Published in final edited form as:
Curr Biol. 2009 July 14; 19(13): 1128–1132. doi:10.1016/j.cub.2009.05.068.

Dosage-dependent deregulation of an AGAMOUS-LIKE gene cluster contributes to interspecific incompatibility

Harkamal Walia, **Caroline Josefsson**, **Brian Dilkes**, **Ryan Kirkbride**, **John Harada**, **Luca Comai**

Department of Plant Biology and Genome Center University of California at Davis

SUMMARY

Postzygotic lethality of interspecies hybrids can result from differences in gene expression, copy number or coding sequence [1] and can be overcome by altering parental genome dosage [2-5]. In crosses between *Arabidopsis thaliana* and *A. arenosa*, embryo arrest is associated with endosperm hyperproliferation and delayed development similar to paternal-excess interploidy crosses and polycomb repressive complex (PRC) mutants [6, 7]. Failure is accompanied by parent-specific loss of gene silencing including the dysregulation of three genes [1] suppressed by PRC [8, 9]. Increasing the maternal genome dosage rescues seed development and gene silencing [2]. A gene set upregulated in the failing seed transcriptome encoded putative AGAMOUS-LIKE MADS domain transcription factors (AGL) that were expressed in normal early endosperm and were shown to interact in a previous yeast-2 hybrid analysis [10]. Suppression of these AGLs expression upon cellularization required PRC. Preceding seed failure, expression of the PRC member *FIS2* decreased concomitant with over-expression of the AGL cluster. Inactivating two members, *AGL62* and *AGL90*, attenuated the postzygotic barrier between *A. thaliana* and *A. arenosa*. We present a model where dosage-sensitive loss of PRC function results in a dysregulated AGL network, which is detrimental for early seed development.

Results and Discussion

Expression of a gene cluster during incompatibility

We wished to determine the dosage-sensitive pathways associated with failure of *A. thaliana* and *A. arenosa* crosses. *A. thaliana* Col-0 is a natural diploid, but colchicine tetraploidized strains are available. Diploid Col-0 and tetraploid Col-0 are isogenic and differ only in total chromosome number (10 and 20, respectively). They were used as seed mother plants. *A. arenosa* accession *Strecno* is a natural diploid and was used as the pollen parent. By changing the seed mother ploidy from tetraploid to diploid, we defined compatible (4×2) and incompatible (2×2) crosses, which produce, respectively ~70% and ~1% live seeds [2]. By comparing the seed transcriptomes of 5-day old siliques of compatible and incompatible crosses we identified genes differentially expressed during interspecies hybrid failure.

Among others, incompatibility was associated with the activation of seven genes encoding AGAMOUS-LIKE Type-1 MADS-box genes (AGL). Induction of these genes in globular embryo stage seed was confirmed by RT-qPCR (Figure 1). One of these, *PHERES1*, is a direct PRC target[8], which was previously shown to be both activated in incompatible crosses [2]. Another induced gene is *AGL62*. In *agl62* mutant seeds, early syncytial endosperm over-proliferates resulting in death [11]. In addition to *AGL62* and *PHERES1*, the five additional *AGL* genes upregulated in the incompatible crosses were *PHERES2*, *AGL35*, *AGL36*, *AGL40*, *AGL90*. Parent-specific primers were developed for the *AGL*s and induction was observed for both homeologous copies (Supplemental Table 2). The role of these *AGLs*, other than *AGL*62 and *PHERES*1, is unknown. Based on the *A. thaliana* expression atlas [12], six of these seven *AGLs* are coexpressed in pollen and during early seed development and down regulated at the transition from syncytial to cellular endosperm growth, corresponding to the transition from mid-globular to early-heart stage embryos. Moreover, the expression levels of these *AGLs* with the exceptions of *PHERES1* and *PHERES2* are reduced in heart-stage samples. Expression of these and other *AGL* genes in endosperm has been recently reported [13]. Their coexpression with the two known seed regulators *PHE*1 and *AGL*62 suggest that the remaining five *AGL*s are important in early seed development. The sensitivity of their expression levels to a change in genomic dosage in the interspecies crosses indicates that this perturbation might play a role in hybrid lethality.

