Pivotal role of STIP in ovule pattern formation and female germline development in *Arabidopsis thaliana*

Rosanna Petrella, Flavio Gabrieli, Alex Cavalleri, Kay Schneitz, Lucia Colombo and Mara Cucinotta

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Original submission

First decision letter

MS ID#: DEVELOP/2022/200989

MS TITLE: Pivotal role of WUSCHEL-RELATED HOMEOBOX 9/STIMPY in ovule pattern formation and female germline development in Arabidopsis thaliana.

AUTHORS: Rosanna Petrella, Flavio Gabrieli, Kay Schneitz, Lucia Colombo, and Mara Cucinotta
ARTICLE TYPE: Research Article

Dear Dr. Cucinotta,

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see from their reports, the referees recognise the potential of your work, but they also raise significant concerns about it. Given the nature of these concerns, I am afraid I have little choice other than to reject the paper at this stage.

However, having evaluated the paper, I do recognise the potential importance of this work. I would therefore be prepared to consider as a new submission an extension of this study that contains new experiments, data and discussions and that address fully the major concerns of the referees. The work required goes beyond a standard revision of the paper. Please bear in mind that the referees (who may be different from the present reviewers) will assess the novelty of your work in the context of all previous publications, including those published between now and the time of resubmission.

Reviewer 1

Advance Summary and Potential Significance to Field

This work is very nicely done, very informative regarding STIP/WOX9, a gene that was previously reported to be expressed in embryo and reproductive and vegetative organs; its mutation affects embryo development, but that has received little attention. Here a complete analysis of the expression pattern during ovule development and the characterization of null mutants during ovule development, clearly indicates that STIP/WOX9 is playing a role in the control of symmetry in the

ovule, to facilitate the correct curvature of the Arabidopsis ovule. The data provide has enough novelty to be reported.

Comments for the author

The only major issue is regarding he regulation of STIP on PHB. In the model of Figure 7b, where STIP regulates INO by PHB, no direct data on this molecular mechanism is provided. They have shown that STIP does not directly regulate INO, but does it regulate PHB instead? I wonder if a ChIP-PCR could be done to identify STIP activity on PHB regulatory regions. A sequence analysis of the putative STIP binding sites may also help (and should be provided) to have a better and more complete view of the role of STIP on ovule development.

Minor comments.
The first sentence in the introduction is overstated “Sexual reproduction of higher plants relies upon ovules, the precursors of seeds”. Ovules are indeed key in plant reproduction, but other factors (like pollen) are also crucial for seed development. I recommend to downstate the first sentence.

Line 42, this statement should be corrected. Asymmetrical integument development is important in curved anatropous ovules (curved ovules but with straight nucellus), but it is not a general feature. Some plants develop symmetrical orthotropous ovules or even campylotropous ovules, where the nucellus is also curvated.

Line 55-65. Most (is not all) of this information is gathered from Arabidopsis, and these are Arabidopsis genes. It is most probably that ortholog genes in other plant species have similar roles, but this is yet to be totally demonstrated. Therefore, I recommend clarifying that the information in this section of Introduction is in Arabidopsis.

Line 69-70. Despite there is limited text space, it is important to provide in the Introduction what is exactly known about the role of WUS in ovule integument development, so more precise information form Groß-Hardt et al. (2002) and Sieber et al. (2004) should be provided in the introduction.

Line 78-79. It seems that STIP has been reported to play a role in ovule development “STIP acts redundantly with its paralog WOX8 to define the apical-basal axis in the embryo (Haecker et al., 2004)”. But in this paper, only expression pattern of WOX9 in embryo development is shown. I recommend rewriting the conclusion from Haecker et a. (2004) regarding the expression of STIP or provide a reference that support the statement in line 78-79.
Following this, the reference Haecker et al. (2004) should be add to line 95 along with reference Wu et al. (2005), as it is in Haecker where detailed STIP expression in embryo is provided.
Line 120. What does “n” indicate here (n=12 and n=17)? It is not clear to me (or I am missing something obvious).

