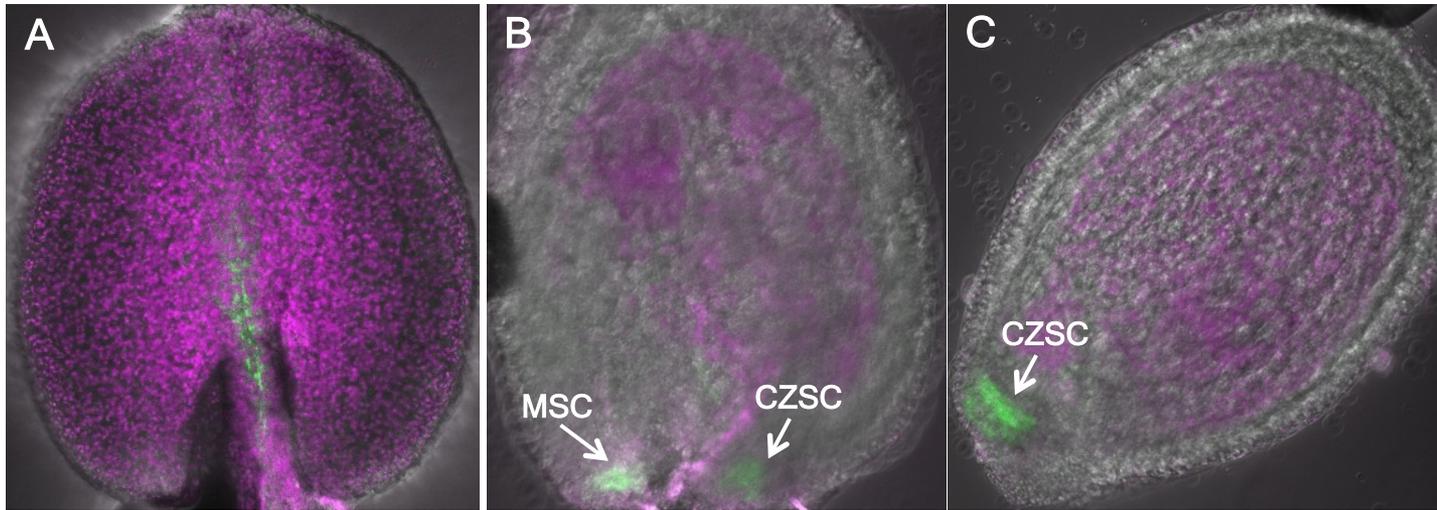
Figure S3. Expression pattern of the PHOH1-GFP fusion protein in anthers and developing seeds of *A. thaliana*, related to Figure 1.
PHOH1-GFP is expressed in the connecting tissue and the vascular strand of the anthers (**A**), the micropylar seed coat (MSC) and the chalazal seed coat (CZSC) at the globular stage (**B**) and the CZSC at the mature green stage (C) of the seed development.
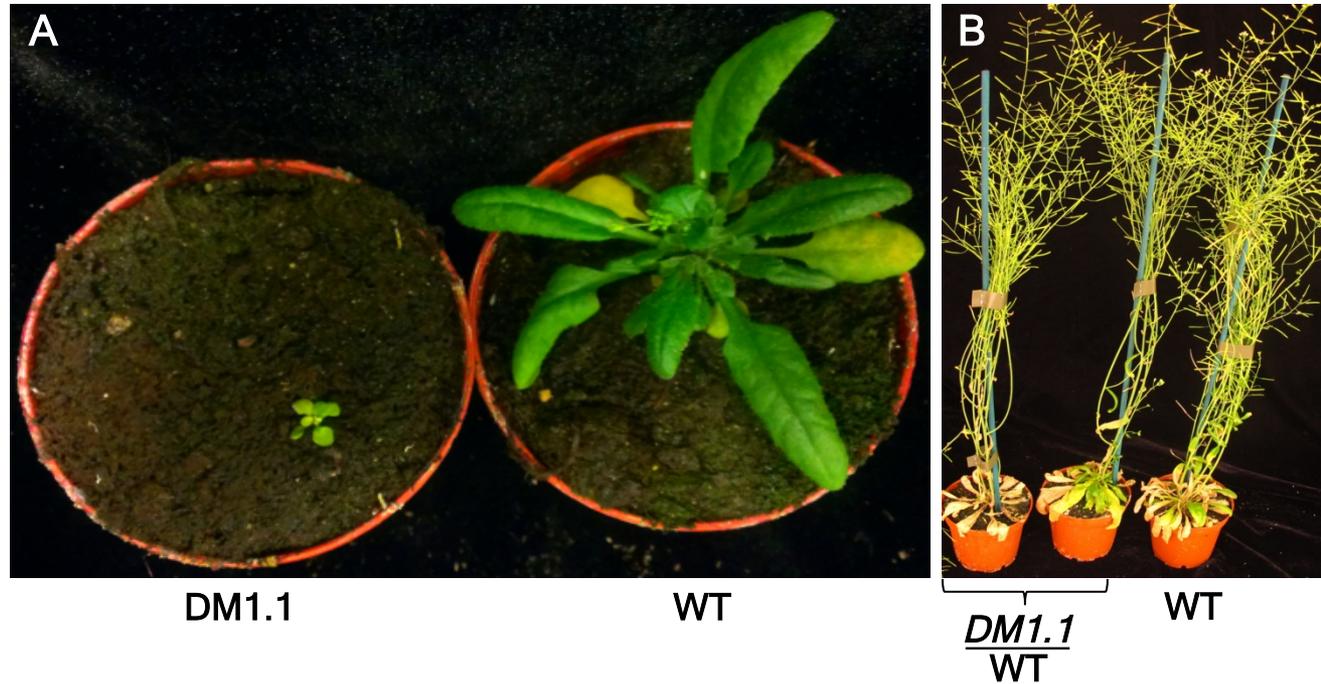