We investigated the expression of these genes during early hybrid seed development. RNA was extracted from whole siliques from 2×2 and 4×2 hybrid crosses at 4, 5 and 6 days after pollination (DAP). The relative expression levels of the AGLs were determined by quantitative RT-PCR (Figure 1). We found no significant differences in the relative expression levels of the AGL cluster genes at 4 DAP between incompatible and compatible crosses. However at 5 DAP, the expression of the AGL genes in the 4×2 compatible cross remained low, while increasing in the 2×2 incompatible cross. The relative differences between compatible and incompatible crosses increased further by 6 DAP. Thus, AGLs become deregulated in synchrony between DAP 4 and 5, concurrent with abnormal syncytial endosperm proliferation and a lack of cellularization of endosperm. Our data and those of others suggest that suppression of the AGL-cluster at 5 DAP is critical for restricting endosperm proliferation in hybrids and for a successful transition from the syncytial to cellularized stages of seed development.

We further refined our analysis of the coexpressed *AGL* genes by examining the spatial expression pattern of *AGL*35/90 (which in this experiment could not be distinguished), *AGL*36, *AGL*40, *AGL*62, *PHE*1, and *PHE*2. We used a microarray dataset (GSE11262) that reports expression in different regions of wild type seed at the globular stage embryo (about DAP3). We found that these genes were expressed in the endosperm and absent in the embryo. Within the endosperm, *AGL*36, *PHE*1, and *AGL*62 were expressed in both the chalazal and peripheral endosperms, while *AGL*35/90 and AGL40 were primarily expressed in the chalazal endosperm.

Co-expressed genes often play a role in a common pathway or encode members of protein complexes. Yeast-two hybrid interactions between the proteins encoded by the coexpressed

AGL genes were examined previously [10]. Six of the seven coexpressed genes in the set were included in the interactome study and while none homodimerized all six AGLs formed heterodimers with at least two others. The interactions are depicted as lines connecting each protein in Figure 2. AGL62 interacted with the other five AGL proteins, suggesting that AGL62 is a central hub of the network. PHERES1 was the second-most connected member and interacted with three other AGL proteins, but not with PHERES2. The interaction network and co-expression of these co-AGLs are consistent with a common role of this networked cluster during early endosperm development.

Two of the genes in the AGL-biomodule namely *PHERES*1 and *AGL*62 were reported to be regulated by members of the PRC [8, 11]. To test whether the PRC regulated the AGL-biomodule in its entirety, we determined the expression levels of the seven *AGL* genes in seeds deficient in the PRC member FIS2. From a selfed heterozygous *fis*2/+ individual we sampled *fis*2/+ and +/+ seeds, which when harvested 5 DAP cannot be distinguished, and compared them to wild-type Col-0 seeds. We found the expression of all members of the coexpressed set to be very high in the seed of the *FIS*2/*fis*2 plant compared to wild-type seeds (Figure 3-A). This indicates that *FIS*2 directly or indirectly regulates the temporal expression of the AGL-biomodule during early seed development.

The MEDEA gene encodes another member of the PRC, is dosage sensitive in the hybrid seed development [2], is known to be paternally imprinted [14] and is autoregulated, either with [15][16] or without PRC participation [9]. The timing of the MEDEA response to hybridization may help elucidate the mechanisms at work. We measured MEDEA's transcript aboundance in two independent samples from compatible and incompatible crosses using quantative RT-PCR assays. MEDEA's levels in incompatible crosses at day 4 showed no difference from the compatible levels. However, progressive induction at day 5 and 6 was observed as for the AGLs (Figure 3-B). To distinguish the parental source of the MEDEA transcript, we used species-specific primers. We found that transcripts in the incompatible hybrid seed were derived from both the *A. thaliana* and the *A. arenosa* parent. MEDEA's aberrant induction in this system is consistent with alteration of PRC activity.

