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Peer Review Report for 10.1105/tpc.16.00981

Ethylene-Inhibited Jasmonic Acid Biosynthesis Promotes Mesocotyl/Coleoptile Elongation of Etiolated Rice Seedlings

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Review timeline:

TPC2016-00981-RA	Submission received:	Dec. 30, 2016
	1st Decision:	Feb. 10, 2017 *revision requested*
TPC2016-00981-RAR1	1st Revision received:	Mar. 27, 2017
	2nd Decision:	Apr. 17, 2017 *acceptance pending, sent to science editor*
	Final acceptance:	May 2, 2017
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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2016-00981-RA 1st Editorial decision – *revision requested* **Feb. 10, 2017**

We have received reviews of your manuscript entitled "Ethylene-Inhibited JA Biosynthesis Promotes Mesocotyl/Coleoptile Elongation of Rice Etiolated Seedlings." Thank you for submitting your best work to The Plant Cell. The editorial board agrees that the work you describe is substantive, falls within the scope of the journal, and may become acceptable for publication pending revision, and potential re-review.

In addition to experimentally addressing all reviewer's critics please pay attention to the following points in preparing your revision. Provide clarification on how the gy1 mutant was identified as concerns are raised by reviewer 2. Provide biochemical evidence that GY1 catalyzes the initial step of the JA synthesis and also carry on activity tests with the GY1376T mutant. Demonstrate that the ethylene inhibition of JA under JA-induced conditions.

However, I should stress that we are reluctant to see manuscripts undergoing multiple rounds of revision and would be unlikely to offer you more than one chance to satisfy the reviewers.

Note that the sampling and nature of "biological replicates" should be described precisely (i.e., different plants, parts of plants, pooled tissue, independent pools of tissue, sampled at different times, etc.). The reader should know exactly what was sampled; what forms the basis of the calculation of any means and statistical parameters reported. This is also necessary to ensure that proper statistical analysis was conducted.
Please contact us if there are ambiguous comments or if you wish to discuss the revision.

The supplemental materials must be in Arial or Helvetica (legends to supplemental figures are not). Please see comments on the attached files to improve presentation of tables and figures. You will need to download and view in comments mode in Acrobat. In addition, note that supplemental figures must support the main figures in the paper (e.g., replicates, large datasets), and not introduce new results. Please ensure that this is the case and if not move the figure into the main paper.

Given the nature of the comments, we are offering you 60 days to complete the revision. If a revision is not returned within this time frame, and if you have not been granted an extension, we will withdraw the manuscript, which will leave you free to submit the work elsewhere. If you need an extension, we encourage you to contact us at any point before submitting your revision.

---------- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2016-00981-RAR1 1st Revision received Mar. 27, 2017

Reviewer comments and **author responses**:

Reviewer #1:

Xiong et al., reported the role of one jasmonic acid (JA) biosynthetic enzyme GaoYao1 (GY1), a PLA1-type phospholipase, in repressing the elongation of mesocotyl and coleoptile during the emergence of rice seedlings from soil. The authors identified the *GY1* gene through map-based cloning and it localizes in chloroplasts and catalyzes the initial step of JA biosynthesis. The *gy1* mutant exhibits an increased length of mesocotyl and coleoptile during the germination. The expression of *GY1* and other JA biosynthetic genes could be suppressed by ethylene, and thus ethylene promotes the growth of mesocotyl and coleoptile. The authors showed that GY1 genetically acts downstream of OsEIN2-mediated ethylene signaling pathway during the seedling growth. Moreover, the authors revealed one single natural variation of the *GY1* gene, GY1376T, that is associated with mesocotyl elongation in rice varieties. This work provides insights into how plants regulate mesocotyl/coleoptile elongation, and has the potential to be used for breeding.

Point 1. The authors mentioned that GY1 has close homologs in Arabidopsis and other plants. How about the evolutionary importance of GY1-assocated JA synthesis for seed germination in other species? Please cite some papers and highlight the similarity and difference of this finding with previous papers.

