RESEARCH NOTE

REVISED Pollen tube contents from failed fertilization contribute to seed coat initiation in *Arabidopsis* [version 2; peer review: 3 approved]

Xiaoyan Liu [1,2], Parakash Babu Adhikari [1,2], Ryushiro D. Kasahara [1,2]

[1]School of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002, China
[2]FAFU-UCR Joint Center and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002, China

v2 **First published:** 28 Mar 2019, **8**:348 (https://doi.org/10.12688/f1000research.18644.1)
Latest published: 30 Apr 2019, **8**:348 (https://doi.org/10.12688/f1000research.18644.2)

Abstract
Plant seeds are essential for human beings, constituting 70% of carbohydrate resources worldwide; examples include rice, wheat, and corn. In angiosperms, fertilization of the egg by a sperm cell is required for seed formation; therefore, fertilization failure results in no seed formation, except in the special case of apomixis. Initially, plants produce many pollen grains inside the anthers; once the pollen grain is deposited onto the top of the pistil, the pollen tube elongates until it reaches the ovule. Generally, only one pollen tube is inserted into the ovule; however, we previously found that if fertilization by the first pollen tube fails, a second pollen tube could rescue fertilization via the so-called fertilization recovery system (FRS). Our previous reports also demonstrated that failed fertilization results in pollen tube-dependent ovule enlargement morphology (POEM), enlarged seeds, and partial seed coat formation if the pollen tube releases the pollen tube contents into the ovule. However, we have not determined whether all the ovules enlarge or produce seed coats if an ovule accepts the pollen tube contents. Therefore, we conducted a partial seed coat formation experiment taking into account both the FRS and POEM phenomena. Notably, the ratios of failed fertilization and the ovules with partial seed coats matched, indicating that all ovules initiate seed coat formation if the fertilization fails but the pollen tube contents enter the ovule. In addition, we confirmed that the *agl62* mutant , defective in early endosperm formation, showed seed coat initiation with and without fertilization, indicating that for a normal seed coat initiation, fertilization is not required; however, for the completion of normal seed coat formation, both normal fertilization and endosperm formation are required. Further molecular evidence is required to understand these phenomena because very few factors related to FRS and POEM have been identified.

Keywords
Arabidopsis, Pollen tube, fertilization recovery system, pollen tube-dependent ovule enlargement morphology, GCS1, pollen tube contents, Seed coat initiation, AGL62

Open Peer Review

Referee Status: ✓ ✓ ✓

	Invited Referees 1	2	3
REVISED version 2 published 30 Apr 2019	✓ report		
version 1 published 28 Mar 2019	? report	✓ report	✓ report

1 **Tomoko Igawa**, Chiba University, Japan

2 **Nobutaka Mitsuda**, National Institute of Advanced Industrial Science and Technology (AIST), Japan

3 **Tomokazu Kawashima**, University of Kentucky, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Ryushiro D. Kasahara (kasahara@fafu.edu.cn)

Author roles: Liu X: Data Curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Adhikari PB**: Conceptualization, Data Curation, Formal Analysis, Investigation, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Kasahara RD**: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by start-up funds from the School of Life Sciences, Fujian Agriculture and Forestry University (Grant #: 114-712018008) and the FAFU-UCR Joint Center and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Liu X, Adhikari PB and Kasahara RD. **Pollen tube contents from failed fertilization contribute to seed coat initiation in *Arabidopsis* [version 2; peer review: 3 approved]** F1000Research 2019, **8**:348 (https://doi.org/10.12688/f1000research.18644.2)

First published: 28 Mar 2019, **8**:348 (https://doi.org/10.12688/f1000research.18644.1)

REVISED **Amendments from Version 1**

We have changed Figure 1E. Blue bars indicate SF (Seed formation ratio). Dark red bars indicate VSW (vanillin stained in whole seed coat). Gray bar indicates SA (Seed abortion ratio). Pink bars indicate VSP (vanillin stained in partial seed coat).

See referee reports

Introduction

In angiosperms, seed formation begins with pollination[1,2]. Once the pollen grain lands on the stigma at the top of the pistil, pollen tubes from the grain elongate toward the ovule. Fusion of the two gametes is required for seed formation. The male gametophyte is the pollen grain and the female gametophyte is the embryo sac[3]. Immediately after arrival at the ovules, the pollen tube bursts and the pollen tube contents (PTC) are released to the female gametophyte[4].