Line 301-302. The last part of the sentence “suggesting a movement of the protein” should be removed (or moved to later). The difference between mRNA and protein localization observed in Figure 1 is not sufficient to rise such conclusion. It makes sense that this is the case based n the data on other WOX genes, as mentioned a little bit later in the text. The conclusion of protein movement should be then moved later in the text. For example, in line 305, I

would suggest beginning the sentence with “The different mRNA and protein localization observed in Figure 1 is consistent with the previous suggestion that STIP functions a non-cell autonomous transcription factor in the embryo, probably by the movement of the STIP protein…”
Finally, the hypothesis that STIP function in ovule is thorough CKs is quite interesting. It is a pity that this has not been experimentally addresses in this paper. In addition, it would be necessary to know whether the regulation of CKs genes by STIP is also mediated by WUS/PHB (or where CKs are in the scheme shown in Figure 7).
In the ChIP-PCR shown in Supplementary Figure S2, it is not clear why they used these DNA regions for the analysis. Are they addressing specific cis elements that are potential targets of bHLH proteins? Data regarding the cis elements addresses in the ChIP-PCR would be helpful. These regions-elements could be provided in the same Figure S2.

Other minor issues:
Line 460-461, it seems that there is a problem with this text.

Line 42, missing period after reference.

Supplementary Figure 1S. Panel F, there is a triangle pointing to a T-DNA insertion in the genomic region of STIP for stip-2, identical as for STIP-D. I guess that this is a mistake in stip-2.

Feedback on other Reports

I did not go into detail on comments and issues raised by reviewer 2. I assume that she/he is right, and therefore these issues must be addressed before publication.
There is a strong discrepancy on the novelty of the work and whether it can be published in Development. I still believe that this work, especially after completed all requirements by both reviewers, has enough relevance for Development.

Reviewer 2

Advance Summary and Potential Significance to Field

Review

The manuscript by Petrella et al., addresses the role of STIP or WOX9 in regulating ovule development. They do so by describing the phenotypes of a stip/wox9 mutant and of STIP/WOX9 overexpression. Furthermore, they report altered expression of three genes, WUS, PHB, and INO, the roles of which in the ovule have been reported before, dependent on STIP/WOX9.

Overall, although the analyses are carefully done, the amount of novel insight into mechanisms of ovule development is very limited and far below the current average paper in DEVELOPMENT. Therefore, I regret to say that I cannot recommend this manuscript for publication in DEVELOPMENT.

Comments for the author

Detailed comments and open questions

- The sense probe control is not shown in any of the in-situ hybridization results. Please include appropriate controls.

-Line #100. (1) Indicate the frequency of STP-GFP detection in nucellus or chalaza. (2) The authors infer the pSTIP:STIP-GFP expression in the nucellar epidermis in contrast to the in-situ data as a consequence of protein movement. However, it could be also due to the length of the promoter used to drive the STIP-GFP or the movement of split off GFP. An experiment with mobile (eg.1xGFP) and non-mobile (eg.3xGFP) versions of STIP could address if STIP protein moves and if so, whether the movement is functionally relevant.

-Line #156. How did the authors confirm the arrest of FG in 94% of stip-2 ovules? Is it based on anatomical observation? If so, please provide representative images and describe the phenotype.

-Line #274 & Figure 6. How could STIP, which is majorly expressed in the funicular epidermis, repress the PHB expression in the nucellus? Their spatio-temporal overlap seems to be minimal. The same question applies to WUS expression as well.

-Line #447. Please describe the method used for ChIP-qPCR normalization.

- Do phb and wus mutants share the integument/ FG phenotype of STIP/INO? Is there genetic or molecular evidence to suggest that they act in the same pathway? If not, STIP-mediated regulation of PHB & WUS expression might be irrelevant to this story.
Minor Comments

-Line #93. Expression in the transmitting tract (tt) is not readily noticeable when compared to other tissues. The strength of expression could be mentioned in the text. Moreover, the authors mention that a similar expression pattern was described previously. However, the reference is missing. Is it Wu et al., 2005? Please give a reference.

-Line #118. Spelling: 'thicker'?

-Line #128. Figure '2G' and not Figure '3G'.

-Line #190. Figure '1E' and not Figure '1D'.

-Line #197. Typo: '33s' CAMV instead of 35s CAMV

-Line #190. Figure reference: Figure '5I' and not Figure '5M'.

-Line #233. The term 'tip of the nucellus' could be misleading. Technically, the tip of the nucellus is the epidermis. However, the pKNU expression is not seen in the epidermis of the stip-D ovules rather the altered size and shape of the stip-D MMC suggest a parenchyma-like appearance.