Figure S4. Phenotype of ungrafted and grafted *pho1-1 phoh1-1* double mutant, related to Figure 3.
The double mutant *pho1-1 phoh1-1* (DM1.1) and WT plants grown in soil for 5 weeks. Grafted plants between WT roots and *pho1-1 phoh1-1* scion (**A**) compared to WT plants grown in soil for 8 weeks (**B**).
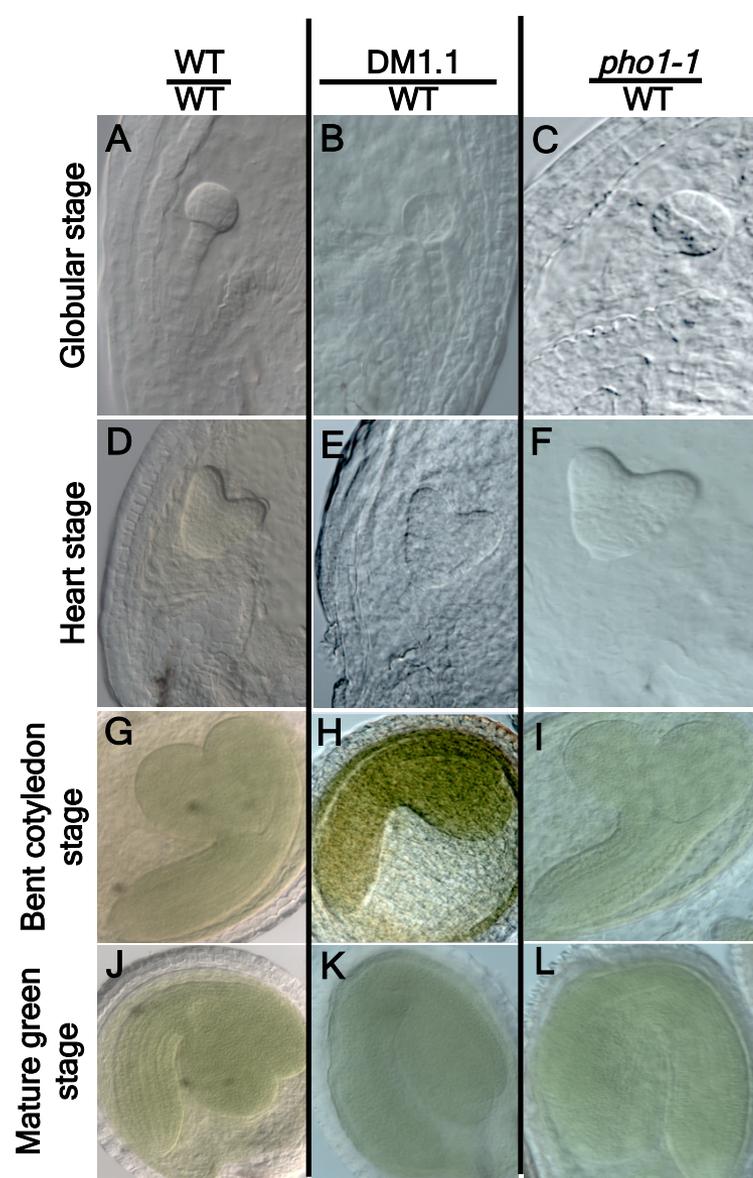
Figure S5. Phenotype of developing embryos, related to Figure 3.
