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News and Views

Double the action: multimodal action of a CONSTANS-LIKE protein enhances stress tolerance in soybean

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Climate change has intensified the damage caused to agricultural production by abiotic stress conditions such as drought, salinity, freezing, and flooding. More comprehensive understanding of plant stress tolerance mechanisms is crucial for developing future-proof crop varieties.

CONSTANS-LIKE (COL) proteins, belonging to the B-box (BBX) family of transcription factors, are well known for their role in light-regulated developmental responses such as photomorphogenesis and photoperiodic flowering (Yadav et al., 2020). However, the role of these proteins in abiotic and biotic stress responses is poorly understood. In this issue of *Plant Physiology*, Xu et al. (2022) report that GmCOL1a, a COL/BBX protein that promotes flowering in soybean (*Glycine max*), also enhances tolerance against drought and salinity stress.

Xu et al. (2022) found increased transcript and protein levels of GmCOL1a in soybean plants stressed with drought or salinity or treated with abscisic acid (ABA). To study the role of GmCOL1a in stress responses, the authors generated overexpression (*GmCOL1a-ox*) and loss-of-function mutant (*co-9*) soybean lines. *GmCOL1a-ox* lines showed remarkable resistance to salt-mediated inhibition of seed germination, whereas the germination rate of the loss-of-function mutant *co-9* was lower as compared with the wild-type. Hypocotyl elongation and root growth also increased in *GmCOL1a-ox* seedlings as compared with wild-type plants under salinity stress. Moreover, the overexpressor lines retained higher water content as compared with the wild-type under salinity-induced osmotic stress, whereas the relative water content further decreased in the *co-9* mutant. In the absence of stress conditions, both the overexpression and mutant lines grew similar to the wild-type.

To understand the mechanism by which GmCOL1a promotes salinity tolerance, the authors measured the ratio of Na^+ and K^+ in the overexpressor and mutant lines. The Na^+/K^+ ratio in the shoot (but not roots) was lower in the overexpressors and higher in the loss-of-function mutant as compared with the wild-type, suggesting that GmCOL1a plays a role in maintaining N^+/K^+ ratios in the shoots. Concurrently, *SALT OVERLY SENSITIVE 1* (*GmSOS1*) and *SALT TOLERANCE-ASSOCIATED GENE ON CHROMOSOME 3* (*GmSALT3*), genes that encode two key salt tolerance-associated Na^+ transporters, showed enhanced expression in the overexpressor lines under salinity stress. The authors speculate that the enhanced salt tolerance of the *GmCOL1a-ox* plants is a consequence of elevated expression of these salt transporters, likely through reducing transport of Na^+ from roots to shoots.

When grown under drought conditions, the *GmCOL1a-ox* lines showed extended greening, greater recovery upon rewatering, and overall higher relative water content as compared with the wild-type, whereas all these attributes were oppositely affected in the *co-9* mutant. ABA plays a key role in managing plant water content during drought by controlling stomatal movements (Hsu et al., 2021). Although there was no difference in stomatal aperture between the different lines in control conditions, stomatal apertures were smaller in the *GmCOL1a-ox* lines when ABA was applied, whereas *co-9* showed larger stomatal apertures as compared with the wild-type, indicating that GmCOL1a prevents water loss during drought stress by enhancing ABA-induced stomatal closure.

Drought conditions induce the production of reactive oxygen species (ROS). Staining with nitro blue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB) revealed that the H_2O_2 level in *GmCOL1a-ox* was lower as compared with the wild-type. Biochemical analyses indicated that *GmCOL1a-ox* accumulated greater levels of protective compounds like proline

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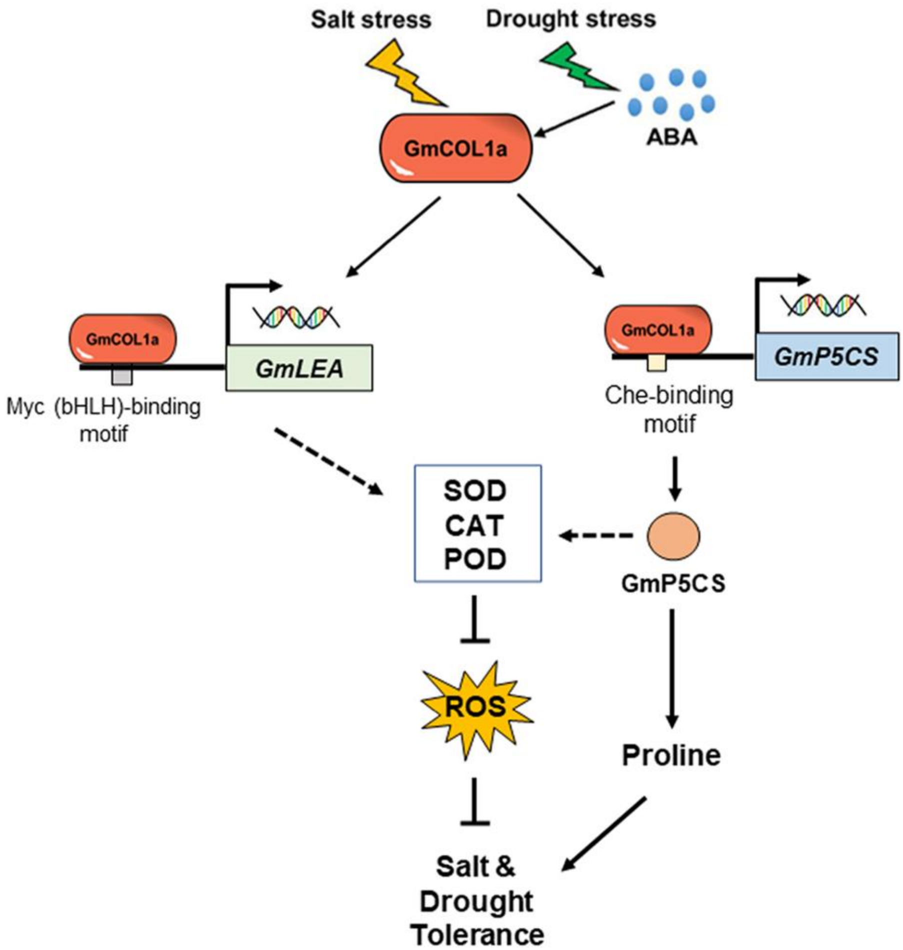