To investigate possible causes of aberrant PRC function, we surveyed the expression of *FIS*2 in siliques 4, 5, and 6 DAP by RT-qPCR. At all three time points, *FIS*2 expression was decreased 2 to 10 fold in the incompatible response (Figure 3-B). Higher level of *FIS*2 expression in the compatible 4×2 cross could be explained by the increased maternal dosage of this imprinted factor[17]. This hypothesis was tested by measuring the expression of *FWA*, which being imprinted and maternally expressed[18] should also respond to dosage. Contrary to the above hypothesis, we did not find increased expression of *FWA* in the 4×2 compatible cross (Supplemental Table 2). Although we do not know the level of *FIS*2 expression required for wild-type function, this reduction in expression of a critical regulator is consistent with its deficiency being a cause of the reduced PRC activity.

To find evidence for chromatin modification by PRC complex associated with the AGL biomodule genes, we attempted chromatin immunoprecipitation (ChIP) on 5 DAP seed but found the chromatin yield from the microdissected seed to be problematically low. As a proxy, we examined the data from a whole-genome ChIP study in *A. thaliana* seedlings that profiled chromatin marked by trimethylation of lysine 27 of histone H3 (H3K27me3) [19]. We searched for H3K27me3-positive regions closely linked to six AGL genes of the biomodule, but found no evidence for PRC mediated histone modification marks. *PHERES*1, on the other hand, is a direct target of the PRC silencing complex and is marked by H3K27me3 in the flower and silique [20]. Since deficiency in *FIS*2 results in loss of regulation of the AGL-biomodule genes in hybrid and wild-type seeds we asked if *PHERES*1 is the upstream regulator of the biomodule. We measured the expression levels of the *AGL* gene set in homozygous *phe1* knock-out mutant seeds and in the corresponding parental accession, L*er*. We found that only the expression of *PHERES*2 increased 5 to 11 fold in the absence of a functional copy of *PHERES1*. The expression of the other five AGL genes remained unchanged. Based on this data, we conclude that suppression of AGL genes by *FIS*2 does not require *PHERES*1.

We wished to determine whether the expression of this AGL module had an effect on the incompatibility phenotype. Notably, seeds of interspecies crosses can be grouped into four phenotypes. The first, shriveled, results in small and collapsed seed consistent with early failure and death. The next two phenotypes, viviparous and green, correspond to seed that has avoided early death but failed to complete normal development. The last phenotype, normal or plump, describes normal seed. We obtained mutants in the Col-0 accession with T-DNA insertions[17] in *AGL*62 and *AGL*90. Wild-type Col-0 crossed to diploid *A. arenosa* produced 1.6% live seed (of these, 0.5% were normal, 0.3% viviparous, 0.8% were green, n=358). Consistent with a previous report[11] plants heterozygous for the *AGL*62 knock out produced 1/4 dead seed when selfed, indicating homozygous lethality. When the heterozygotes were crossed to diploid *A. arenosa*, 10.9% of the seeds were alive at maturity (6.4% normal, 0.6% viviparous, 3.7% were green; n = 848, P-value = 4.01e-06). Plants homozygous for the *AGL*90 knock-out mutation produced normal seed when selfed, indicating that this gene is either redundant or not essential for embryogenesis. Nevertheless, when the diploid mutant was crossed to diploid *A. arenosa*, the homozygote significantly improved seed set and produced a higher fraction of green seed than the *AGL*62 mutant (10.9% live seeds of which 3% were normal, 0.6% viviparous, and 7.2% green; n = 1221, P-value = 1.03e-07). The beneficial effect of the knock-out alleles of both AGL62 and AGL90 should result in segregation distortion. We germinated seed from crosses of heterozygous *A. thaliana* to *A. arenosa* and genotyped the hybrid seedlings. The knock-out alleles were preferentially inherited by the progeny (AGL62, 17:5, P = 0.058; AGL90, 14:3, P = 0.046). The AGL62 and AGL90 effects are notable considering that a previously characterized knock-out of *PHERES*1 failed to ameliorated the strongly incompatible 2 × 2 cross used here, while it had a distinct effect on the compatible 4 × 2 cross [2].

