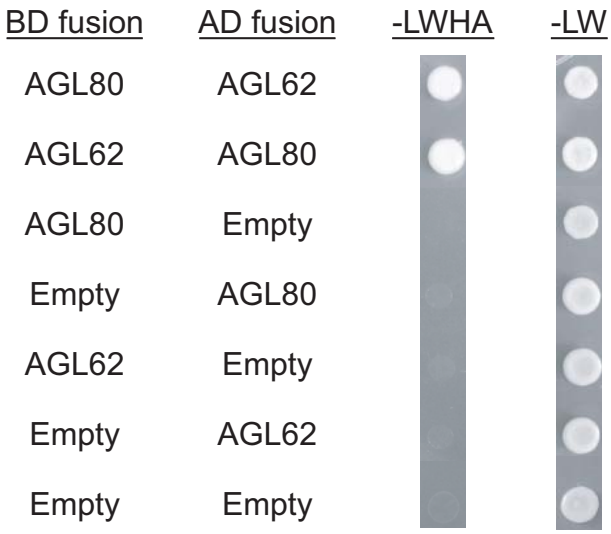
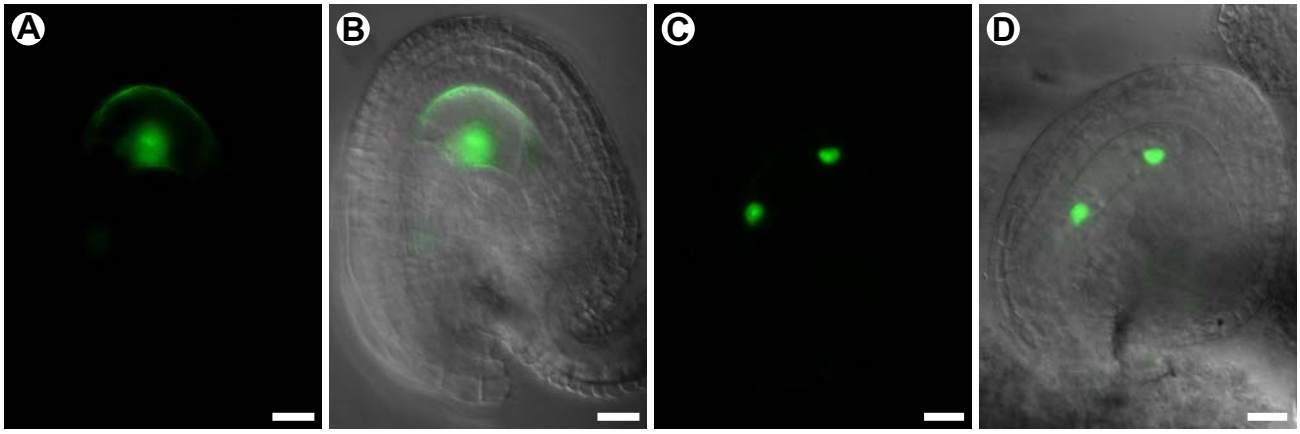


Supplemental Figure 1. Yeast Two-Hybrid Analysis of AGL62-AGL80 Interaction.

Growth occurs only when cells contain both AGL80-BD and AGL62-AD (row 1) or both AGL62-BD and AGL80-AD (row 2). Cells containing AGL80-BD only (row 3), AGL80-AD only (row 4), AGL62-BD only (row 5), AGL62-AD only (row 6), and neither AGL62 nor AGL80 (row 7) do not grow.

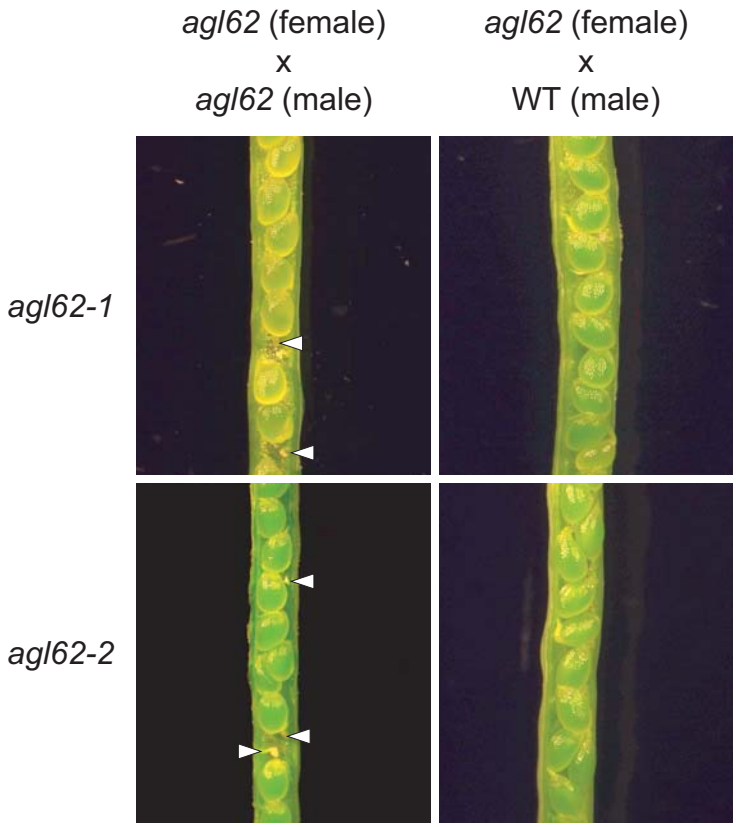
Abbreviations: AD, GAL4 activation domain; BD, GAL4 DNA-binding domain; -LW, growth medium lacking leucine and tryptophan; -LWHA, growth medium lacking leucine, tryptophan, histidine and adenine.