Embryos of WT/WT, DM1.1/WT and *pho1-1*/WT grafted plants at various developmental stages (**A-L**).
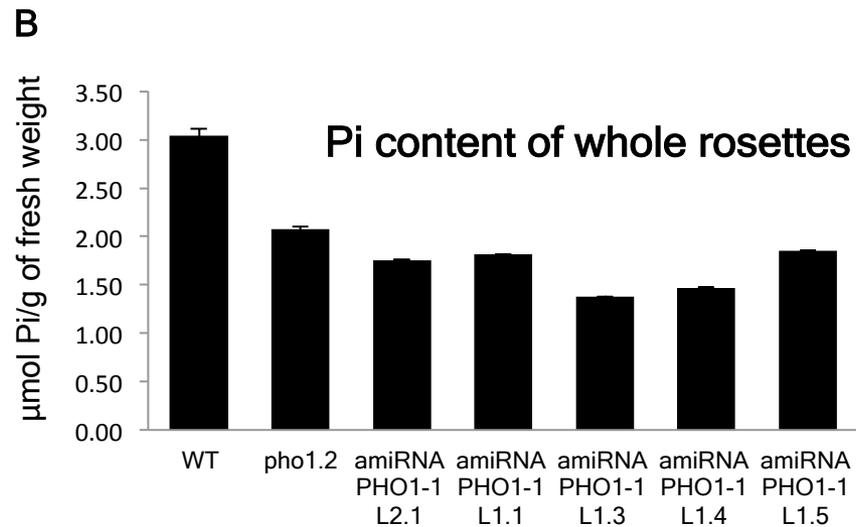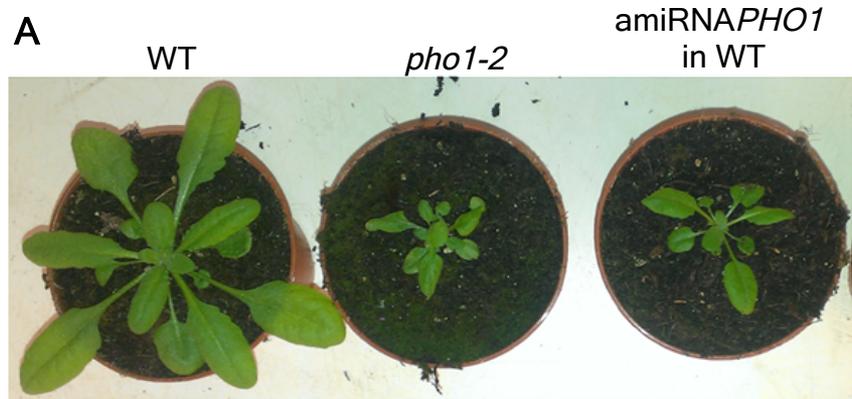
Figure S6. Expression of amiRNA*PHO1* construct targeting *PHO1* phenocopies the null *pho1* mutant, related to Figure 6.
(A) General appearance of 4-week-old plants grown in soil. (B) Analysis of Pi content of rosettes from wild type, *pho1-2* and 5 independent Col-0 transgenic plants transformed with the amiRNA*PHO1* construct. Error bars denote standard deviations with n=4.