-Supplementary Figure 2A: Please consider labelling the black lines with the region numbers, 2B: Colour code (WT vs pSTIP:STIP-GFP) for the bar chart is missing.

-Please consider marking the adaxial & abaxial specific gene expression in the in-situ figures with different arrow marks.

-Line #424&425. Please consider re-writing the sentence for clarity.

Author response to reviewers' comments

Rebuttal to Reviewers.

Reviewer 1 Advance Summary and Potential Significance to Field:

This work is very nicely done, very informative regarding STIP/WOX9, a gene that was previously reported to be expressed in embryo and reproductive and vegetative organs; its mutation affects embryo development, but that has received little attention. Here a complete analysis of the expression pattern during ovule development and the characterization of null mutants during ovule development, clearly indicates that STIP/WOX9 is playing a role in the control of symmetry in the ovule, to facilitate the correct curvature of the Arabidopsis ovule. The data provide has enough novelty to be reported.

We thank the reviewer for the punctual analysis which helped to improve our manuscript. In this revised version we performed experiments to address the raised points and to improve the overall presentation of the work according to the suggestions received.

Reviewer 1 Comments for the Author:

The only major issue is regarding the regulation of STIP on PHB. In the model of Figure 7b, where STIP regulates INO by PHB, no direct data on this molecular mechanism is provided. They have shown that STIP does not directly regulate INO, but does it regulate PHB instead? I wonder if a ChIP-PCR could be done to identify STIP activity on PHB regulatory regions. A sequence analysis of the putative STIP binding sites may also help (and should be provided) to have a better and more complete view of the role of STIP on ovule development.

We thank the reviewer for the suggestion. In the revised version of the manuscript we performed a sequence analysis, using PlantPan 3.0; we found six putative binding regions on *PHB* locus, presenting WOX HOMEODOMAIN consensus sites (Supplementary Figure S3A,B). Then, we performed

a ChIP-PCR on these regions, using a GFP antibody to immunoprecipitated WOX9-GFP fusion protein (Figure 6G). We could indeed find two enriched regions. We agree with the reviewer, and we think that these results contribute to better dissect the *STIP-PHB-INO/WUS* interplay in the chalaza and in the nucellus, as presented in Figure 7G,H.

Minor comments.

The first sentence in the introduction is overstated "Sexual reproduction of higher plants relies upon ovules, the precursors of seeds". Ovules are indeed key in plant reproduction, but other factors (like pollen) are also crucial for seed development. I recommend to downstate the first sentence.

We edited the sentence in the text "Ovules, which develop into seeds upon fertilization, are fundamental for sexual reproduction".

Line 42, this statement should be corrected. Asymmetrical integument development is important in curved anatropous ovules (curved ovules but with straight nucellus), but it is not a general feature. Some plants develop symmetrical orthotropous ovules or even campylotropous ovules, where the nucellus is also curvated.

We apologized with the reviewer, and we corrected the sentence "In Arabidopsis, an important role of the OI is the establishment of the curvature (anatropy) of the ovule".

Line 55-65. Most (is not all) of this information is gathered from Arabidopsis, and these are Arabidopsis genes. It is most probably that ortholog genes in other plant species have similar roles, but this is yet to be totally demonstrated. Therefore, I recommend clarifying that the information in this section of Introduction is in Arabidopsis.

We edited the text "In *Arabidopsis thaliana*, the activities of several transcription factors ensure proper formation of integuments and correct embryo sac development".

Line 69-70. Despite there is limited text space, it is important to provide in the Introduction what is exactly known about the role of WUS in ovule integument development, so more precise information form Groß-Hardt et al. (2002) and Sieber et al. (2004) should be provided in the introduction.

We agree with the reviewer, and we added the information in the text "As matter of fact, lack of *WUS* expression determines ovules that develop without integuments (Groß-Hardt et al., 2002).".

Line 78-79. It seems that STIP has been reported to play a role in ovule development "STIP acts redundantly with its paralog WOX8 to define the apical-basal axis in the embryo (Haecker et al., 2004)". But in this paper, only expression pattern of WOX9 in embryo development is shown. I recommend rewriting the conclusion from Haecker et al. (2004) regarding the expression of STIP or provide a reference that support the statement in line 78-79.