In a previous study, we reported that once the ovule accepts the PTC inside the female gametophyte, it begins enlargement and seed coat formation, irrespective of fertilization[5,6]. We named this phenomenon pollen tube-dependent ovule enlargement morphology (POEM). We also reported that if fertilization of the ovule fails, a partial seed coat is still produced, even though a complete seed coat cannot be formed. However, we have not confirmed whether all the ovules have the partial seed coat phenotype when ovule fertilization fails but PTC is accepted. To address this question, statistical experiments were conducted that included the fertilization recovery system (FRS), where a second pollen tube rescues the fertilization if fertilization by the first pollen tube fails, which we previously identified[7,8]. We reported that the seed formation ratio of *gcs1*/+ mutants[9–11] was approximately 65%; the remaining mutants were unable to produce seeds because fertilization of these ovules failed. Therefore, matching of the ratio of the ovules with the partial seed coat phenotype to the seed abortion ratio suggests that there was fertilization failure for all ovules with a partial seed coat. We also conducted experiments to determine whether *agl62* mutants[12] had the partial seed coat phenotype. In *agl62* seeds, the endosperm cellularizes prematurely, indicating that AGL62 is required for suppression of cellularization during the syncytial phase. During seed development, *AGL62* is exclusively expressed in the endosperm. Because *agl62* mutants have an abnormal endosperm phenotype after central cell fertilization, these mutants are ideal for investigating the relationship between endosperm formation and seed coat initiation and formation.

Methods

Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia (Col-0) plants were used as the wild-type (WT) plants. Test cross experiments were conducted in *gcs1*/+[9–11], *agl62*/+[12], and WT plants. Seeds were sterilized with 5% sodium hypochlorite containing 0.5% Triton X-100 and germinated on plates containing 0.5× Murashige and Skoog salts (pH 5.7) (Wako Pure Chemical), 2% sucrose, Gamborg's B5 vitamin solution (Sigma), and 0.3% Gelrite (Wako Pure Chemical) in a growth chamber at 21.5°C under 24 h of light after cold treatment (4°C) for 2 days. Next, 10-day-old seedlings were transferred to Metro-Mix 350 soil (Sun Gro) and grown at 21.5°C under 24 h of light.

Phenotypic analyses

For staining the silique tissue, the WT flowers were emasculated at stage 12c[13] and pollinated with *gcs1*/+ pollen grains. For *agl62* experiments, the *agl62* mutant flowers were emasculated at stage 12c and pollinated with WT, *gcs1*/+, and *agl62* pollen grains. The siliques were collected at 3 days after pollination (DAP).

For vanillin staining, the ovules were manually dissected from the ovaries and mounted on slides in 1% (wt/vol) vanillin (4-hydroxy-3-methoxybenzaldehyde; Sigma) in 6 N HCl solution. Slides were analyzed after 20 min of incubation. Samples were analyzed with a Leica DM2500 microscope using differential interference contrast optics. Images were recorded with a Leica DFC 300 FX digital camera at a magnification of 5×, 10× and 20×. The microscopic protocols followed were as previously described[5].

Results and discussion

First, the WT plants as both the female and male parent (Figure 1A) were crossed and the silique after vanillin staining at 3DAP was observed. The ratio of full seed coat formation was 98.7±1.2% (mean ± SD; n=10 pistils), which was consistent with our previous WT fertilization data[7,8]. By contrast, when the WT plants as the female parent and *gcs1*/+ as the male parent were crossed, the ratio of full seed coat formation was 68.7±5.8% (n=10 pistils) and the ratio of partial seed coat formation was 32.2±6.5% (n=10 pistils), which also was consistent with our previous *gcs1*/+ fertilization data. These data suggest that all successfully fertilized ovules produce a full seed coat and all unfertilized but PTC accepted ovules produce a partial seed coat.

