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LETTER TO THE EDITOR

Reply: Brassinosteroid Regulates Gibberellin Synthesis to Promote Cell Elongation in Rice: Critical Comments on Ross and Quittenden's Letter[OPEN]

Brassinosteroid (BR) and gibberellin (GA) are two important hormones regulating plant cell elongation. A defect in either of these hormone pathways leads to reduced plant growth and dwarfism. Because an early attempt in pea (*Pisum sativum*) failed to correlate BR level with active GA level (Jager et al., 2005), it was presumed that BR does not regulate GA synthesis in plants, despite the fact that BR promotes expression of GA biosynthetic genes in *Arabidopsis thaliana* (Bouquin et al., 2001; Sun et al., 2010; Stewart Lilley et al., 2013). In 2012, three studies in Arabidopsis reported the physical interaction between GA repressors DELLA proteins and BZR1 in mediating BR-GA crosstalk (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012). In Tong et al. (2014), we published results showing that BR modulates GA levels to regulate cell elongation in rice (*Oryza sativa*). In our article, we suggest that rice may have a different mechanism from Arabidopsis, as we found that rice BR-deficient mutants (*d11-2*) showed a normal response to GA, in contrast to Arabidopsis BR-deficient or -insensitive mutants (*det2-1*, *bri1-5*, and *bri1-119*) that were reported to be insensitive to GA application at early seedling stages (Bai et al., 2012). Unterholzner et al. (2015) subsequently reported similar results in Arabidopsis, confirming our finding in rice that BR promotes GA biosynthesis to regulate plant growth. They also showed that BR-deficient mutants (*cpd*, *bri1-1*, and *bri1-301*) have normal responses to GA at different growth stages. Integrating all of these results, Unterholzner et al. (2015) proposed an updated model for BR-GA crosstalk: BR promotes BZR1/BES1 to induce GA biosynthesis and the increased GA level promotes DELLA degradation to further release BZR1/BES1 activity.

Ross and Quittenden (2016) attempt to split this integrated model into two separate ones: a "synthesis" model and "signaling" model. They dispute our evidence showing that BR regulates GA biosynthesis and argue that the signaling model is more dominant than the synthesis model. However, neither Tong et al. (2014) nor Unterholzner et al. (2015) make a claim that a synthesis model is more important than the signaling model of GA-BR interactions. Ross and Quittenden overlook the fact that prior to our study (Tong et al., 2014), neither model had been proposed in rice. Some of our findings are indeed inconsistent with a signaling-only model (see below), and we suggested that rice might have a different mechanism from that of Arabidopsis (Tong et al., 2014). Although we didn't add the DELLA-BZR1 interaction in our model, we reported that the DELLA protein SLR1 interacts with BZR1 in yeast, and we are open to the possibility that this interaction plays a role in BR-GA crosstalk. The synthesis model does not exclude the signaling model and vice versa, but both may be operating together with complex and differing interactions in different tissues, stages of development, environments, and species, which reflects the complexity of hormonal regulation in plants. Indeed, Unterholzner et al. (2015) integrated the two models into one working model (see Figure 8 in Unterholzner et al., 2015).

Ross and Quittenden made three arguments for the notion that BR regulation of GA biosynthesis is not of significant importance in BR-GA interactions. The first argument is centered on the GA responsiveness of BR mutants and the idea that BR loss-of-function mutants, especially strong mutants, should not show a growth response to GA in the signaling model (as there is no active BZR1/BES1 to respond to GA-mediated degradation of DELLA repressors in the strong BR mutants). This argument is supported by results of Bai et al. (2012), who found that three moderate BR mutants (*det2*, *bri1-5*, and *bri1-119*) show basically no response to exogenous GA. However, both Unterholzner et al. and Tong et al. showed that moderate to strong BR mutants can have largely normal GA growth responses. Ross and Quittenden claim that the severe BR-deficient mutants are insensitive to GA in both Arabidopsis and rice; however, we think that this is a misinterpretation of our data and others showing that GA can rescue many of the BR response phenotypes of strong BR mutants (Tong et al., 2014; Unterholzner et al., 2015, 2016).

Ross and Quittenden argued that *brd1*, the severe rice BR-deficient mutant, has "no response to GA" because GA application does not restore the phenotype to wild type. However, GA application is not necessarily expected to restore the elongation to wild-type levels; it is only expected that the ratio of the response will be the same or similar. We note that even BR application cannot rescue the *brd1* height to wild type as shown in two original articles (Hong et al., 2002; Mori et al., 2002). Importantly, our data (Tong et al., 2014, Supplemental Figure 7) showed that the *brd1* mutant has the same elongation ratio in response to GA as that of the wild type, suggesting that *brd1* has normal GA response.

In our model (Tong et al., 2014, Figure 10), we proposed that the GA pathway is only one of the downstream branched pathways through which BR regulates cell elongation. BR can also regulate shoot apical meristem development, cell division, and microtubule formation and orientation to affect plant height (Yamamuro et al., 2000; Hong et al., 2002; Mori et al., 2002). For example, *brd1*, the most severe BR-deficient mutant, failed to form microtubules, whereas *d18*, the most severe GA-deficient mutant, retains normal microtubule formation (Mori et al., 2002). GA application apparently cannot complement this kind of defect in severe BR mutants. Moreover, in our article and the one by Mori et al. (2002), the leaf blade of *brd1* elongated normally in response to GA. We believe that

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when testing for hormone sensitivity, we must compare the elongation ratio, rather than absolute lengths, to exclude the possible effect of endogenous background and other indirect effects of the mutation, such as microtubule disorganization.

