

## Article Addendum

# Is boron involved solely in structural roles in vascular plants?

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It is very well proved that boron (B) plays a primary structural role in the plant cell wall. In addition, this micronutrient has been involved in a great variety of physiological processes in vascular plants. It has been reported that B deficiency induces stress-responsive genes and, in tobacco plants, it seems to decrease net nitrate uptake by repressing expression of root plasmalemma H<sup>+</sup>-ATPase gene. Moreover, root asparagine concentration is clearly increased under B deficiency, as also observed for other abiotic stresses. Accumulation of asparagine in response to abiotic stresses could be an ammonium detoxification mechanism when high amounts of ammonium are internally generated by deamination of soluble amino acids released from enhanced proteolysis under stress conditions. Nevertheless, the mechanisms underlying the several effects caused by B deficiency are unknown. Although a mechanism has been reported to explain B effects based on signals via the cell wall-plasma membrane-cytoskeleton continuum, we propose and discuss the possible role of B as a cellular signal through transcription factors. This hypothetical mechanism could explain not only its diverse effects on so many physiological processes, but also that a negligible amount of boron into the protoplast can be decisive for the normal development of such events.

### Structural Role of Boron in the Cell Wall and its Involvement in Other Physiological Functions

Boron (B), an essential nutrient for vascular plants, is located mostly (about 80–90% of dry weight) in the plant cell wall forming borate ester cross-linked rhamnogalacturonan II (RG-II) dimer, this complex being essential to the structure and function of the cell wall.<sup>1,2</sup> To date, the primordial function of this micronutrient is undoubtedly its structural role in the cell wall.<sup>3,4</sup> Nonetheless, it has been reported that B seems to exert different effects on very diverse

processes in vascular plants, such as root elongation, indole-3-acetic acid oxidase, sugar translocation, carbohydrate metabolism, nucleic acid synthesis, and pollen tube growth.<sup>3,5,6</sup> Boron also appears to affect membrane potential, plasmalemma-bound enzymes and ion fluxes across membranes,<sup>7-9</sup> cytoskeletal proteins,<sup>10,11</sup> accumulation of phenolics and polyamines,<sup>8,12,13</sup> and nitrogen metabolism.<sup>14,15</sup> Although the symptoms due to B deficiency are rapid and clear,<sup>5,6,9</sup> however, results from long-term B deficiency hinder the progress in the knowledge on the primary physiological roles of B in vascular plants. Often, the observed effects are consequence of secondary effects of B deficiency and this fact, along with the very small amount required of this element, become B probably the least understood of all essential nutrients in vascular plants.<sup>16,17</sup> The mechanisms underlying the various metabolic disorders caused by cell wall defects under B deficiency are indeed unknown.<sup>18</sup>

Very recently our group has shown that a short-term boron-deficient treatment led to a decline in root and, especially, leaf nitrate concentrations in tobacco plants<sup>15</sup> as a result of a lower net nitrate-uptake rate. B deficiency seems to decrease net nitrate uptake by repressing expression of root plasma membrane H<sup>+</sup>-ATPase gene<sup>15</sup> and, consequently, by decreasing the proton electrochemical gradient across the plasma membrane necessary to cotransport protons and nitrate inwards. Root asparagine concentration increased under B deprivation,<sup>15</sup> which was also observed for other mineral deficiencies.<sup>19</sup> Moreover, asparagine synthetase (AS) genes are also overexpressed under osmotic and salt stresses,<sup>20</sup> and the levels of AS transcripts and free asparagine increased in plants stressed by excess of cadmium.<sup>21</sup> All these data point out that induction of AS could be a common response of plants to cope with various stresses in order to reassimilate, in cooperation with glutamine synthetase-glutamate synthase cycle,<sup>15,22</sup> the ammonium released by the enhanced protein and amino acid catabolism in stressed plants, thus preventing ammonium accumulation and toxicity in the plant tissues.

Therefore, it is really surprising that the deficiency of B—an element whose primary role is in the cell wall structure—is able to trigger diverse effects on so ample number of physiological processes in plants, particularly taking into account that B is very largely located at the cell wall that is, outside the protoplast.<sup>23,24</sup>

### Does Boron Act as a Cellular Signal Too?

It has been suggested that changes in B concentrations may lead to a mechanical cascade of signals starting by an altered conformation of

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membrane-bound proteins, and extending into the cytoplasm via the cell wall-plasma membrane-cytoskeleton continuum<sup>4</sup> (Fig. 1). This proposal is supported by the fact that B deprivation enhanced rapidly levels of cytoskeletal proteins (actin and tubulin) in *Arabidopsis*<sup>10</sup> and maize<sup>11</sup> roots, which led to an altered polymerization pattern of their cytoskeletal assemblies. Accordingly, the primary action of boron deprivation would be on an apoplastic target, which signals deeper into the cell via endocytosis-mediated pectin signaling along putative cell wall-plasma membrane-cytoskeleton continuum.<sup>25</sup>

Interestingly, it has been also reported that B deficiency induces the expression of stress-responsive genes<sup>18</sup> and *NIP5;1* gene<sup>26,27</sup> that codes a protein essential for B uptake when this micronutrient is limiting.<sup>26</sup> It has been proposed that a quick signal transfer from the cell wall to the cytoplasm could be involved for gene induction after B removal.<sup>18</sup>