Because the *agl62* mutant had an abnormal and arrested endosperm formation phenotype after fertilization, this mutant was ideal for investigating the relationship between endosperm formation and seed coat initiation and formation. The *agl62*/+ plants as the female parent and the WT as the male parent were crossed (Figure 1) and the silique after vanillin staining at 3DAP was observed. The ratio of full seed coat formation was 97.6±2.1% (n=10 pistils), which was consistent with our previous WT fertilization data. By contrast, when *agl62*/+ plants as the female parent and *agl62*/+ as the male parent were crossed, the ratio of full seed coat formation was 74.7±3.9% (n=10 pistils) and the ratio of partial seed coat formation was 25.2±5.4% (n=10 pistils), which was consistent with previous *agl62* data[12]. These results suggest that normal endosperm development is required for completion of seed coat formation, irrespective of fertilization. When *agl62*/+ plants as the female parent and *gcs1*/+ as the male parent were crossed, the ratio of full seed coat formation was 66.9±6.2% (n=10 pistils) and the ratio of partial seed coat formation was 33.2±5.9% (n=10 pistils), which also was consistent with our previous *gcs1*/+ fertilization data. These results suggest that *agl62*/+ abnormal endosperm prevents normal seed coat formation, but these

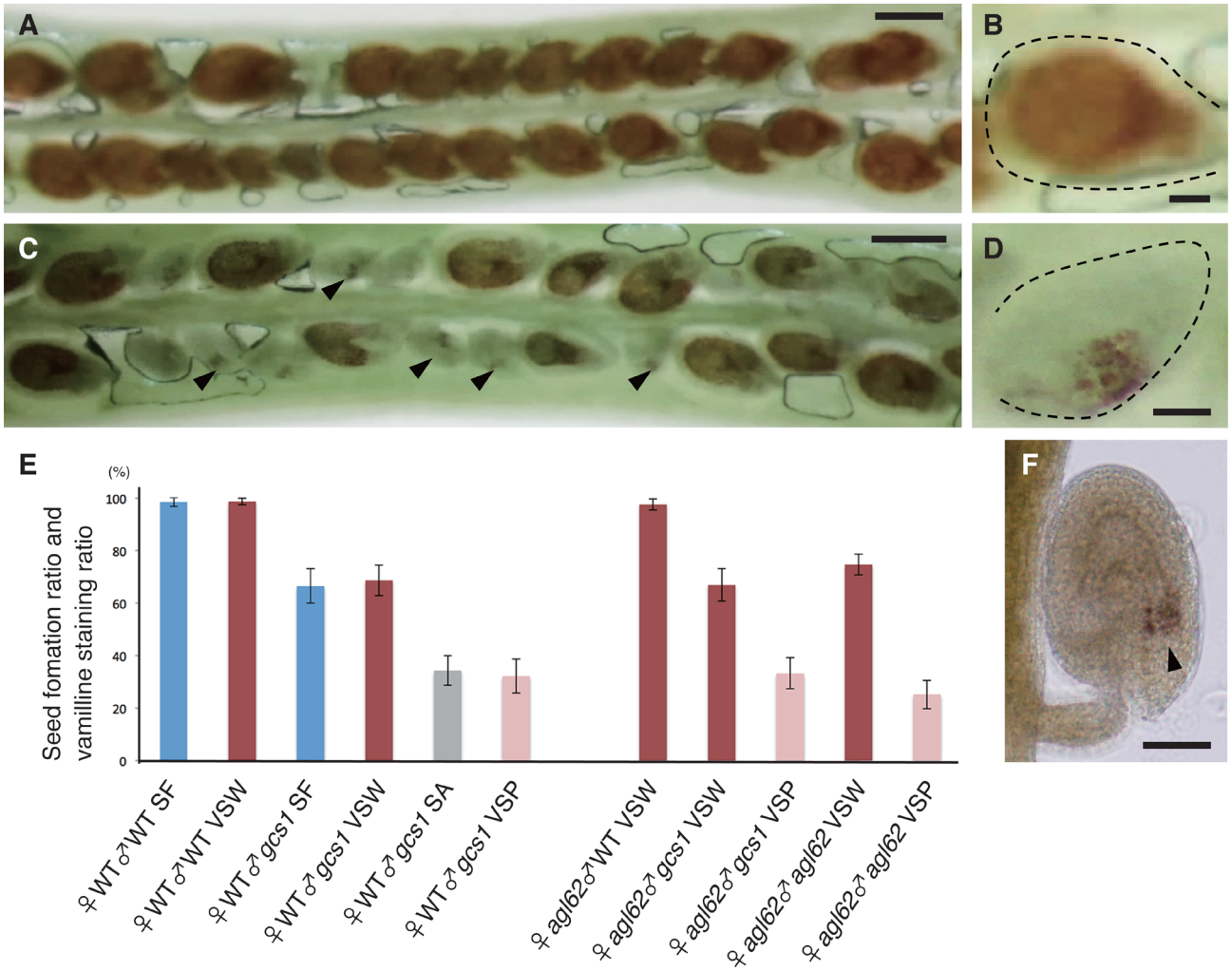

Figure 1. Pollen tube content (PTC) is sufficient to initiate seed coat formation. (**A**) Wild-type (WT) silique crossed with WT pollen and stained with vanillin. Almost all ovules were stained. Bar: 300μm. (**B**) Representative image of whole seed coat staining. Bar: 50μm. (**C**) WT silique crossed with *gcs1* pollen and stained with vanillin. Several ovules had partial seed coat (arrowhead) staining. Bar: 300μm. (**D**) Representative image of partial seed coat staining. Bar: 50 μm. (**E**) Comparison of seed formation ratio and vanillin staining ratio. ♀WT♂WT seed formation ratio (SF) indicates that a WT silique was crossed with WT pollen and the seed formation ratio was calculated. ♀WT♂WT vanillin stained in whole seed coat (VSW) indicates that a WT silique was crossed with WT pollen and the whole seed