

## Commentary

# MYC2-SUMO protease feedback loops boost salt tolerance in wheat

Salt tolerance enables plants to withstand the toxicity of high concentrations of soluble salts, particularly NaCl. Increasing soil salinity slows plant growth and ultimately affects productivity with varying levels of impact depending on the plant species, duration of exposure, and stage of development. Therefore, engineering plant salt tolerance, defined as maintaining superior growth performance under high soil salinity, would be a valuable trait in response to the global salinisation of arable land (Munns *et al.*, 2020). In a study recently published in *New Phytologist*, Xiao *et al.* (2024; doi: 10.1111/nph.20171) developed stable salt-tolerant wheat lines (*Triticum aestivum* L.) by overexpressing the small ubiquitin-like modifier (SUMO) protease-encoding gene *TaDSU*.

Salt tolerance has independently evolved multiple times across different angiosperm lineages and typically involves a complex set of gene functions (Moray *et al.*, 2015). After uptake in the root, Na<sup>+</sup> is transported across different cell types, loaded into xylem, and delivered to the shoot via the transpiration stream (Munns & Tester, 2008; van Zelm *et al.*, 2020). While channel proteins help limit the transport of Na<sup>+</sup> to the shoot, other cellular protective mechanisms rely on transcriptional and post-translational reprogramming. This involves the combined action of transcription factors and specific enzymes, which in turn regulate ion transport, osmotic adjustment, and detoxification of reactive oxygen species (ROS), thereby preventing tissues from oxidative stress and cell damage (Jiang *et al.*, 2012).

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The ability to quickly reprogram protein functions in response to salt stress becomes crucial to activate cellular protection mechanisms. The covalent attachment of SUMO on target proteins is a well-recognised post-translational modification that can change the localisation, stability and activity of target proteins. In brief, SUMO is ligated by a specialised set of enzymes to lysine residues usually embedded within a canonical consensus of amino acids (Benlloch & Lois, 2018). After SUMO conjugation, SUMO

deconjugating enzymes (belonging to different SUMO protease families, each encoded by multiple genes in plants) quickly remove SUMO from their targets to maintain a dynamic equilibrium between SUMOylated and nonSUMOylated target levels (Ghosh *et al.*, 2024). The study by Xiao *et al.* contributes to the expanding list of abiotic stress responses mediated by SUMO proteases by highlighting the role of *TaDSU*. The authors observed increased shoot and root growth in wheat lines overexpressing *TaDSU* (*TaDSU OX*) compared with the wild-type (WT), specifically under saline soil conditions. High salt concentrations in the soil also cause osmotic stress, which reduces water uptake and impairs growth. Supporting the role of *TaDSU* in protecting plant cells from the osmotic aspect of salt stress, *TaDSU OX* lines showed reduced growth inhibition when cultivated in a medium containing mannitol, a nonmetabolisable sugar that induces osmotic stress. Metabolic and cation profiling of plants subject to salt stress revealed that *TaDSU OX* lines had reduced levels of Na<sup>+</sup> content in the shoot (accompanied by increased K<sup>+</sup>), increased contents of soluble sugar and proline, and reduced ROS accumulation, which can be regarded as metabolic hallmarks for augmented salt tolerance.

