

## Addendum

## Tomato BRI1 and systemin wound signalling

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Brassinosteroids (BRs) are perceived by Brassinosteroid Insensitive 1 (BRI1), that encodes a leucine-rich repeat receptor kinase. Tomato BRI1 has previously been implicated in both systemin and BR signalling. The role of tomato BRI1 in BR signalling was confirmed, however it was found not to be essential for systemin/wound signalling. Tomato roots were shown to respond to systemin but this response varied according to the species and growth conditions. Overall the data indicates that mutants defective in tomato BRI1 are not defective in systemin-induced wound signalling and that systemin perception can occur via a non-BRI1 mechanism.

Brassinosteroids (BRs) are steroid hormones that are essential for normal plant growth. The most important BR receptor in Arabidopsis is BRASSINOSTEROID INSENSITIVE 1 (BRI1), a serine/threonine kinase with a predicted extracellular domain of ~24 leucine-rich repeats (LRRs).<sup>1,2</sup> BRs bind to BRI1 via a steroid-binding domain that includes LRR 21 and a so-called "island" domain.<sup>2,3</sup> In tomato a BRI1 orthologue has been identified that when mutated, as in the *curl3* (*cu3*) mutation, results in BR-insensitive dwarf plants.<sup>4</sup> Tomato BRI1 has also been purified as a systemin-binding protein.<sup>5</sup> Systemin is an eighteen amino acid peptide, which is produced by post-translational cleavage of prosystemin. Systemin has been implicated in wound signalling and is able to induce the production of jasmonate, protease inhibitors (PIN) and rapid alkalization of cell suspensions (reviewed in ref. 6).

To clarify whether tomato BRI1 was indeed a dual receptor it was important to first confirm its role in BR signalling. Initially this was carried out by genetic complementation of the *cu3* mutant phenotype.<sup>7</sup> Overexpression of tomato BRI1 restored the dwarf phenotype and BR sensitivity and normalized BR levels (Table 1). The plants also exhibited additional phenotypic characteristics such as reduced serration of leaf margins. When tomato BRI1 was expressed using its endogenous promoter the complemented lines did not exhibit such dramatic differences in their phenotype and were morphologically

Table 1 **BR content**

	<b>35S:TomatoBRI1 complemented line</b>	<b>Wt*</b>	<b>cu3*</b>
6-deoxocathasterone	566	964	676
6-deoxoteasterone	nd	47	48
3-dehydro-6-deoxoteasterone	87	62	69
6-deoxytyphasterol	nd	588	422
6-deoxocastasterone	1,755	6,247	26,210
castasterone	255	637	17,428
brassinolide	nd	nd	nd

BR content ng/kg fw. \*Montoya et al.<sup>4</sup> nd, not detected.

similar to wild-type plants. Interestingly, when trying to confirm orthologous function of tomato and arabidopsis BRI1 genes, arabidopsis BRI1 was able to complement both the tomato *cu3* and Arabidopsis *bri1-5* mutant phenotypes. However, transformation of tomato BRI1 into the arabidopsis *bri1-5* mutant did not fully restore the dwarf phenotype. Chimeric BRI1 genes indicated that the lack of effective complementation is due to the LRR region of tomato BRI1 and further work is required to discern the reason for this. The lack of BR binding to microsomes from *cu3* mutants provided further proof that the *cu3* mutants are defective in BRI1 and BR signalling.

To show the role of tomato BRI1 in systemin signalling tomato BR mutants and the complemented line were tested for their systemin response. Tomato *cu3* mutants were shown not to be defective in systemin-induced proteinase inhibitor (PIN) gene induction, nor were they defective in PIN gene induction in response to wounding. Cell suspensions made from *cu3* mutant tissue exhibited an alkalization of culture medium similar to wild-type cell suspension. These data taken together indicated that BRI1 was not essential for systemin signalling. However, Scheer et al.<sup>8</sup> demonstrated that the overexpression of tomato BRI1 in tobacco suspension cultures results in an alkalization in response to systemin, which was not observed in untransformed cultures. This suggests that BRI1 is capable of eliciting systemin responsiveness and that in tomato BRI1 mutants another mechanism is functioning to enable systemin signalling.

Root elongation is a sensitive bioassay for BR action with BRs inhibiting root growth. *Solanum pimpinellifolium* roots elongate in response to systemin, in a BRI1-dependent fashion. In *Solanum lycopersicum* root length was reduced in response to systemin and BR and jasmonate synthesis mutants indicated that the inhibition did not

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require jasmonates or BRs. Normal ethylene signalling was required for the root response to systemin. When a tobacco, *Nicotiana benthamiana*, BRI1 orthologue was transformed into *cu3* both the dwarfism and systemin-induced root elongation was restored to that of wild type. Tobacco plants however do not respond to systemin. This is puzzling as the introduction of tomato BRI1 into tobacco enabled systemin responsiveness.<sup>8</sup> Further investigation as to how tomato BRI1 elicits this response is therefore required.

Systemin has been demonstrated to bind to two tomato proteins BRI1/SR160<sup>5</sup> and SBP50.<sup>9</sup> The data presented by Holton et al.<sup>7</sup> indicates that tomato BRI1 is not essential for systemin-induced wound responses and that a non-BRI1 pathway is present that is able to facilitate a systemin response. Whether this is via a related LRR receptor kinase or by another protein remains to be elucidated.

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