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News and Views

A benzoxazinoid twist to boron homeostasis story in maize

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Boron is an essential micronutrient for plants, but too much boron is toxic to plants. Both boron deficiency and toxicity in soil significantly affect agricultural productivity worldwide. Boron plays crucial roles in developmental processes that influence yield traits in crops, partly because it is essential for maintaining cell wall integrity (Matthes et al. 2020). Although the mechanisms of boron uptake, transport, and utilization are well-characterized (Yoshinari and Takano 2017), the regulation of boron homeostasis in plants remains poorly understood. Unraveling how plants manage cellular boron levels and adapt to both deficiency and toxicity is essential for improving crop productivity and resilience under suboptimal boron conditions.

Hormones regulate boron uptake, transport, and overall homeostasis in plants (Eggert and von Wiren 2017; Aibara et al. 2018). Variations in phenotypic responses across species and genetic backgrounds to different boron conditions have been observed, suggesting that additional factors are involved in regulating boron homeostasis. In this issue of *Plant Physiology*, Chu et al. (2024) used a combination of quantitative genetics and analytic approaches to uncover genetic factors influencing boron content in maize.

The authors analyzed boron concentrations in leaves of over 270 maize inbred lines from the Goodman-Buckler association panel, a collection of maize inbreeding lines worldwide (Flint-Garcia et al. 2005). Under low-boron soil conditions, leaf boron levels significantly varied among the inbred lines, suggesting that genetics plays an important role in this trait. Thus, they conducted a genome-wide association study and discovered that the trait was significantly linked to 2 single nucleotide polymorphism markers: one on chromosome 4 and one on chromosome 7. Six and 8 candidate genes were identified in the 2 locations, respectively. Notably, 2 candidate genes on chromosome 4, *BENZOXAZINLESS3* (*BX3*) and *BX4*, are involved in the biosynthesis of benzoxazinoids—defense metabolites found in grasses and some eudicots (Morant et al. 2008) (Fig. 1A). Further investigation into the maize boron transporter mutant *Zmtassel-less1* (*Zmtls1*), which displays boron deficiency symptoms, revealed a significant upregulation of the 2 *BX* genes (Fig. 1B). The authors analyzed publicly available transcriptome data from leaf tissues of the Goodman-Buckler lines and identified a significant correlation for 5 out of the 14 candidate genes linked to boron content. Out of these candidates, they observed significant correlations between *BX3* gene expression and leaf boron concentration, and *bx3* is most upregulated in Zmtls1 in that dataset, further supporting BX3 important role in boron-related functions in maize. BX3 catalyzes the conversion of Indolin-2-one to 2-Hydroxy-indoline-2-one (Fig. 1A).

To test the association between *BX3* and boron, the authors analyzed *bx3* loss-of-function maize mutants under varying soil boron conditions. The mutants showed elevated boron concentrations in mature leaves, without the typical leaf tip necrosis as seen in boron toxicity. Intriguingly, seedling leaves also had elevated boron levels but exhibited leaf tip necrosis that worsened as soil boron increased (Fig. 1B). This phenotype persisted even in boron-free media, suggesting that factors other than boron may contribute to the necrosis. Decoupling these *bx3* phenotypic responses between the mature and seedling stages would be an important question for future studies.

To further investigate the link between the early steps of benzoxazinoid biosynthesis and boron content, the authors used *Arabidopsis thaliana* (Arabidopsis) plants that overexpress *BX1* and *BX2*, leading to the accumulation of indolin-2-one, the substrate of BX3 (Fig. 1A). The elegance of this experiment is that Arabidopsis lacks benzoxazinoid biosynthesis pathways and thus provides a clean background. Consistent with their hypothesis, plants overexpressing *BX1* and *BX2* also accumulated more boron than wild-type *Col-0* plants. However, overexpressing *BX3* in Arabidopsis did not increase boron levels. These findings suggest that indolin-2-one accumulation plays a key role in elevating boron content, even though it does not act as a chelator for boron. A plausible explanation is that derivatives of indolin-2-one may have chelating properties, but the actual mechanism remains unclear.