A second argument of Ross and Quittenden against the GA synthesis model relates to the significance of changes of GA levels in BR mutants. First, they consider that in the rice mutant *m107*, which overaccumulates BR, an increase in GA in the mutant should promote plant growth. As reported by Tong et al. (2014), *m107* shows greatly increased seedling growth (statistically significant data were shown in Tong et al., 2014, Supplemental Figure 3B). In addition, we showed that active GA levels were consistently decreased in both BR biosynthesis- and BR signaling-deficient mutants (*d11*, *GSK2* overexpression plant, and *dlt*) (Tong et al., 2014, Figure 4A). We also showed that in three *DLT* overexpression lines, GA levels correlated well with plant seedling height (Tong et al., 2014, Supplemental Table 2 and Supplemental Figure 3A). Moreover, we showed that when seedling elongation was inhibited by exogenous high BR application, GA levels decreased accordingly (Tong et al., 2014, Supplemental Table 5). The correlated changes between bioactive GA content and plant height in both BR-deficient and BR-enhanced mutants as well as BR-treated plants strongly support the conclusion that changes in GA levels in BR mutants or BR-treated plants contribute to plant growth. In Arabidopsis, it was shown that active GAs were reduced in several BR loss-of-function mutants (Unterholzner et al., 2015). Ross and Quittenden argued that the observed reduction (in some cases only 2-fold) could not adequately explain the strong dwarf phenotype of the BR mutant. However, as discussed above, GA regulation is only one branch of BR-regulated growth.

The third argument of Ross and Quittenden is based on the assumption that DELLA-deficient mutants should have an enhanced BR response if the signaling model is correct and should have reduced responses to BR under the synthesis model. We believe that this is an oversimplification of the situation and falsely sets up one model against the other. What is expected if BR-GA crosstalk happens at both biosynthesis and signaling levels? What about the other branches downstream of BR-regulated growth? Although Bai et al. (2012) showed that there is dramatic increase of BR response in a DELLA-deficient mutant in Arabidopsis, our data showed that there is no such increase in BR sensitivity in a DELLA-deficient rice mutant. In the rice DELLA-deficient mutant *slr1*, BR sensitivity is increased (from 1.7- to 1.9-fold) at lower BR concentration and decreased (from 3.0- to 2.7-fold) at higher BR concentration (Tong et al., 2014, Figure 3C). These data argue for the existence of the integrated model of BR-GA crosstalk in that a decreased BR response from the biosynthesis model is likely offset by an increased BR response from the signaling model.

It is important to emphasize that the difference between BR and GA functions depends on environmental conditions and tissue specificities in rice, and likely in other species as well. For example, only BR, but not GA, is essential for rice skotomorphogenesis in the dark (Yamamuro et al., 2000). In addition, BR controls lamina bending and grain size, whereas GA has very subtle effects on these processes: BR is not believed to influence these processes by regulating either GA synthesis or GA signaling (Tong et al., 2014; Che et al., 2015; Sun et al., 2015). Ross and Quittenden listed several results from other species to suggest that BR and GA levels show no consistent changes across different species. However, early studies clearly showed that different species have different BR responses (Bishop, 2003). For example, BR-deficient mutants of both Arabidopsis and rice show a deetiolated phenotype in the dark (defective skotomorphogenesis), whereas BR-deficient mutants of both pea and tomato (*Solanum lycopersicum*) appear to have a relatively normal etiolated phenotype in the dark (Bishop, 2003). We note that, coincidentally, all the examples cited by Ross and Quittenden to show the inconsistency between BR and GA levels come from pea and tomato. Ross and Quittenden fail to consider many other factors, including tissue specificity, hormone concentration, developmental stage, environmental condition, cell type, and species, when discussing these complex results. Differential contexts are well known to be critical factors for understanding BR functions, as highlighted in a recent review (Singh and Savaldi-Goldstein, 2015).

Our understanding of BR functions is moving forward, accompanied by debates and many unanswered questions. The present integrated model regarding BR-GA crosstalk is applicable to certain processes (cell elongation) in certain tissues under certain conditions in certain species. Although the biological significance of the SLR1-BZR1 interaction needs to be further verified in rice, GA synthesis and signaling must be coordinated or form a positive loop for full BR function, at least in Arabidopsis (Unterholzner et al., 2015).

Hongning Tong
State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing)
Institute of Genetics and Developmental Biology
Chinese Academy of Sciences
Beijing 100101, China

Chengcai Chu
State Key Laboratory of Plant Genomics and National Center for Plant Gene Research (Beijing)
Institute of Genetics and Developmental Biology
Chinese Academy of Sciences
Beijing 100101, China
ccchu@genetics.ac.cn
ORCID ID: 0000-0001-8097-6115

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AUTHOR CONTRIBUTIONS

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