coat staining ratio was calculated. ♀*agl62*♂WT VSW indicates that an *agl62*/+ silique was crossed with WT pollen and the whole seed coat staining ratio was calculated. ♀WT♂*gcs1* SF indicates that a WT silique was crossed with *gcs1*/+ pollen and the seed formation ratio was calculated. ♀WT♂*gcs1* VSW indicates that a WT silique was crossed with *gcs1*/+ pollen and the whole seed coat staining ratio was calculated. ♀*agl62*♂*gcs1* VSW indicates that an *agl62*/+ silique was crossed with *gcs1*/+ pollen and the whole seed coat staining ratio was calculated. ♀WT♂*gcs1* seed abortion ratio (SA) indicates that a WT silique was crossed by *gcs1*/+ pollen and the seed abortion ratio was calculated. ♀WT♂*gcs1* (vanillin stained in partial seed coat) indicates that a WT silique was crossed with *gcs1*/+ pollen and the partial seed coat staining ratio was calculated. ♀*agl62*♂*gcs1* VSP indicates that an *agl62*/+ silique was crossed with *gcs1*/+ pollen and the partial seed coat staining ratio was calculated. ♀*agl62*♂ *agl62* VSW indicates that an *agl62*/+ silique was crossed with *agl62*/+ pollen and the whole seed coat staining ratio was calculated. ♀*agl62*♂*agl62* VSP indicates that an *agl62*/+ silique was crossed with *agl62/+* pollen and the partial seed coat staining ratio was calculated. Blue bars indicate SF (Seed formation ratio). Dark red bars indicate VSW (vanillin stained in whole seed coat). Gray bar indicates SA (Seed abortion ratio). Pink bars indicate VSP (vanillin stained in partial seed coat). (**F**) A ♀*agl62*♂*agl62* VSP ovule. The arrowhead indicates the vanillin-stained zone. Bar: 50 μm. For normal seed coat initiation, fertilization is not required; however, for completion of normal seed coat formation, both normal fertilization and normal endosperm development are required.

ovules still produce a partial seed coat because these ovules had accepted the PTC. In summary, for normal seed coat initiation, fertilization is not required; however, for completion of normal seed coat formation, both normal fertilization and normal endosperm development are required.

Data availability

Open Science Framework: Vanillin staining project. https://doi.org/10.17605/OSF.IO/6U73H[14].