RESPONSE: Thanks for the comment. In Arabidopsis, the function of three GY1 homologs has been reported, i.e., DAD1, DGL, and AtDLAH (At1g30370). All of them exhibited PLA activity. DAD1 and DGL regulated floral development by JA synthesis, and AtDLAH is positively associated with seed viability. These have been incorporated into the discussion, in lines 471–485.

Point 2. The authors claimed that GY1 catalyzes the initial step of JA biosynthesis. However, beside the genetic data and JA measurement, no direct biochemical activities analysis. Further, it would be great to do the activity analysis of the native one and the mutated version of GY1376T among natural cultivars.

RESPONSE: Thanks for the comment. The genetic data and lipidomic analysis indicated PC may be the preferred substrate of GY1. Thus, we purified the recombinant proteins of GY1, gy1 ($GY1^{518A}$) and $GY1^{376T}$, and measured PLA activity for all of them by using dye-labeled PC. The result indicated that GY1 exhibits strong PLA activity, and the allelic mutation in gy1 and $GY1^{376T}$ reduced this activity. Moreover, the PLA activity of $GY1^{376T}$ is higher than that of gy1. This may be the reason why the natural allelic variation of $GY1^{376T}$ still exhibited normal flower development. These results have been incorporated into the Figure 8B, and the corresponding result (see lines 389–393) and method sections (see lines 687–707) were also revised accordingly.

Point 3. The role of JA in rice development has not been fully clarified. It will be informative if the authors compare GY1 with EG1, EG2 and JA in flower development and flower opening.

RESPONSE: Thanks for the comment. The roles of GY1/EG1 and EG2 have been compared in the discussion part pertaining to floral development; see lines 486–505.

Point 4. Some papers should be considered for citation:
-Liu et al., Functional diversity of jasmonates in rice, Rice, 2015 8:5 DOI: 10.1186/s12284-015-0042-9;
-Song et al., Jasmonate signaling and crosstalk with gibberellin and ethylene. Current Opinion in Plant Biology 2014, Pages 112–119;
-Yuan and Zhang. Roles of jasmonate signalling in plant inflorescence and flower development. Current Opinion in Plant Biology， 2015, 44–51

RESPONSE: Thanks for the comment. These studies have been cited in the introduction and discussion.

Reviewer #2:

Point 1. This reviewer may have got it completely wrong, but the authors state that the mutation was identified from among a population of T-DNA insertion mutagenesis mutants in Nipponbare (Page 29, Line 526–527).

This should have led to a large insertion in the gene and not to a SNP. Genes with such mutations are identified through one of various PCR techniques that help clone the flanking sequence at the insertion site. However, the authors present full sections in 'Results' and in 'Methods' on rough and fine mapping followed by sequencing the 3 candidate genes within the fine mapped region to identify the SNP in one of them as causative to the altered coleoptile and mesocotyl phenotype. This is more like cloning the causative gene of a QTL or one from a mutagenized population.

This major discrepancy in how the gene and mutation was identified puts a question mark on the entire manuscript.

Given such a discrepancy, unfortunately it is imprudent to assess the rest of the manuscript at this stage.

RESPONSE: Thanks for the comment. Unlike Arabidopsis, production of rice T-DNA-tagged populations underwent mass production of embryogenic calli and longer callus growth periods (Wei et al., 2016). Thus the tagging efficiency of T-DNA-tagged populations is only 5–10% in rice (Wei et al., 2013), and the relationship between flanking sequence and phenotype is very weak in rice (Wei et al., 2016). In our previous research, none of the mutant phenotype of *mhz* co-segregated with T-DNA insertion, and causal genes in these mutants exhibited a point or InDel mutation in the coding sequence (Ma et al., 2013; Ma et al., 2014; Yang et al., 2015; Yin et al., 2015). For these reasons, we used positional mapping to clone causal gene in the *gy1* mutant. These statements have been incorporated into the results section; see lines 152–159.