*TaDSU*, a homologue of OVERLY TOLERANT TO SALT1/2 (OTS1/2) from *Arabidopsis* and OsOTS1 from rice (Conti *et al.*, 2008; Srivastava *et al.*, 2016), has SUMO protease activity *in vitro*. Its overexpression in *Arabidopsis* leads to a global reduction in the SUMOylated proteome under salt stress, indicating deSUMOylation activity also *in vivo*. However, an important question raised by Xiao *et al.* concerns the specific target(s) of *TaDSU*-mediated deSUMOylation, which are responsible for the increased salt tolerance. A yeast two-hybrid screen followed by independent pairwise interaction assays identified the transcription factor *TaMYC2* as a *bona fide* substrate for *TaDSU* SUMO protease activity. This was shown in transient assays and by using stable *Arabidopsis* lines, since *Arabidopsis* MYC2 also appears to interact with *TaDSU* and *TaDSU* overexpression confers increased salt tolerance to *Arabidopsis*. This suggests a conserved mechanism of salt tolerance mediated by *TaDSU* through MYC2 deSUMOylation. Exposure of WT *Arabidopsis* seedlings to high salt conditions resulted in increased levels of MYC2 accumulation accompanied by a corresponding increase of MYC2 SUMOylation. Conversely, when *TaDSU* is overexpressed in *Arabidopsis*, the MYC2 SUMOylated pool was reduced specifically under salt conditions, with no corresponding reduction in the nonSUMOylated pool of MYC2 relative to the WT. These data support a correlation between the reduced accumulation of SUMOylated MYC2 and the acquisition of salt tolerance traits. *TaDSU OX* lines (in wheat and *Arabidopsis*) also displayed increased levels of MYC2 transcripts but limited to salinity conditions. Supporting the role of *TaMYC2* in conferring salt tolerance downstream of *TaDSU*, virus-induced gene-silencing (VIGS) of *TaMYC2* significantly

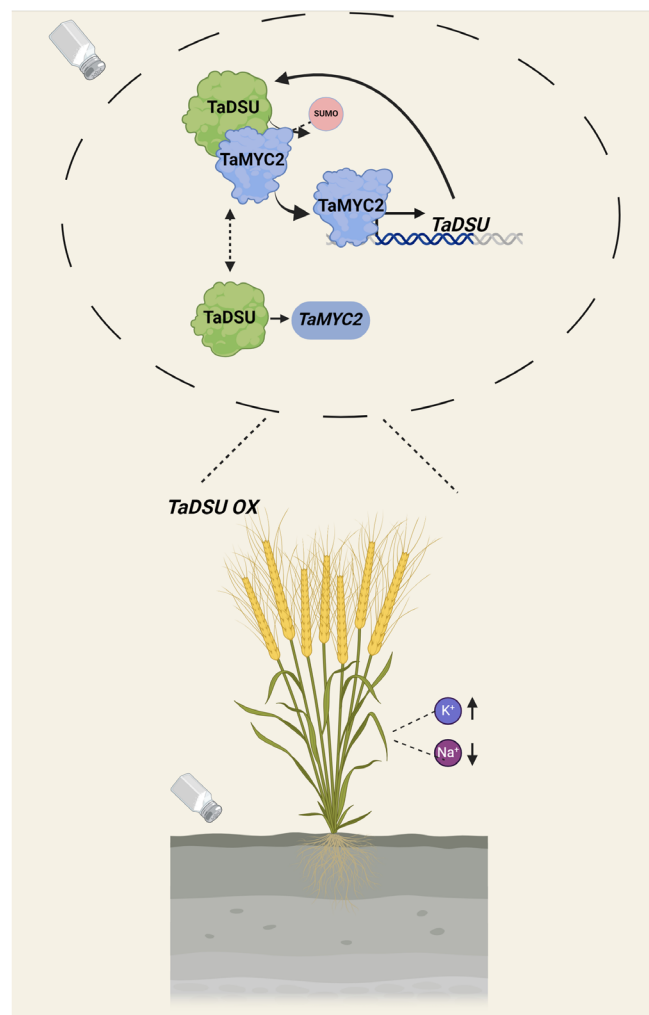
This article is a Commentary on Xiao *et al.* (2024), doi: 10.1111/nph.20171.

weakened the salt-tolerant phenotype of *TaDSU* OX lines in wheat. Therefore, the enhanced salt tolerance conferred by *TaDSU* overexpression can be attributed to an increase in *TaMYC2* transcript accumulation and a reduced SUMOylation level of *TaMYC2*.