In summary, a cluster of coregulated and interacting AGLs is induced in a dosage-sensitive manner in the postzygotic incompatibility response. The central role of the AGL biomodule in seed failure is consistent with the observation that two members are required for incompatibility. PRC activity is required for suppression of the AGL genes. Concomitant with the AGL induction, expression of the gene encoding the PRC member FIS2 is decreased several-fold. These observations suggest a model in which sensitivity of PRC, perhaps of *FIS2* itself, to the dose of maternal contributions misregulates factors such as the

AGL, which coordinate endosperm development. Therefore, this work suggests a molecular mechanism for the role of endosperm in the interspecific barrier.

Experimental procedures

Plant Material

The *A. thaliana* accession Columbia-0 was used as the wild type for all crosses. Wild-type Ler accession was used for the q-PCR expression analysis comparison with *phe1* knockout line. *Phe1* knock out line (ET189) was obtained from Cold Spring Harbor Collection and is in Ler background. The diploid line of *A. thaliana* was tetraploidized with a modified protocol from Santos et al.[21] and is described in Josefsson et al.[2]. Diploid *A. arenosa* seeds, collected from Strecno (Slovakia) were a gift from Dr. M. Lysak. The Arabidopsis T-DNA insertion knock-out lines used for AGL62 were SALK_137707 and SALK_013792. Insertion lines used for AGL90 were homozygous for insertion in promoter, SALK_092748 and SALK_008897. All four lines were obtained from the SALK collection. The *fis2-8* mutant was a gift from Ramin Yadegari (University of Arizona, Tucson). All crosses were performed by emasculating flowers before anthesis and pollinating healthy stigmas the following morning. For analysis of segregation distortion, seed produced by crossing the *A. thaliana agl62* (+/-) and *agl90* (+/-) mutants to *A. arenosa* was germinated on nutrient salt agar. Seedlings were used for DNA purification and genotyped for the relevant T-DNA [22] using wild-type and insertion-specific PCR products. Many green seeds and some apparently normal seed produced by the AGL90 heterozygous seed mother failed to germinate.

Plant Growth Conditions

Plants were grown in a growth room with 16 h of light period at 22°C and 8 hours of dark at 18°C.

RNA Extraction

RNA for q-RT-PCR was extracted using the hot borate method described in Wilkins and Smart[23]. We used whole siliques for the time series analysis of the compatible and incompatible hybrid crosses. For all other experiments, RNA was derived from developing seeds. Unless otherwise specified, all materials were collected 5 DAP. RNA was purified and using the Qiagen RNA columns and DNAseI treated.

For reverse transcription, we used SuperScript VILO cDNA synthesis kit (Invitrogen, Carlsbad, California) and followed the manufacturer's protocol. The cDNA was diluted 1:20 and then used for subsequent q-PCR reactions.

Quantitative-PCR

Quantitative-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystem Inc.) in 20 uL volume reactions. We used an Opticon 2 (MJ Research) for q-PCR. We used 2 uL of the cDNA template and 20 nmoles of gene specific primers. PCR conditions were as follows: 2 min incubation at 50 °C, 10 min denaturation at 90 °C followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. A melting curve analysis was performed for all primer pairs to ensure that signal was derived from a single product. All reactions were conducted at least in triplicates. We used *ROC1* as control gene and employed the relative quantification feature in Opticon 3 (Bio-Rad Inc) for determination of relative quantities of transcript for any given gene. The data were exported to MS-EXCEL for statistical analysis (mean and standard error) and for graphing. The sequences for gene specific primers used in the expression assays are listed in Supplemental Table 1.

Analysis of segregation distortion

Pearson's Goodness of Fit Chi-square was used to calculate probabilities with one degree of freedom.

Phenotyping

Hybrid seeds from the compatible and incompatible crosses were harvested 4, 5 and 6 DAP. The seeds were removed from the siliques and cleared with Hoyer's solution. Images were obtained using Leica DM-6000 microscope equipped with Nomarski optics.