In conclusion, Chu et al. (2024) identified *BX3* as a key gene in boron homeostasis and described a novel connection between

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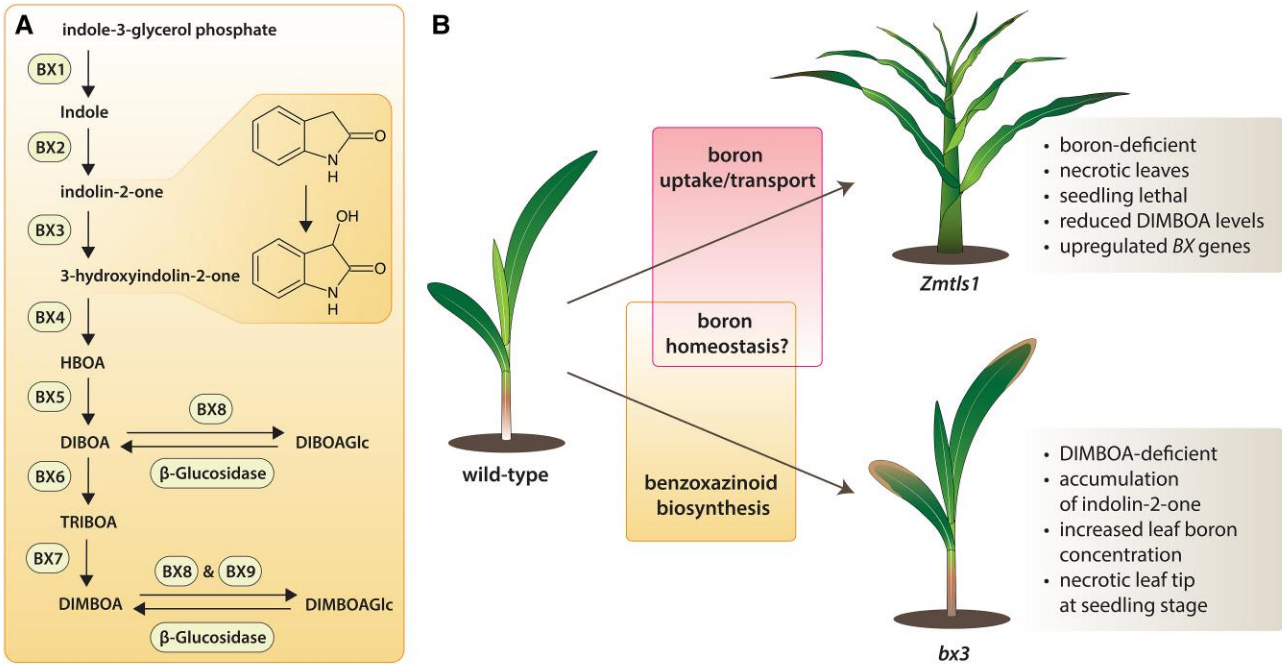

Figure 1. Benzoxazinoid biosynthesis and boron homeostasis in maize. **A)** Benzoxazinoid biosynthesis pathway in maize (adapted from Morant et al. 2008). **B)** Schematic depiction of early phenotypes in boron-deficient *Zmtls1* and DIMBOA-deficient *bx3* mutants in maize. Under low-boron conditions, the *Zmtls1* mutant exhibits boron deficiency symptoms and developmental defects, leading to seedling lethality. In contrast, the *bx3* mutant exhibits boron toxicity symptoms, including necrotic leaf tips during the seedling stage. These phenotypes support the hypothesis that *BX3* may act as a non-boron transporter-related gene associated with boron homeostasis in maize. Abbreviations: HBOA, 2-hydroxy-3,4-dihydro-1,4-benzoxazin-3-one; DIBOA, 2,4-dihydroxy-1,4-benzoxazin-3-one; DIBOAGlc, DIBOA-glucoside; TRIBOA, 2,4,7-trihydroxy-1,4-benzoxazin-3-one; DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; DIMBOAGlc, DIMBOA-glucoside.

boron homeostasis and benzoxazinoid metabolism in maize. Although the *bx3* mutants exhibited subtle toxicity symptoms at the seedling stage, they showed enhanced reproductive traits, suggesting that elevated boron could help maize adapt to low-boron soil conditions without causing toxicity. As boron is one of plants' least understood micronutrients, these findings expand our understanding of boron homeostasis and its roles in plant development. This study also highlights the potential of manipulating benzoxazinoid pathways to engineer crops better suited to soils with nonoptimal boron levels.

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Conflict of interest statement. None declared.

Data availability

No new data were generated or analysed in support of this.

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