Supplemental Figure 2. Expression of the Paternally Derived Allele of *AGL62-GFP* During Early Endosperm Development.

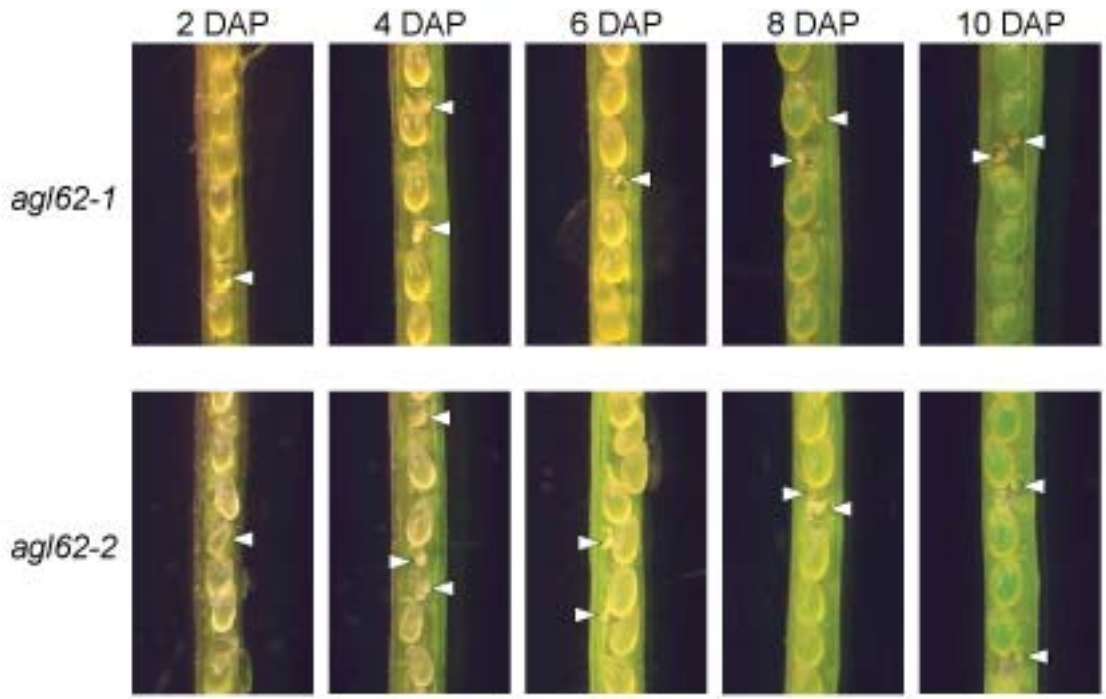
(A) and **(C)** Fluorescence images of the GFP signals.
(B) and **(D)** Fluorescence-brightfield overlay images.
(A) and **(B)** Expression of paternally derived *AGL62-GFP* in stage I endosperm.
(C) and **(D)** Expression of paternally derived *AGL62-GFP* in stage II endosperm.

Scale Bars = 20 μm.



Supplemental Figure 3. Silique Phenotypes of *agl62-1* and *agl62-2* Mutants at 10 Days After Pollination.

Siliques of self-crossed heterozygous plants (left panels) have defective seeds. Arrowheads point to defective seeds. Siliques resulting from crosses of heterozygous females with wild-type (WT) males (right panels) do not exhibit defective seeds.



Supplemental Figure 4. Silique Phenotypes of Self-Pollinated *agl62-1/AGL62 and agl62-2/AGL62* Plants at 2-10 Days After Pollination (DAP).

With both alleles, defective seeds (arrowheads) are observed as early as two days after pollination (2 DAP).

SUPPLEMENTAL TABLES

Supplemental Table 1. Expression of the Maternal and Paternal Alleles of *AGL62-GFP* During Endosperm Development.

Endosperm Developmental Stage	No. of Endosperm Nuclei	Embryo Developmental Stage	Expression of Paternal Allele	Expression of Maternal Allele
I	1	Zygote	++	++
II	2	Zygote	+++	+++
III	4	Zygote	+++	+++
IV	8	Elong. Zygote	+++	+++
V	14-16	Elong. Zygote, 1-celled	+++	+++
VI	26-30	1-2 celled	+++	+++
VII	~50	2-4 celled	+++	+++
VIII	~100	Octant	+	+
IX	~200	Dermatogen-globular	-	-
X	~300	Heart	-	-

Endosperm developmental stages defined in Boisnard-Lorig, et al. (2001) and Ingouff et al. (2005). Cellularization in the PEN is initiated during stage IX (Sorensen et al., 2002).

SUPPLEMENTAL METHODS

Yeast Two-Hybrid Analysis

We used the Clontech Matchmaker GAL4 Two-Hybrid System 3 for the yeast two-hybrid analysis. The AGL80 and AGL62 open reading frames (without introns) were fused to the GAL4 activation domain and GAL4 DNA-binding domain in pGAD-T7 and pGBK-T7. Yeast strain AH109 was cotransformed with combinations of pGAD-T7 and pGBK-T7 constructs (AGL80 plus AGL62 or controls containing one or both empty vectors) and selected on synthetic dropout (SD) medium lacking leucine and tryptophan (SD-LW). Co-transformants were then assayed for interaction and activation of the histidine and adenine reporter genes on SD medium lacking leucine, tryptophan, histidine and adenine (SD-LWHA). For this, fresh colonies were grown in SD-LW at 30°C overnight to an OD of 1-2, the cells were pelleted and resuspended in 0.5 M sorbitol to an OD of 0.5, and 3 µl of each cell suspension was spotted on SD-LWHA plates using a multi-channel pipetor and grown at 30°C for 2-3 days.