This project contains the following underlying data:

- # of seeds data (Sheet2 contains the number of seeds stained out of the total number of seeds; Sheet1 the data summary used to produce Figure 1E)
- agl62-3v.tif (raw image of stained *agl62*/+ seeds)
- gcs1 vaniline.tif (raw image of stained *gcs1*/+ seeds)
- WT vaniline.tif (raw image of stained wild-type seeds)

Grant information

This work was supported by start-up funds from the School of Life Sciences, Fujian Agriculture and Forestry University (Grant #: 114-712018008) and the FAFU-UCR Joint Center and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Haixia Institute of Science and Technology, Fujian Agriculture and Forestry University.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We thank Shaowei Zhu and Xiaoyan Wu for technical assistance.

References

1. Maheshwari P: **An Introduction to the Embryology of Angiosperms.** (New York: McGraw-Hill). 1950.
Publisher Full Text
2. Kasahara RD: **The Regulation of Sperm Cells Delivery to the Embryo Sac.** *Pollination in plants.* 2018.
Publisher Full Text
3. Drews GN, Koltunow AM: **The female gametophyte.** *Arabidopsis Book.* 2011; **9**: e0155.
PubMed Abstract | Publisher Full Text | Free Full Text
4. Hamamura Y, Saito C, Awai C, *et al.*: **Live-cell imaging reveals the dynamics of two sperm cells during double fertilization in *Arabidopsis thaliana*.** *Curr Biol.* 2011; **21**(6): 497–502.
PubMed Abstract | Publisher Full Text
5. Kasahara RD, Notaguchi M, Nagahara S, *et al.*: **Pollen tube contents initiate ovule enlargement and enhance seed coat development without fertilization.** *Sci Adv.* 2016; **2**(10): e1600554.
PubMed Abstract | Publisher Full Text | Free Full Text
6. Kasahara RD, Notaguchi M, Honma Y: **Discovery of pollen tube-dependent ovule enlargement morphology phenomenon, a new step in plant reproduction.** *Commun Integr Biol.* 2017; **10**(4): e1338989.
Publisher Full Text | Free Full Text
7. Kasahara RD, Maruyama D, Hamamura Y, *et al.*: **Fertilization recovery after defective sperm cell release in *Arabidopsis*.** *Curr Biol.* 2012; **22**(12): 1084–1089.
PubMed Abstract | Publisher Full Text
8. Kasahara RD, Maruyama D, Higashiyama T: **Fertilization recovery system is dependent on the number of pollen grains for efficient reproduction in plants.** *Plant Signal Behav.* 2013; **8**(4): e23690.
PubMed Abstract | Publisher Full Text
9. Mori T, Kuroiwa H, Higashiyama T, *et al.*: **GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization.** *Nat Cell Biol.* 2006; **8**(1): 64–71.
PubMed Abstract | Publisher Full Text
10. von Besser K, Frank AC, Johnson MA, *et al.*: ***Arabidopsis HAP2* (*GCS1*) is a sperm-specific gene required for pollen tube guidance and fertilization.** *Development.* 2006; **133**(23): 4761–4769.
PubMed Abstract | Publisher Full Text
11. Nagahara S, Takeuchi H, Higashiyama T: **Generation of a homozygous fertilization-defective *gcs1* mutant by heat-inducible removal of a rescue gene.** *Plant Reprod.* 2015; **28**(1): 33–46.
PubMed Abstract | Publisher Full Text | Free Full Text
12. Kang IH, Steffen JG, Portereiko MF, *et al.*: **The AGL62 MADS domain protein regulates cellularization during endosperm development in *Arabidopsis*.** *Plant Cell.* 2008; **20**(3): 635–647.
PubMed Abstract | Publisher Full Text | Free Full Text
13. Christensen CA, King EJ, Jordan JR, *et al.*: **Megagametogenesis in *Arabidopsis* wild type and the *Gf* mutant.** *Sexual Plant Reproduction.* 1997; **10**(1): 49–64.
Publisher Full Text
14. Kasahara R: **Vanillin staining project.** 2019.
http://www.doi.org/10.17605/OSF.IO/6U73H

Open Peer Review

Current Referee Status: ✔ ✔ ✔

Version 2

Referee Report 07 May 2019

https://doi.org/10.5256/f1000research.20759.r47869

✔ **Tomoko Igawa**
Graduate School of Horticulture, Chiba University, Chiba, Japan