Point 2. Although the manuscript well elucidates the role of GY1 and its interaction with ethylene and JA for a role in coleoptile and mesocotyl elongation, it does not amount to a prelude to a 'very precious resource for breeding new rice varieties'. There is enough natural variation in rice varieties for coleoptile elongation under water and under soil. That said, anaerobic germination which depends a lot on coleoptile elongation to overcome submergence stress does not seem to be affected by GY1. Interestingly, the other two genes in the fine mapped region are also phospholipases. Generally, a tandem set of genes at a genomic location is the result of gene duplication. It would be interesting to know if the other two phospholipases are related closely enough to GY1 to also influence JA synthesis. If this is indeed the case, there may be additional alleles for similar effects and hence natural variants of these may again contribute to the resources already available for whatever breeding value the gene may have.

RESPONSE: Thanks for the comment. Actually, based on our analysis, among the three genes, LOC_Os01g6720 is a triacylglycerol lipase identified by Phytozome database, and its sequence and expression in shoot did not change between Nip and the *gy1* mutant (Figure 1F and Supplemental Figure 1D). *LOC_Os01g67450* should be a tandem replicate of *GY1* (*LOC_Os01g67430*), and no expression of *LOC_Os01g67450* was detected in the shoot (Supplemental Figure 1D). In addition, the mutant *gy1* phenotype (including mesocotyl/coleoptile in Figures 1G, 1H and seed phenotype, Supplemental Figures 3A, 3B) can be fully complemented by the genomic sequence of *GY1* (*LOC_Os01g67430*), indicating that *GY1* corresponds to *LOC_Os01g67430* but not the other two genes. As the reviewer suggested, some other loci related to coleoptile and mesocotyl elongation have been obtained through GWAS analysis. And *GY1* overlapped with one major QTL (Lee et al., 2012). Through variation analysis of rice cultivars, some rice varieties already adopted an elite allele of GY1 (376T) for long mesocotyl. This implied that GY1 should be valuable for breeding new rice varieties. Identification and functional analysis of the additional loci from GWAS analysis should further disclose the mechanism controlling mesocotyl elongation in rice seedlings. Comparison of the three gene expressions in Nip and *gy1* mutant have been added in Supplemental Figure 1D and briefly mentioned in result (see lines 164–165). Some of the discussions have also been incorporated into the discussion part; see lines 542–551.

Point 3. Fig 1G lower panel presents a conundrum. If gy1 and GY1 are different in one SNP, why are the band sizes different, this has not been explained.
The mention of CAPS marker has not been elaborated to name the restriction enzyme used and how and when the marker may have been utilized in differentiating GY1 from gy1 in these studies.

RESPONSE: Thanks for the comment. We used dCAPS (Derived Cleaved Amplified Polymorphic Sequences) marker to genotype the *GY1* and *gy1* (GY1 518A) plants. We generated an *Aor*51H I (AGCGCT, the underlined indicates mutation site 518A) site in PCR product of *gy1* (G518A) by reverse primer 5'-acgagacgagcacgtAGCGC-3' (519th nucleotide), whereas we will get an AGCGCC (the underlining indicates normal nucleotide G) site in PCR product of

***GY1* (G518G) by the same reverse primer (see Supplemental Table 7). Then the PCR product was cleaved by *Aor*51H I, and the resulting product of *gy1* is shorter than that of *GY1*. These descriptions have been incorporated into the method section; see lines 636–643.**

Point 4. Figure 5 A to C shows that ethylene inhibits JA formation and the expression of JA-synthesis related genes. However, under the conditions used, the production of JA and the expression of JA synthesis genes is known to be weak. More pertinent would be to assess JA and the JA synthesis gene expression under JA-inductive conditions i.e., de-etiolated seedling (1 hour of light is sufficient) or wounding of plants. If ethylene is suppressing JA, it should be very obvious and more pertinent through such an experiment.

RESPONSE: Thanks for the comment. As the reviewer suggested, we evaluated the expression of the JA synthesis gene and JA content under 10 ppm ethylene treatment in the shoot of the de-etiolated seedling (etiolated seedlings exposed to 1 hour of light). In de-etiolated seedlings, the JA content and expression of most JA biosynthesis genes appeared to be higher than that in etiolated seedlings, and a clear reduction in JA content and expression of JA biosynthesis genes was still noted after ethylene treatment (Supplemental Figures 8A and 8B), supporting that ethylene also inhibits JA biosynthesis in de-etiolated seedlings. These results have been incorporated into the result section (seeing lines 283-288) and Supplemental Figures 8A and 8B.