Microarray Data and Analysis

Differentially expressed genes in siliques of compatible and incompatible hybrids at 5 DAP were identified by employing the *A. thaliana* Whole-Genome Tiling Array from Affymetrix. The array covers ~97 % of the *A. thaliana* genome at a 35-bp resolution. To identify significant expression differences we employed two independent statistical approaches. We combined probe-level t-statistics generated by a Hidden Markov Model implemented in TileMap [24] with a second approach, which used a Wilcoxon-Signed Rank test implemented in Tiling Array Software (TAS) from Affymetrix. Any genomic region corresponding to a gene model in TAIR and called by both softwares was hereafter termed as a differentially expressed gene. The experiment for microarray analysis was performed in three independent biological replicates. We found 315 genes to be dosage responsive, 155 genes showed increased transcript abundance in the incompatible hybrids and 160 genes displayed higher transcript levels in compatible hybrids. Among the 155 genes, the seven Type 1 AGAMOUS-LIKE (AGL) genes were highly induced in the incompatible hybrids. The expression of AGLs was further validated by quantitative-PCR using several independent biological replicates. The NCBI GEO accession for the data is GSE14090.

We used the NCBI GEO microarray data portal for downloading the raw (CEL files) Arabidopsis Atlas data set and the LCM data from globular stage seed compartments (GSE11262). The data set was independently imported into DChip[25], the analysis software used for hierarchical clustering. Briefly, the data was normalized using the invariant set approach, and expression values calculated for each probe set by Model-based Expression Index (MBEI). Unsupervised hierarchical clustering was performed using the mean of a given probe set for calculating the relative signal and the color for the gene in the heat map. The clustering was limited to the probe sets representing the AGL module members. The p-value threshold used for clustering genes was 0.005.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge helpful suggestions from anonymous reviewers and Brian Watson for reading the reading the revised manuscript. This work was supported by the National Institute of Health (1R01GM076103 to LC).

References

1. Dilkes BP, Comai L. 2004; A differential dosage hypothesis for parental effects in seed development. Plant Cell. 16:3174–3180. [PubMed: 15579806]
2. Josefsson C, Dilkes B, Comai L. 2006; Parent-dependent loss of gene silencing during interspecies hybridization. Curr Biol. 16:1322–1328. [PubMed: 16824920]
3. Nishiyama I, Yabuno T. 1978; Casual relationships between the polar nuclei in double fertilization and interspecific cross-incompatibility. Cytologia. 43:453–466.
4. Johnston SA, Hanneman REJ. 1982; Manipulations of Endosperm Balance Number Overcome Crossing Barriers Between Diploid Solanum Species. Science. 217:446–448. [PubMed: 17782980]
5. Mable BK, Bogart JP. 1995; Hybridization between tetraploid and diploid species of treefrogs (Genus hyla). J Hered. 86:432–440. [PubMed: 8568210]
6. Bushell C, Spielman M, Scott RJ. 2003; The basis of natural and artificial postzygotic hybridization barriers in Arabidopsis species. Plant Cell. 15:1430–1442. [PubMed: 12782734]
7. Guitton AE, Page DR, Chambrier P, Lionnet C, Faure JE, Grossniklaus U, Berger F. 2004; Identification of new members of Fertilisation Independent Seed Polycomb Group pathway involved in the control of seed development in Arabidopsis thaliana. Development. 131:2971–2981. [PubMed: 15151989]
8. Kohler C, Page DR, Gagliardini V, Grossniklaus U. 2005; The Arabidopsis thaliana MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. Nat Genet. 37:28–30. [PubMed: 15619622]
9. Baroux C, Gagliardini V, Page DR, Grossniklaus U. 2006; Dynamic regulatory interactions of Polycomb group genes: MEDEA autoregulation is required for imprinted gene expression in Arabidopsis. Genes Dev. 20:1081–1086. [PubMed: 16651654]
10. de Folter S, Immink RG, Kieffer M, Parenicova L, Henz SR, Weigel D, Busscher M, Kooiker M, Colombo L, Kater MM, et al. 2005; Comprehensive interaction map of the Arabidopsis MADS Box transcription factors. Plant Cell. 17:1424–1433. [PubMed: 15805477]
11. Kang IH, Steffen JG, Portereiko MF, Lloyd A, Drews GN. 2008; The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis. Plant Cell. 20:635–647. [PubMed: 18334668]
12. Laubinger S, Zeller G, Henz SR, Sachsenberg T, Widmer CK, Naouar N, Vuylsteke M, Scholkopf B, Ratsch G, Weigel D. 2008; At-TAX a whole genome tiling array resource for developmental expression analysis and transcript identification in Arabidopsis thaliana. Genome Biol. 9:R112. [PubMed: 18613972]
13. Day RC, Herridge RP, Ambrose BA, Macknight RC. 2008Transcriptome analysis of proliferating arabidopsis endosperm reveals biological implications for the control of syncytial division, cytokinin signalling, and gene expression regulation. Plant Physiol.
14. Choi Y, Gehring M, Johnson L, Hannon M, Harada JJ, Goldberg RB, Jacobsen SE, Fischer RL. 2002; DEMETER, a DNA glycosylase domain protein is required for endosperm gene imprinting and seed viability in arabidopsis. Cell. 110:33–42. [PubMed: 12150995]
15. Jullien PE, Katz A, Oliva M, Ohad N, Berger F. 2006; Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. Curr Biol. 16:486–492. [PubMed: 16527743]
16. Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, Fischer RL. 2006; DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. Cell. 124:495–506. [PubMed: 16469697]
17. Jullien PE, Kinoshita T, Ohad N, Berger F. 2006; Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. Plant Cell. 18:1360–1372. [PubMed: 16648367]