Figure 1 GmCOL1, activated by drought and salinity stress, binds to the promoters of *GmLEA* and *GmP5CS* and activates their transcription. Increased levels of GmLEA and GmP5CS enhance the neutralization of hazardous reactive oxygen species (ROS) and increase the production of protective compounds like proline, conferring drought and salt tolerance in soybean. COL1—CONSTANS-LIKE 1; ABA—Abscisic acid; *LEA—LATE EMBRYOGENESIS ABUNDANT*; *P5CS—PYRROLINE-5-CARBOXYLATE SYNTHETASE*; SOD—SUPEROXIDE DISMUTASE; CAT—CATALASE; POD—PEROXIDASE; ROS—Reactive oxygen species. Solid arrows indicate positive regulation through known mechanisms, broken arrows indicate regulation through unknown mechanisms, flat-headed lines indicate negative regulation. Figure adapted from Xu et al. (2022).

and showed enhanced activities of ROS-neutralizing enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

To identify the molecular targets of GmCOL1a, the authors performed ChIP-Seq analysis in one of the *GmCOL1a-ox* lines and discovered the putative binding of GmCOL1a on the promoter regions of the genes encoding the protective antioxidant protein LATE EMBRYOGENESIS ABUNDANT (GmLEA) and the proline biosynthesis enzyme PYRROLINE-5-CARBOXYLATE SYNTHETASE (GmP5CS). Direct binding was subsequently confirmed using chromatin immunoprecipitation-quantitative PCR (ChIP-qPCR) and electrophoretic mobility shift assay (EMSA). Reverse transcription quantitative PCR (RT-qPCR) and luciferase assays further suggested that binding of GmCOL1a leads to transcriptional activation of *GmLEA* and *GmP5CS*.

To validate the functional importance of the higher expression of *GmP5CS* in drought and salt tolerance, the authors used *Agrobacterium rhizogenes*-mediated hairy root transformation to generate composite transgenic plants overexpressing *GmP5CS*. Interestingly, the hairy root transgenic plants exhibited strikingly greater tolerance to drought and salinity, producing lower levels of hazardous ROS like H_2O_2 and higher levels of protective compounds like proline. Furthermore, the overexpression of *GmP5CS* in the *co-9* mutant background rescued its drought hypersensitive phenotype, suggesting that *GmP5CS* acts downstream of *GmCOL1a* in promoting drought and salinity tolerance in soybean.

In recent years, genome-wide analyses have identified BBX proteins in several economically important crop species (Talar and Kiełbowicz-matuk, 2021). While several studies have observed that *COL/BBX* genes undergo dynamic transcriptional changes under various abiotic stress conditions, their actual functions in stress responses remain elusive. The study from Xu et al. (2022) is an advancement toward the molecular-level understanding of the role of BBX proteins

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in abiotic stress responses (Figure 1). The authors demonstrate that GmCOL1a confers drought tolerance in soybean by ameliorating hazardous ROS. Similarly, IbBBX24, a BBX protein in sweet potato (*Ipomoea batatus*), enhances abiotic stress tolerance by activating the transcription of a peroxidase gene to promote ROS scavenging (Zhang et al., 2022). Together, these studies indicate that the regulation of ROS metabolism during adverse conditions is an important facet in the diverse functions of BBX proteins.

B-box proteins have been proposed as important integrators of hormonal, environmental, and developmental signals (Vaishak et al., 2019). Xu et al. (2022) show that GmCOL1a promotes ABA-mediated closure of stomata, suggesting that GmCOL1 might act through multiple pathways to promote drought tolerance besides ROS scavenging. Previous studies in Arabidopsis (*Arabidopsis thaliana*) have shown that BBX proteins can regulate the ABA signaling pathway by regulating the transcription of *ABSCISIC ACID INSENSITIVE 5* (*ABI5*) (Xu et al., 2014; Bai et al., 2019). However, the precise mechanism by which GmCOL1a modulates ABA-mediated stomatal movements remains to be delineated.

Conflict of interest statement. None declared.

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