We added Breuninger et al., (2008) in the text, and in the reference list in which they clearly showed the phenotype of *wox8 wox9* double mutant during embryo patterning.

Following to this, the reference Haecker et al., 2004 should be added along with reference Wu et al. (2005), as it is in Haecker where detailed STIP expression in embryo is provided.

We added the reference to the text: "To assess whether STIP protein accumulation pattern reflects transcript localization we analysed the expression of *pSTIP:STIP-GFP* reporter (Haecker et al., 2004; Wu et al., 2007).".

Line 120. What does "n" indicate here (n=12 and n=17)? It is not clear to me (or I am missing something obvious).

We apologized for the lack of clarity. "n" stands for number of siliques analysed. We moved this information in figure legend and we rephrased the text in the manuscript: "We therefore compared seed set in siliques of *stip- 2* and wild-type".

Line 301-302. The last part of the sentence “suggesting a movement of the protein” should be removed (or moved to later). The difference between mRNA and protein localization observed in Figure 1 is not sufficient to rise such conclusion. It makes sense that this is the case based on the data on other WOX genes, as mentioned a little bit later in the text. The conclusion of protein movement should be then moved later in the text. For example, in line 305, I would suggest beginning the sentence with “The different mRNA and protein localization observed in Figure 1 is consistent with the previous suggestion that STIP functions as non-cell autonomous transcription factor in the embryo, probably by the movement of the STIP protein…”

We thank the reviewer for the suggestion. First, to support the movement of the protein we checked the expression of GFP by in situ hybridization probing *stip-2 pSTIP:STIP-GFP* ovules (Supplementary Figure 1). In addition, as suggested, we edited the text in the discussion section: “The observed discrepancy between *STIP* transcript accumulation and protein pattern is consistent with the previous suggestion that STIP acts as a non-cell autonomous transcription factor in the embryo”.

Finally, the hypothesis that STIP function in ovule is thorough CKs is quite interesting. It is a pity that this has not been experimentally addresses in this paper. In addition, it would be necessary to know whether the regulation of CKs genes by STIP is also mediated by WUS/PHB (or where CKs are in the scheme shown in Figure 7).

We understand the reviewer’s observation, and we would really like to investigate in detail this interesting connection in a future work. Also, adding CKs in the model of Figure 7 would be too speculative at this point.

In the ChIP-PCR shown in Supplementary Figure S2, it is not clear why they used these DNA regions for the analysis. Are they addressing specific cis elements that are potential targets of bHLH proteins? Data regarding the cis elements addresses in the ChIP-PCR would be helpful. These regions-elements could be provided in the same Figure S2.

We agree with the reviewer. In the revisited the ChIP-PCR on *INO* locus was moved to Figure 4T manuscript. Moreover, we added the analysis that we did on the locus of *INO* to look for WOX HOMEODOMAIN putative consensus sequences, using PlantPan 3.0 web tool in Supplementary Figure 3.

Other minor issues:

Line 460-461, it seems that there is a problem with this text.

Line 42, missing period after reference.

We corrected the typos throughout the text

Supplementary Figure 1S. Panel F, there is a triangle pointing to a T-DNA insertion in the genomic region of STIP for stip-2, identical as for STIP-D. I guess that this is a mistake in stip-2.

The scheme shown in Supplementary Figure S1 is correct; *stip-2* mutation has the same genetic background of *stip-D* (it harbors the T-DNA in the 3’UTR); but it presents a mis-match in the coding region, generating a premature stop codon, leading to a knock-out mutation (Wu et al., 2005).

Reviewer 2 Advance Summary and Potential Significance to Field:

The manuscript by Petrella et al., addresses the role of STIP or WOX9 in regulating ovule development. They do so by describing the phenotypes of a stip/wox9 mutant and of STIP/WOX9 overexpression. Furthermore, they report altered expression of three genes, WUS, PHB, and INO, the roles of which in the ovule have been reported before, dependent on STIP/WOX9. Overall, although the analyses are carefully done, the amount of novel insight into mechanisms of ovule development is very limited and far below the current average paper in DEVELOPMENT. Therefore, I regret to say that I cannot recommend this manuscript for publication in DEVELOPMENT.