Reviewer #3:

The manuscript identified a novel rice mutant exhibiting longer mesocotyl/coleoptile named *gaoyao1* (*gy1* for short). Further, the authors revealed that *GY1* encodes a PLA1-type phospholipase functioning at the initial step of JA biosynthesis. With several lines of evidence, the authors proposed that soil coverage-induced ethylene production inhibits the biosynthesis of JA mainly through decline of *GY1* gene expression (also other genes that are responsible for JA production), in which ethylene-stablized transcription factor OsEIL1 directly binds to the *GY1* promoter and repress its transcription. In order to fix the problem that *gy1* (*Oryza sativa* as the original variety) exhibited floral abnormality at the maturity stage, the authors identified a single natural variation of GY1376T, which contributes to mesocotyl elongation in rice varieties and avoids yield penalty. With thorough experiments and clear points, this manuscript illustrates the molecular interplay between ethylene signaling and JA biosynthesis in rice, and more importantly provides a very precious resource for breeding to produce new rice cultivars.

Point 1. Given that the growth difference between the *gy1* mutant and Nip was much larger when seedlings were covered with soil than those in water, it seems that the *gy1* mutant was more sensitive to ethylene than was Nip. Also, there are several other lines of evidence implying that ethylene promotes mesocotyl/coleoptile growth in a GY1-independent manner. Please discuss the role of GY1-mediated JA biosynthesis pathway and the alternative pathway in mesocotyl/coleoptile growth.

RESPONSE: Thanks for the comment. It has been reported that ethylene induces cell wall acidification and increases extensibility of cell in *Rumex palustris* (Vreeburg et al., 2005). This can be largely attributed to the acidic pH optimum for expansin activity (McQueen-Mason et al., 1992). Furthermore, ethylene can regulate stability of intracellular microtubule through WDL5 to mediate etiolated hypocotyl cell elongation in Arabidopsis (Sun et al., 2015). In addition, many loci controlling mesocotyl elongation have been identified through GWAS analysis of rice cultivars, and identification and functional analysis of these loci may finally lead to a full understanding of mesocotyl elongation. These have been incorporated into the discussion part, seeing lines 533–551.

Point 2. Application of 1 μM MeJA did not inhibit the length of mesocotyl or coleoptile in Nip, please explain the possible reason.

RESPONSE: Thanks for the comment. This concentration was selected through test of a series of JA concentrations (Supplemental Figure 7), and is the maximal JA concentration tolerated by etiolated shoot of Nip. This 1 μM MeJA did not inhibit wild type Nip but rescued mutant *gy1* phenotype, probably due to that application of this concentration can elevate the internal physiological concentration in *gy1* to the normal JA level but did not heavily enhance the internal JA level in Nip. This may be the reason that 1 μM MeJA rescued the phenotype of the *gy1* mutant but did not inhibit the phenotype of Nip.

Point 3. Previous genetic evidence showed OsEIL2 is the major TF to mediate ethylene-induced mesocotyl and coleoptile elongation in rice. However, in this study, OsEIL2 seemed dispensable, and the authors suggested the existence of other TFs. What could they be?

RESPONSE: Thanks for the comment. We have revised the discussions about this. The discrepancy may be explained by the fact that although *OsEIL2* expression was reduced in the *OsEIL2*-RNAi plants, significant residual *OsEIL2* transcripts were still left (Yang et al., 2015b) and the resulting OsEIL2 activity may be enough to inhibit *GY1* expression but not reach the threshold to promote coleoptile elongation in response to ethylene. Alternatively, differential sensitivity to ethylene may be present for different responses. Other explanations/possibilities cannot be excluded. These have been incorporated into the discussion part, seeing lines 555–565.

TPC2016-00981-RAR1 2nd Editorial decision – *acceptance pending* **Apr. 17, 2017**

We are pleased to inform you that your paper entitled "Ethylene-Inhibited JA Biosynthesis Promotes Mesocotyl/Coleoptile Elongation of Rice Etiolated Seedlings" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff.

Final acceptance from Science Editor **May 2, 2017**