The author revisions are sufficient for the comments by the reviewers. The revised paper is acceptable for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gamete imaging during double fertilization in flowering plants, cytological and morphological analysis of sexual plant reproduction processes, molecular biology focusing on proteins regulating fertilization

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 15 April 2019

https://doi.org/10.5256/f1000research.20411.r46516

✔ **Tomokazu Kawashima**
Department of Plant and Soil Sciences, University of Kentucky, Kentucky, USA

This paper describes that the pollen tube content is sufficient to partially initiate seed coat without fertilization in Arabidopsis. This work appears the extension and further clarification of the previously published work (Kasahara et al., Science Advances 2016[1], and Roszak and Kohler, PNAS, 2011[2]). During plant fertilization, many cellular processes are happening, and it is important to integrate the identified phenomena together for comprehensive understanding of plant sexual reproduction. The authors studied the relationships among FRS, POEM, seed coat initiation, and endosperm development, and I think the work reported in this paper is an important piece of information in sexual plant reproduction.

I have a few suggestions:

1. The first paragraph of the introduction, the author stated that "gametophytes" fuse. I believe the authors mean "gametes".
2. From the authors previous work in Science Advances, 2016[1]), it is clear that PTC initiates seed coat development (transcriptionally and anatomically). It is important to state these details in the introduction to remind the readers. Along with this, please describe what vanillin staining detects (proanthocyanidin accumulation) and why this can be used for seed coat visualization.
3. In Roszak and Kohler, 2011[2], the authors reported that not *fis2* and *mea*, but *fie* and *msi1* autonomously developing seeds differentiate seed coat, suggesting that fertilization is not required for seed coat initiation. The authors also described seed coat phenotypes in *agl62*. Please refer this publication and highlight and/or discuss new findings/hypotheses in this paper, then this paper will be more impactful.
4. As other reviewers suggested, Figure 1E is difficult to dissect information. I believe "vamiline" in the Y-axis label should be "vanillin".

References

1. Kasahara RD, Notaguchi M, Nagahara S, Suzuki T, Susaki D, Honma Y, Maruyama D, Higashiyama T: Pollen tube contents initiate ovule enlargement and enhance seed coat development without fertilization. *Sci Adv*. 2016; **2** (10): e1600554 PubMed Abstract | Publisher Full Text
2. Roszak P, Köhler C: Polycomb group proteins are required to couple seed coat initiation to fertilization. *Proc Natl Acad Sci U S A*. 2011; **108** (51): 20826-31 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sexual plant reproduction, cellular dynamics, molecular biology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 15 Apr 2019

Kasahara Ryushiro, Fujian Agriculture and Forestry University, China

Dear Dr. Tomokazu Kawashima,

Thank you very much for your critical comments.
We will improve our paper based on your suggestion.

Again, thank you very much.

Ryushiro Dora Kasahara

Competing Interests: No competing interests were disclosed.

Author Response 15 Apr 2019

Kasahara Ryushiro, Fujian Agriculture and Forestry University, China

Dear Dr. Tomokazu Kawashima,

Thank you very much for your critical comments.
We will improve our paper based on your suggestion.

Again, thank you very much.

Ryushiro Dora Kasahara

Competing Interests: No competing interests were disclosed.

Referee Report 11 April 2019

https://doi.org/10.5256/f1000research.20411.r47023

Nobutaka Mitsuda
Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

This paper showed basically the confirmation of the previous report of the POEM paper. However, because this report showed that the relationships between pollen tube contents and seed coat formation statistically, including the data for agl62 mutants, they clearly confirmed that almost all ovules started seed coat formation without fertilization but just required for the pollen tube contents. I believe this report is a sort of important discoveries in the plant science field and will contribute to further research for seed coat formation in angiosperms.

Introduction:

- At the introduction part, it is clear what they need to understand and what sorts of approach were taken. I believe their approach conducted here is adequate and leads to a neat statistics for seed coat initiation and completion study.

Results and Discussion:

- I agree with the first reviewer's comment. Authors could improve the paper if they change the colors for Fig. 1. However, I think this change is not mandatory because if authors change the color dramatically, it could be too showy. I thought current style looks fair enough.
- I also agree with the first reviewer's comment that because the study of agl62 was not the authors study, "our previous agl62 data" sounds strange. But this is just a very minor correction to be done.