The study of Xiao *et al.* also describes a direct transcriptional regulation of *TaDSU* mediated by MYC2. *TaDSU* promoter contains multiple MYC2 binding motifs and *TaMYC2* can directly bind to these regions to activate *TaDSU*. The *TaMYC2*-dependent regulation of *TaDSU* expression was further investigated in wheat plants in which *TaMYC2* was knocked down through VIGS. As expected, VIGS-treated plants had reduced levels of *TaMYC2* and correspondingly decreased levels of *TaDSU* transcript accumulation, specifically under salt stress conditions. Interestingly, a similar MYC2-OTS1/2 direct regulation is conserved in *Arabidopsis* because *OTS1/2* transcripts were upregulated in *MYC2* overexpression lines. Additionally, a chromatin immunoprecipitation assay supports the direct binding of MYC2 to the *OTS1/2* promoters. Therefore, under salt stress conditions, *TaMYC2* accumulation feeds back into the transcriptional activation of *TaDSU*, which in turn promotes deSUMOylation of *TaMYC2* (Fig. 1).

Gains in stress tolerance measured under controlled environments are often less clear under field trials due to the higher complexity of natural stressors. Xiao *et al.* verified the performance of *TaDSU* wheat overexpression lines under saline soils through multiyear experiments. Overall, transgenic lines had more spikes per plant than the untransformed background, with significant increases in yield under salt stress conditions. *TaDSU* OX lines also performed better in another location characterised by saline-alkaline soil, whereas no yield penalty was observed in the presence of low salt.

The study by Xiao *et al.* thus provides a path to an effective genetic strategy for wheat improvement under an agriculturally relevant scenario. It also raises intriguing questions across three interconnected areas, ranging from broader biological and physiological aspects of hormone signalling to more detailed molecular insights. First, it highlights the utility of SUMO modifications and, more generally, post-translational modification to modify key traits in crops. It also reveals important connections between *TaDSU*-MYC2 and other hormonal gene networks, mainly abscisic acid (ABA), since *TaDSU* OX lines had reduced ABA sensitivity. ABA and other phytohormones are known to play important roles in regulating ionic homeostasis and plant growth under salt stress conditions (Achard *et al.*, 2006). Second, the relative paucity of the SUMO conjugation; encoding genes suggests a major role for SUMO deconjugating enzymes in providing specificity to adaptive responses in plants (Ghosh *et al.*, 2024). *TaDSU*, like OTS1/2, falls in a subclade of cysteine proteases, which evolved mostly in angiosperms, perhaps in conjunction with the expansion and adaptation of flowering plants to an increasingly challenging environment or the evolution of more complex plant structures and functions. However, questions remain about how these proteases are post-translationally activated, particularly in response to salt stress. Third, SUMOylation is a fast and dynamic mode of influencing protein function. Here, the discovery of the



**Fig. 1** Depiction of the findings described in Xiao *et al.* (2024). Under salt stress conditions, the small ubiquitin-like modifier (SUMO) protease *TaDSU* interacts with *TaMYC2* to reduce its SUMOylation levels. *TaMYC2* directly activates *TaDSU* expression, thus favouring further *TaMYC2* DeSUMOylation, which is associated with increased salt tolerance. Concurrently, when *TaDSU* is overexpressed, *TaMYC2* transcript levels are also increased. In wheat, overexpression of *TaDSU* leads to lower Na<sup>+</sup> and higher K<sup>+</sup> accumulation, thus conferring improved growth and yield under saline soil conditions in the field.

*TaDSU*-*TaMYC2* regulation opens new questions regarding the direct role of SUMOylation on MYC2 function. The recruitment of MYC2 to chromatin appears to be reduced in *TaDSU* OX lines. How does SUMOylation regulate MYC2 DNA binding? Does SUMOylation also influence MYC2-dependent transcriptional activation at its regulated promoters?

Future studies will be required to better understand the *TaDSU*-*TaMYC2* interplay and the broader role of SUMOylation in plant stress responses. Nevertheless, from an agriculture perspective, insights from this study, combined with advancements in detecting SUMOylation targets *in vivo* (Sang *et al.*, 2024), enhance our ability to engineer SUMOylation for boosting salinity tolerance in crop species.



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