



Sugar cravings during stress: Abscisic acid-mediated starch degradation promotes plant drought tolerance

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Drought represents a major climatic condition that adversely affects agricultural yield and ultimately food prices and food safety. Extreme weather events, such as drought and heat waves, are expected to become increasingly common effects of climate change (Dai, 2013; Lesk et al., 2016). A detailed understanding of molecular mechanisms involved in drought responses is crucial to improve plant resilience to drought. The roles of abscisic acid (ABA) and soluble sugars in abiotic stress tolerance are well established, yet our knowledge of molecular mechanisms of starch catabolism under drought stress remains incomplete.

In this issue of *Plant Physiology*, Zhang and co-workers (Zhang et al., 2022) uncovered the molecular underpinnings of soluble sugar production via ABA-mediated starch breakdown during drought stress. They employed several molecular biology techniques, analytical methods, and in vivo assays in leaves of three-month-old trifoliate orange (*Poncirus trifoliata* (L.) Raf.) seedlings that led to a complete picture of the synergic role of two ABA-responsive transcription factors (TFs) in starch catabolism.

The authors initially observed enhanced transcript levels of *BETA-AMYLASE 3* (*BAM3*) within several hours of water withdrawal. Moreover, the transcriptional induction was accompanied by increased β -amylase enzymatic activities. Consistent with the current understanding of the metabolic processes under stress (e.g. Ma et al., 2017; Thalmann and Santelia, 2017), β -amylase induction was followed by starch depletion and soluble sugar accumulation in drought-stressed plants.

To further investigate the effects of starch catabolism during drought stress, the authors generated *BAM3* overexpression (OE) and silenced (via virus-induced gene silencing,

VIGS) lines in lemon and trifoliate orange, respectively. OE lines showed increased β -amylase activity under normal and drought stress conditions with decreased starch content and increased soluble sugars compared with wild-type (WT) plants. After two weeks of water withdrawal, the OE lines outperformed WT plants in stress-related parameters such as electrolyte leakage, accumulation of malondialdehyde (a biomarker of oxidative stress), generation of reactive oxygen species, and chlorophyll fluorescence. As expected, the VIGS lines had significantly lower levels of soluble sugars, which translated into poorer plant performance under limited water availability.

In search of molecular mechanisms regulating drought-induced β -amylase activity, the authors then focused on the *BAM3* promoter sequence. In silico screening of the *BAM3* promoter revealed the presence of an ABA-responsive element (ABRE) recognized by ABRE-binding factors (ABFs) (Yoshida et al., 2010) and a GCC-box, in addition to several other conventional TF-binding sites. GCC-box has been primarily associated with the binding of ethylene-responsive TFs and dehydration-responsive element-binding (DREB) proteins (Donde et al., 2019; Chen et al., 2020). In line with the presence of the ABRE in the *BAM3* promoter, a yeast one-hybrid screen identified the TFs ABF4 and ABA REPRESSOR 1 (ABR1) as potential upstream regulators of *BAM3* expression.

The authors further demonstrated the nuclear localization of ABF4 and ABR1 and their binding to the ABRE and GCC-box, respectively. Given the proximity of both TF binding sites in the *BAM3* promoter, the authors investigated whether ABF4 and ABR1 form a heterodimer. Indeed, a physical interaction between ABF4 and ABR1 was observed

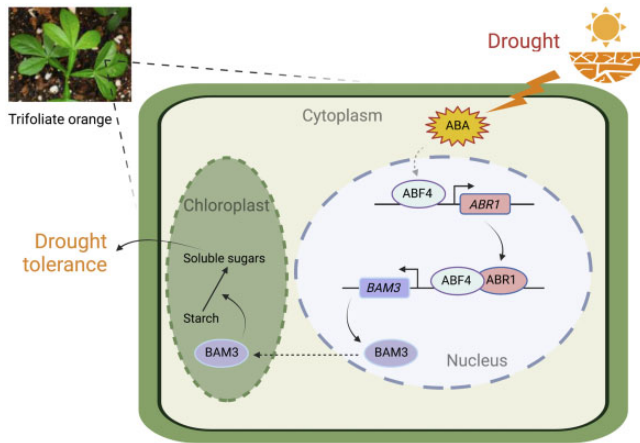


Figure 1 A model showing the co-action of ABA-responsive TFs in regulating starch degradation during drought stress. In trifoliate orange, drought-induced TFs ABF4 and ABR1 act together to activate the expression of the *BAM3* gene. ABF4 transcriptionally activates *ABR1* expression and physically interacts with ABR1, which leads to enhanced *BAM3* expression. The β -amylase enzyme produced as a result of *BAM3* expression catalyses the conversion of starch into soluble sugars in chloroplasts and subsequently promotes drought tolerance. Adapted from Zhang et al. (2022).

in yeast two-hybrid, bimolecular fluorescence complementation, and luciferase complementation imaging assays. Finally, the authors showed improved drought tolerance of *ABF4* and *ABR1* OE lines as well as detrimental effects of loss of either one of the two ABA-responsive TFs upon drought induction. Besides regulating *BAM3* expression synergistically with ABR1, ABF4 also directly controlled *ABR1* expression. Taken together, the authors conclude that drought induces ABF4-mediated expression of *ABR1*, and both TFs jointly induce *BAM3* expression, a key enzyme of starch catabolism (Figure 1).

Overall, the investigations of Zhang et al. (2022) unambiguously identified *BAM3* as the primary β -amylase responsible for the drought-induced starch breakdown. With a series of

complementary assays, the authors provide a complete picture of the regulatory role of ABA-responsive TFs ABF4 and ABR1 in *BAM3*-mediated starch degradation. These findings on stress-induced starch catabolism will be crucial in generating drought-tolerant trifoliate orange, a popular yet drought-susceptible citrus rootstock. In addition, the recently discovered link between starch metabolism and drought tolerance discovered by Zhang et al. (2022) will be of great importance in understanding stress-induced carbohydrate partitioning and will subsequently assist the global efforts to create stress-tolerant crops.

Conflict of interest statement. None declared.

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