18. Kinoshita T, Miura A, Choi Y, Kinoshita Y, Cao X, Jacobsen SE, Fischer RL, Kakutani T. 2004; One-way control of FWA imprinting in Arabidopsis endosperm by DNA methylation. Science. 303:521–523. [PubMed: 14631047]
19. Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE. 2007; The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol. 14:869–871. [PubMed: 17676062]
20. Makarevich G, Leroy O, Akinci U, Schubert D, Clarenz O, Goodrich J, Grossniklaus U, Kohler C. 2006; Different Polycomb group complexes regulate common target genes in Arabidopsis. EMBO Rep. 7:947–952. [PubMed: 16878125]
21. Santos JL, Alfaro D, Sanchez-Moran E, Armstrong SJ, Franklin FC, Jones GH. 2003; Partial diploidization of meiosis in autotetraploid Arabidopsis thaliana. Genetics. 165:1533–1540. [PubMed: 14668400]
22. Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al. 2003; Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science. 301:653–657. [PubMed: 12893945]
23. Wilkins, TA, Smart, LB. Isolation of RNA From Plant Tissue. In: Krieg, PA, editor. A Laboratory Guide to RNA : Isolation, Analysis, and Synthesis. New York: Wiley-Liss; 1996. 21–42.
24. Ji H, Wong WH. 2005; TileMap create chromosomal map of tiling array hybridizations. Bioinformatics. 21:3629–3636. [PubMed: 16046496]
25. Li C, Hung Wong W. 2001; Model-based analysis of oligonucleotide arrays: model validation design issues and standard error application. Genome Biol. 2