We were sorry to read that the reviewer did not appreciate the novelty of our manuscript. We demonstrated, by genetic and molecular data, the importance of STIP/WOX9 in orchestrating the genetic network involving *INO, PHB and WUS* to regulate ovule patterning and female germline development. We thank the reviewer for the detailed analysis of our manuscript and in this revised version we addressed most of their comments.

Reviewer 2 Comments for the Author:

Detailed comments and open questions

- The sense probe control is not shown in any of the in-situ hybridization results. Please include appropriate controls.

We added the sense probe controls for all the *in situ* hybridization experiments in Supplementary Figure 2.

-Line #100. (1) Indicate the frequency of STIP-GFP detection in nucellus or chalaza.
(2) The authors infer the pSTIP:STIP-GFP expression in the nucellar epidermis in contrast to the in-situ data as a consequence of protein movement. However, it could be also due to the length of the promoter used to drive the STIP-GFP or the movement of split off GFP. An experiment with mobile (eg.1xGFP) and non-mobile (eg.3xGFP) versions of STIP could address if STIP protein moves and if so, whether the movement is functionally relevant.

We added the expression pattern of STIP-GFP at earlier stage of ovule development (Figure 1F) and we can confirm that from stage 1-II and 2-I all the ovules present the same pattern of expression in the epidermal layer of the chalaza and nucellus; in contrast, both the *STIP* transcript in wt background and the *GFP* transcript in the *pSTIP:STIP-GFP* plants expressed *STIP* or the *GFP* respectively, solely in the placenta and in the funiculus. We understand the reviewer's suggestion but generating construct with a 3xGFP would require a lot of time. We are willing to address this point in the future to further dissect the mechanism of WOX9 protein activity.

-Line #156. How did the authors confirm the arrest of FG in 94% of stip-2 ovules? Is it based on anatomical observation? If so, please provide representative images and describe the phenotype.

We apologized with reviewer for the lack of clarity. In the revisited version of the manuscript, we better explained our analysis, adding pictures of cleared ovules in Figure 3, as suggested. We performed morphological characterization of cleared ovules. We compared wild-type and *stip-2* ovules at stage 3-II/FG2 (Schneitz et al., 1995) and we determined FG1 block and quantified how many ovules where showing the phenotype (presence of one FM nucleus vs a developing embryo sac with two nuclei. Then, to better understand the identity of the one nucleus in *stip-2* female gametophyte we analysed the *pLC2:3xnlsYFP* (marker of FM and FG2 nuclei).

-Line #274 & Figure 6. How could STIP, which is majorly expressed in the funicular epidermis, repress the PHB expression in the nucellus? Their spatio-temporal overlap seems to be minimal. The same question applies to WUS expression as well.

We understood the reviewer's concern. We therefore added the expression pattern of STIP-GFP in an earlier stage (Figure 1F), showing that at stage 1-II and 2-I STIP-GFP is expressed in the epidermal layer of the chalaza and the nucellus, that is when *WUS* is activated and *PHB* need to be repressed in the nucellus.

-Line #447. Please describe the method used for ChIP-qPCR normalization.

We apologized with the reviewer for the lack of clarity. We added all the information in the Material and methods.

- Do phb and wus mutants share the integument/ FG phenotype of STIP/INO? Is there genetic or molecular evidence to suggest that they act in the same pathway? If not, STIP-mediated regulation of PHB & WUS expression might be irrelevant to this story.

We think that our results corroborate the existence of a STIP-PHB-WUS and STIP-PHB-INO interplay and are in line with already published data. First, lack of regulation of *PHB* expression in the chalaza leads to aberrant integument development (Hashimoto et al., 2018); also, *WUS* lack of expression determines ovules without integuments (Groß-Hardt et al., 2002). Our mutant, despite presenting a strong downregulation of *WUS* expression, still present inner integument development. In the revised version of our manuscript we added the observation of WUS-GFP in *stip-2* background (and wild-type as control) and by quantifying the signal intensity of GFP in nucellar cells of the ovule we could determine that there is still a level of WUS protein that most likely can induce inner integument formation in *stip-2* (Figure 7D-F).

Minor Comments

-Line #93. Expression in the transmitting tract (tt) is not readily noticeable when compared to other tissues. The strength of expression could be mentioned in the text.

In this revised version of the manuscript we removed the information regarding the expression in the transmitting tract since it is not relevant for our study.