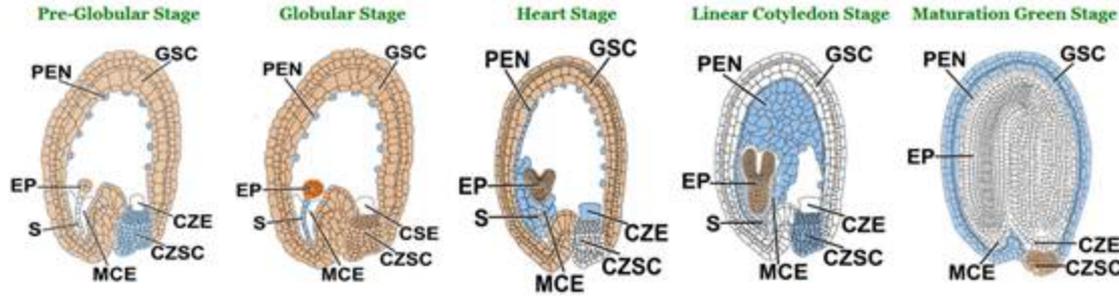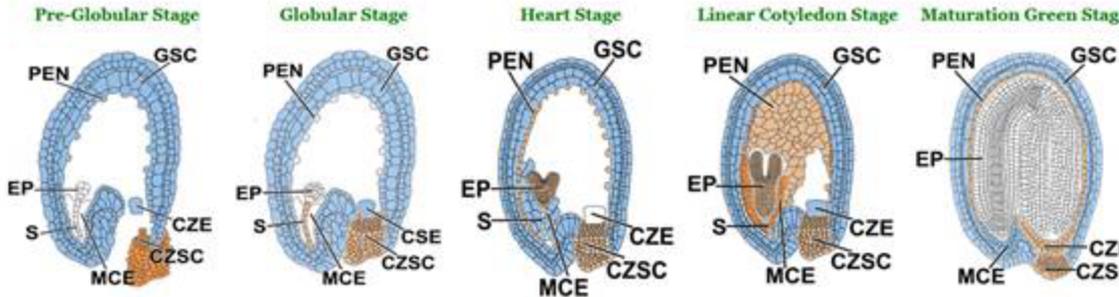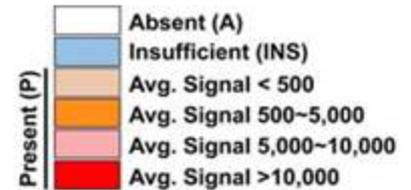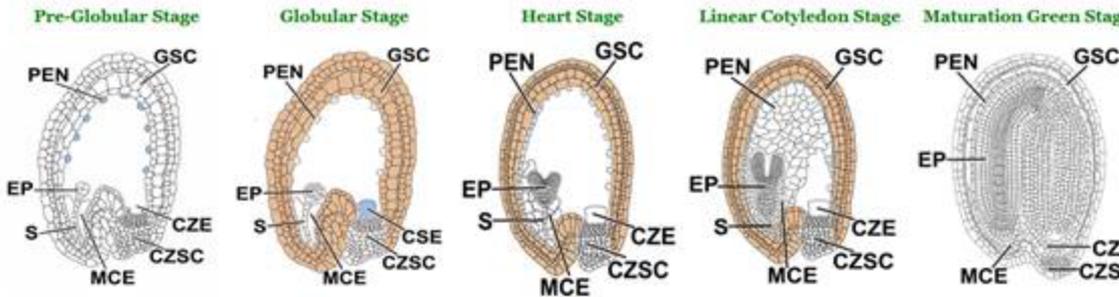
Figure S7. Expression pattern of *PHOH3, PHOH5* and *PHOH8* in developing seeds, related to Figure 1.
The expression pattern shown is according to [S1]. Figure adapted from data obtained on http://seedgenenetwork.net/arabidopsis.