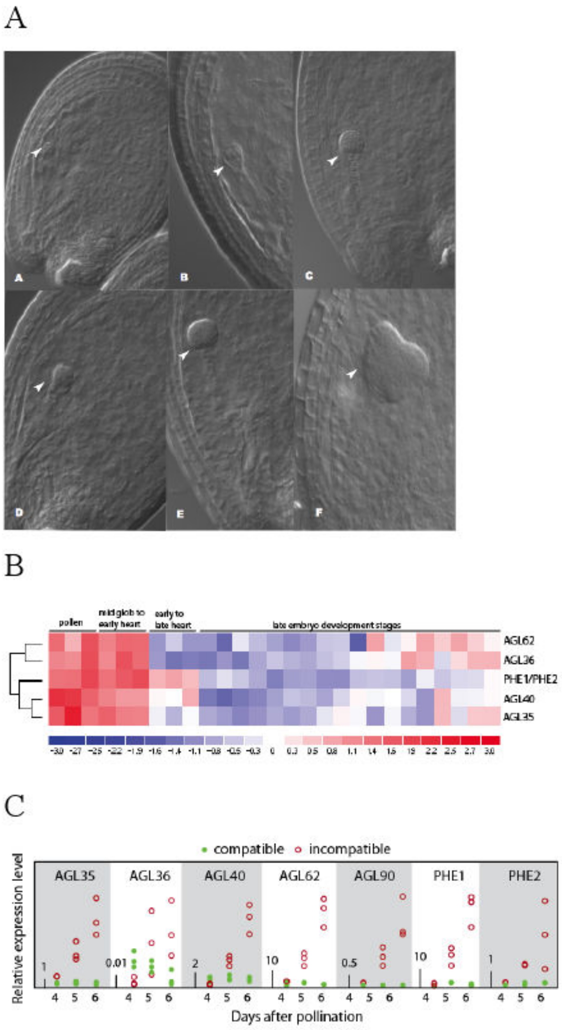

Figure 1.

Expression of incompatibility genes during seed development. A. Images of seeds 4, 5, and 6 day after pollination (left to right) for incompatible (2X mother × 2X father, top) and compatible crosses (4X mother × 2X father, bottom). Delayed embryo development in the incompatible cross is evident at day 5. B. Clustering of expression of the induced AGL in pollen and early embryo. The heat map (red: high relative expression; blue: low relative expression) is derived by comparison of the expression data in the atlas database for Arabidopsis (www.arabidopsis.org). C. Quantitative RT PCR analysis of temporal expression pattern. The relative scale based on the expression of the constitutive *ROC*1 standard is given for each gene.

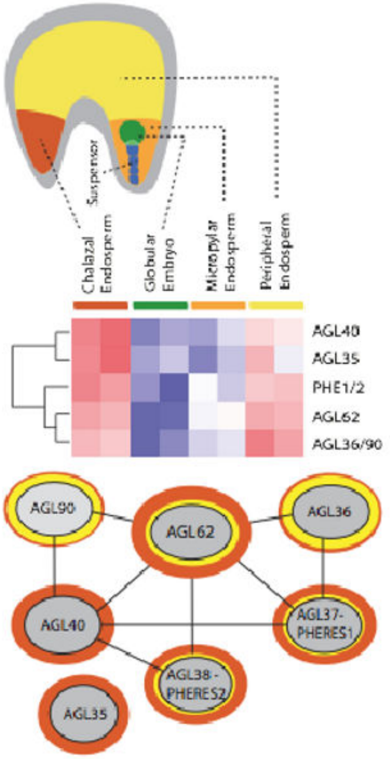

Figure 2.

Spatial expression pattern of the AGL cluster members and interactome. Top: seed developmental zones. Mid: heat maps summarizing relative expression patterns (red: high relative expression; blue: low relative expression) according to GSE11262. *AGL*36 and *AGL*90 are closely related genes and could not be discriminated. Bottom: interactome of AGL proteins derived from published yeast 2-hybrid data. The halo surrounding each protein symbol represents the relative amounts expressed in the different seed zones.

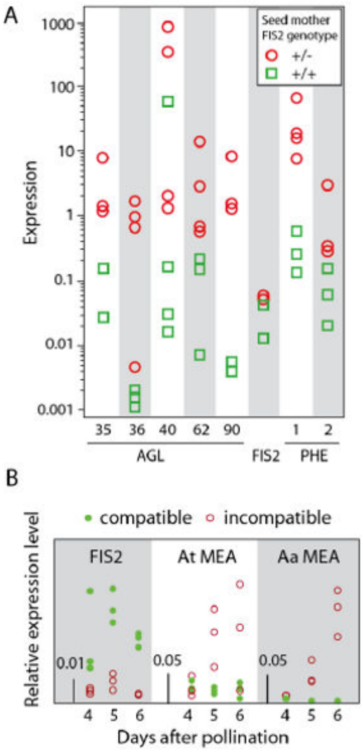

Figure 3.

*FIS*2 regulation of *AGL*s. The graph shows expression of *AGL*s and *FIS*2 in developing 5 day old seeds of a selfed *fis2* heterozygote (circles), in which half of the seeds are FIS2-deficient. A wild-type control is shown for comparison (squares). Each point represents a biological replicate.