Moreover, the authors mention that a similar expression pattern was described previously. However, the reference is missing. Is it Wu et al., 2005? Please give a reference.

Also, we added the reference "Wu et al., 2005 to the text.

-Line #118. Spelling: 'thicker'?
-Line #128. Figure '2G' and not Figure '3G'.
-Line #190. Figure '1E' and not Figure '1D'.
-Line #197. Typo: '33s' CAMV instead of 35s CAMV
-Line #190. Figure reference: Figure '5I' and not
Figure '5M'.

We edited the typos.

-Line #233. The term 'tip of the nucellus' could be misleading. Technically, the tip of the nucellus is the epidermis. However, the pKNU expression is not seen in the epidermis of the stip-D ovules rather the altered size and shape of the stip-D MMC suggest a parenchyma-like appearance.

We changed "tip of nucellus" with "tip of the L2 layer of the nucellus" throughout the text and the legends.

-Supplementary Figure 2A: Please consider labelling the black lines with the region numbers, 2B: Colour code (WT vs pSTIP:STIP-GFP) for the bar chart is missing.

We edited the panel presenting the ChIP results on *INO* locus (see Figure 5T). Also, we added the ChIP on *PHB* locus where we could determine enrichment in two out of six regions tested (Please, see Figure 6G). The region tested and the putative binding sites for WOX9 are described in detail in Supplementary Figure S3.

Please consider marking the adaxial & abaxial specific gene expression in the in-situ figures with different arrow marks.

Unfortunately, in tissue sections it is quite difficult and sometimes impossible to discriminate between adaxial and abaxial side of the ovule.

-Line #424&425. Please consider re-writing the sentence for
clarity.

Typos were edited and corrected.

Resubmission

First decision letter

MS ID#: DEVELOP/2022/201184

MS TITLE: Pivotal role of WUSCHEL-RELATED HOMEOBOX 9/STIMPY in ovule pattern formation and female germline development in Arabidopsis thaliana.

AUTHORS: Rosanna Petrella, Flavio Gabrieli, Alex Cavalleri, Kay Schneitz, Lucia Colombo, and Mara Cucinotta
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

This work is very nicely done, very informative regarding STIP/WOX9, a gene that was previously reported to be expressed in embryo and reproductive and vegetative organs; its mutation affects embryo development, but that has received little attention. Here a complete analysis of the expression pattern during ovule development and the characterization of null mutants during ovule development clearly indicates that STIP/WOX9 is playing a role in the control of symmetry in the ovule, to facilitate the correct curvature of the Arabidopsis ovule.
The data provide has enough novelty to be reported.

Comments for the author

The authors have met all the issues I mentioned in my previous review.
I have no more comments to make

Reviewer 2

Advance summary and potential significance to field

Petrella et al. have done significant work to characterize the role of STIP in ovule development. Other prior studies have studied the role of this gene in earlier aspects of vegetative and reproductive development but the focus on ovules and regulation of ovule development genes is novel.
They show that STIP is expressed in ovules and provide evidence that the protein moves into cellular precursors of the outer integument (OI). The addition in supplemental materials of data on the localization of the STIP-GFP fusion mRNA adequately addresses the reviewers' request for information supporting the protein movement as the mechanism for the expanded pattern of GFP florescence.
Ovules of the stip-2 mutant resemble ovules of the ino mutant due to failure in formation of the OI. INO expression is reduced in stip mutants, and INO expression is increased by ectopic expresson of STIP providing an apparent explanation of the phenotype. ChIP assays did not indicate direct regulation of expression of INO by STIP. However, similar assays on PHB, a known repressor of INO, indicated that STIP could directly repress PHB. This could be the mechanism of stip mutant effect on INO.
They further provide evidence that STIP positively regulates WUS expression. They note that this could be partly or completely through STIP negative regulation of PHB.
Based on these results, the proposed model is completely reasonable and adds another participant in the interplay of genes affecting ovule and integument development.

Comments for the author

Fig. 4 D. The structure labeled “OI” is not actually an OI, but rather a growth particular to strong ino mutants (like ino-5). It is on the wrong side of the ovule to be the OI, the OI would be on the concave side of the curve of the funiculus, not on the convex side where this structure is located. Strong ino mutants do not initiate and OI.
Fig. 5 B labels overlie color boxes making them hard to read (but this could be a result of the .pdf conversion and may not be in the source figure).