Data availability:

- I think the data title agl61-3v. tif is wrong. Title should be "agl62-3v".

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: plant biotechnology, transcription factor, reproductive development, cell wall, seed, cuticle

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Apr 2019

Kasahara Ryushiro, Fujian Agriculture and Forestry University, China

Dear Dr. Mitsuda,

Thank you very much for your critical comments.
We will improve our paper based on your suggestion.

Again, thank you very much.

Ryushiro Dora Kasahara

Competing Interests: No competing interests were disclosed.

Referee Report 08 April 2019

https://doi.org/10.5256/f1000research.20411.r46381

Tomoko Igawa
Graduate School of Horticulture, Chiba University, Chiba, Japan

Normal seed formation required for the birth of the progeny is a critical phenomenon for plant production and breeding field.
This research note reports that the involvements of the fertilization and the normal endosperm development in seed coat formation. First, the involvement of the pollen tube contents was analyzed showing the reproducibility of their previous report. Also, it was indicated that normal endosperm development is required for successive seed coat formation, using agl62 mutant.

Introduction

- Authors described constructively what they wanted to clarify. The experimental design and the statistical analysis performed in this study were enough to examine the questions.

Results and Discussion

- In the present state, the descriptions and the graph (Fig.1E) were confusing to understand the results. How about using different colors for each SF, VSF, AS, and VSP. Also, if the graph-bars obtained with the same female parent-genotype are grouped and indicated, it would be helpful for easy comparing the results.
- As a conclusion, the authors claimed that "for completion of normal seed formation, normal endosperm formation is required in addition to normal fertilization." In the previous studies, the aborted seed caused by the single fertilization of the central cell seemed to have developed the seed coat (kokopelli, gex2, dmp9 mutants, etc...). These studies are supportive of the authors' claim, but the endosperm of these mutants was not normal as the wild type seed. Therefore, how about describing "normal endosperm 'development' is required for seed coat formation."
- As a minor point, authors describe the previous study of agl62 (Kang et al., 2008)[1] as 'our previous....' but no same authors in this paper.

Overall, the content of this research note is logically written. If the points described above are re-considered, it would be improved.

References

1. Kang IH, Steffen JG, Portereiko MF, Lloyd A, Drews GN: The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis.*Plant Cell*. 2008; **20** (3): 635-47 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gamete imaging during doubler fertilization in flowering plants, cytological and morphological analysis of sexual plant reproduction processes, molecular biology focusing on proteins regulating fertilization

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 08 Apr 2019

Kasahara Ryushiro, Fujian Agriculture and Forestry University, China

Dear Dr. Igawa,

Thank you very much for your critical comments. I will improve the paper based on your comments as soon as possible.

Best regard,

Ryushiro Dora Kasahara

Competing Interests: No competing interests were disclosed.

Author Response 11 Apr 2019

Kasahara Ryushiro, Fujian Agriculture and Forestry University, China

Dear Dr. Igawa,

I have finished revising based on your suggestions.

In the present state, the descriptions and the graph (Fig.1E) were confusing to understand the results. How about using different colors for each SF, VSF, AS, and VSP. Also, if the graph-bars obtained with the same female parent-genotype are grouped and indicated, it would be helpful for easy comparing the results.

I have submitted a new version of the Fig.1. Please take a look.

As a conclusion, the authors claimed that "for completion of normal seed formation, normal endosperm formation is required in addition to normal fertilization." In the previous studies, the aborted seed caused by the single fertilization of the central cell seemed to have developed the seed coat (kokopelli, gex2, dmp9 mutants, etc...). These studies are supportive of the authors' claim, but the endosperm of these mutants was not normal as the wild type seed. Therefore, how about describing "normal endosperm 'development' is required for seed coat formation."

I changed the word to normal endosperm development.

As a minor point, authors describe the previous study of agl62 (Kang et al., 2008)[1] as 'our previous....' but no same authors in this paper.

I removed "our". from the text.

Again, thank you very much for your critical comments.

Best regards,

Ryushiro Dora Kasahara

Competing Interests: No competing interests were disclosed.