Table S1. List of oligonucleotides, related to STAR Methods

Oligonucleotides to clone *PHO1* into pENTR2B

gPHO1-F
CCGGTACCGAATTCGATGGTGAAGTTCTCGAAGGAGCTA

gPHO1-R
TGAACCAGATCCACCTCCACCGTCTGAGTCCCTGTCAAG

Oligonucleotides to clone *PHOH1* into pENTR2B

gPHOH1-F
AGGAAAATGATGGTGCGAATTCGGTACCGGATCCAG

gPHOH1-R
GATGAAGAAGACTACTCGCGGCCGCACTCGAGATAT

Oligonucleotides used to clone the *ARR22* promoter

ARR22-R
CGTAGGCGCGCCCTTCGATTTCTTTTCTCTCAAG

ARR22-F
GGCCAGTGCCAAGCTTGATTGATACAACTCTATATGGTT

Oligonulceotides to produce the amiPHO1 construct
miRNA-PHO1-1-F
GGAATATATATGTAGGTACCCATTTAACTCATTCTTTCACAGGTCGTGATATGATTC

miRNA-PHO1-1-R
GAATACAAAAGAGAGGTGCCCATTTAACACATTCTATCAAAGAGAATCAATGATCCA

Table S2 Genotyping of *pho1* and *pho1;h1* alleles, related to STAR Methods

allele to be detected	forward primer	reverse primer	restriction digest	expected fragments, bp
pho1-1	TGTTGAGTTGTTCAGGCGTTG	TGTTGAGTTGTTCAGGCGTTG	BsrI (BseNI)	326 (uncut)
PHO1 (WT)	TGTTGAGTTGTTCAGGCGTTG	TGTTGAGTTGTTCAGGCGTTG	BsrI (BseNI)	143, 183
pho1-2	GGCAGCTCGTTGAATATGATAGC	GACCTCCTTTTATTTTACCTTATCAGAGCTGATCAATT	MfeI (MnuI)	204, 37
PHO1 (WT)	GGCAGCTCGTTGAATATGATAGC	GACCTCCTTTTATTTTACCTTATCAGAGCTGATCAATT	MfeI (MnuI)	241 (uncut)
PHOH1 (WT)	ATGGTCAAGTTCACAAAGCAATTCGA	GCAGCTTATTTCCTCCATCTGGACCATT		1117
phoh1-1 (T-DNA)	ATGGTCAAGTTCACAAAGCAATTCGA	GCGTGGACCGCTTGCTGCAACT		450
phoh1-4 (T-DNA)	ATGGTCAAAGTTCACAAAGCAATTCGA	ATATTGACCATCATACTCATTGC		450

Supplemental Reference

S1. Belmonte, M.F., Kirkbride, R.C., Stone, S.L., Pelletier, J.M., Bui, A.Q., Yeung, E.C., Hashimoto, M., Fei, J., Harada, M., Munoz, M.D., et al. (2013). Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed. Proc. Natl. Acad. Sci. USA *110*, E435-E444.