Another tentative working hypothesis to explain the possible function of B as a cellular signal is illustrated in Figure 1 together with the above-mentioned hypothesis. In addition to the structural role of B stabilizing molecules with cis-diol groups in the cell wall (e.g., cross-linked RG-II dimer)<sup>1,2</sup> or other cis-diol containing molecules located at any cellular place, independently of their function,<sup>17</sup> B could also affect the interaction between transcription factors (TF) and target genes. The TF interaction with the target gene sequence might be modulated by intracellular B levels. This interaction could be by either the direct linkage of B to the TF or through molecules capable of binding B and thereby linking with the TF. So, depending on the target gene and the type of TF (activator or repressor) the linkage or not with B could regulate gene expression (Fig. 1). This hypothesis might be more complicated taking into account the possibility of the networks of TF that can act in plant stress responses.<sup>28</sup> The proteins encoded by these B-modulated genes, or eventually their absence, would act in diverse physiological processes at any cellular level. For instance, B might be essential in the cell wall not only for borate-ester bridges between RG-II monomers, but also as a signal to allow the expression of enzymes needed in the synthesis and assembly of cell wall, such as arabinogalactan proteins and expansins.<sup>4,29</sup> In addition, this tentative hypothesis would be consistent with the results reported by other authors on gene expression under different B supply.<sup>18,27</sup>

How could intracellular B carry out this signaling function from a mechanistic point of view? TF, in the same way that other proteins such as glycoproteins, can contain not only hydroxylated ligands, such as sugar moieties, but also other as serine or threonine residues that might form ester-like complexes with borate.<sup>4</sup> Obviously, the cytosolic concentration of B needed to exert its signaling function would be very low, which is compatible with the negligible levels found into the protoplast for this micronutrient.<sup>23,24</sup>

Curiously, calcium (Ca) is another element very important in the cell wall structure since it is readily bound by the free carboxyl groups and links pectin chains together into expanded, highly hydrated gel networks.<sup>29</sup> Moreover the role of Ca as a cellular signal in many pathways and even as a second messenger in plant responses to pathogen attacks has been highlighted.<sup>30</sup> Therefore, B could share with Ca three physiological features: (a) a structural role in the cell wall, (b) its very scarce mobility and solubility (very low cytosolic concentration), and (c) a signaling function. Hence, despite Ca and B are a macronutrient and a micronutrient, respectively, both of them could play similar roles as structural and signaling elements in plant cells.

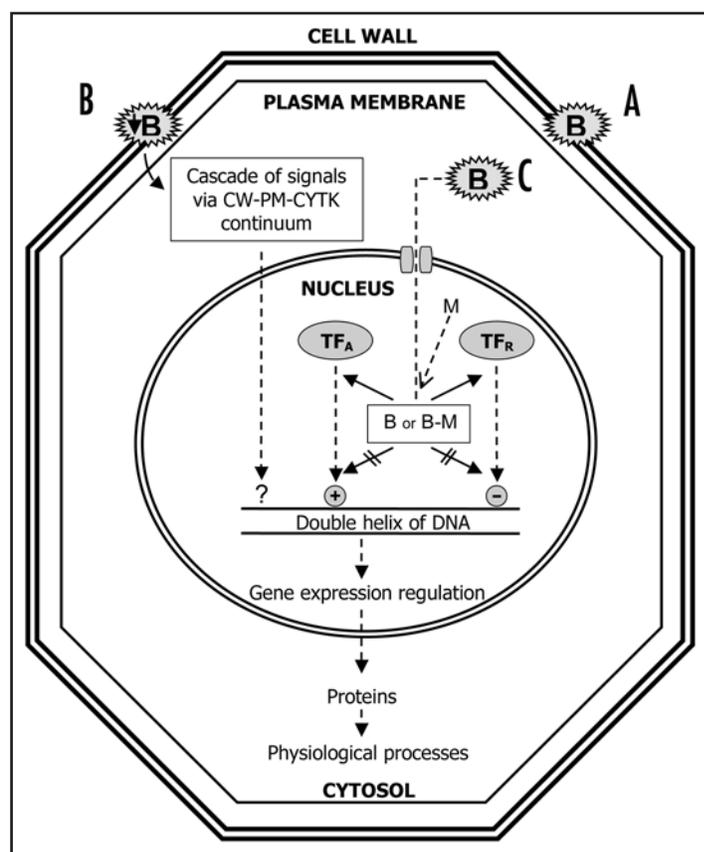


Figure 1. Structural role of B in the cell wall and hypothetical mechanisms of B signaling. (A) B stabilizes molecules with cis-diol groups, either in the cell wall (e.g., a borate-ester bridge between two RG-II monomers forms a cross-linked dimer that provides stability to the cell-wall matrix) or in any other cellular place (plasma membrane, cytosol, or organelles). (B) The B deficiency in cell walls is detected by the cytoplasm and a cascade of signals is transferred via the cell wall-plasma membrane-cytoskeleton continuum. (C) Once B is in the nucleus, this micronutrient could regulate the rate of gene transcription by affecting the interaction between TF and target genes. CW-PM-CYTK, cell wall-plasma membrane-cytoskeleton. M, molecule capable of binding boron. TF<sub>A</sub>, activator transcription factor. TF<sub>R</sub>, repressor transcription factor. For more details see text.

In summary, we propose the hypothesis that B may exert its primary function not only through stabilization of molecules containing cis-diol groups<sup>1,2,16,17</sup> but also as a cellular signal capable of interacting with TF, which could explain why many physiological processes are affected when vascular plants are subjected to B deficiency.

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