

Reducing Stomatal Density by Expression of a Synthetic Epidermal Patterning Factor Increases Leaf Intrinsic Water Use Efficiency and Reduces Plant Water Use in a C₄ Crop

Background/Objective

- Enhancing crop water use efficiency (WUE) is a key target trait for climatic resilience and expanding cultivation on marginal lands. Engineering lower stomatal density to reduce stomatal conductance (g_s) has improved WUE in multiple C₃ crop species. A different response is expected in C₄ plants because they possess specialized anatomy and biochemistry which concentrates CO₂ at the site of fixation.
- To test this hypothesis, genetic strategies were investigated to reduce stomatal density in the C₄ crop, sorghum. This study tested two genetic designs to address the knowledge gap about how engineering reduced stomatal density would impact photosynthetic physiology and whole-plant function in a C₄ species.

Approach

In the first design, the native sorghum epidermal patterning factor 1 (SbEPF1) was constitutively expressed under control of the sugarcane ubiquitin4 (Ubi4) promoter (Wei et al., 2003). In the second design, a fusion element was synthesized that combined elements of the sorghum orthologs of AtEPF2 and AtEPFL9 (SbEPFsyn) and placed under control of the Ubi4 promoter.

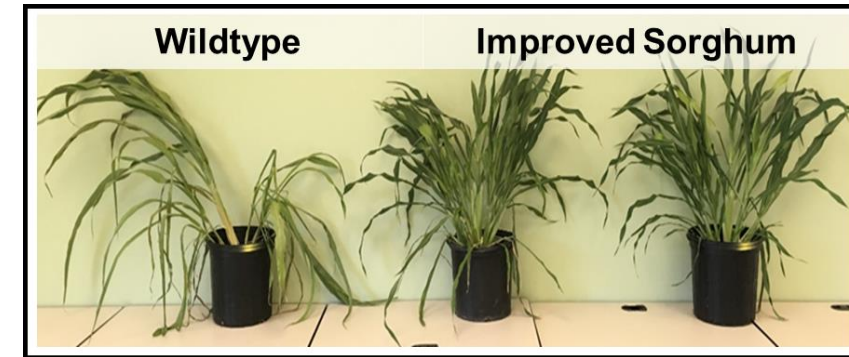
Results

Constitutive expression of a synthetic EPF transgenic allele in sorghum led to reduced stomatal densities, reduced g_s , reduced plant water use and avoidance of stress during a period of water deprivation. In addition, moderate reduction in stomatal density did not increase stomatal limitation to A_N. However, these positive outcomes were associated with negative pleiotropic effects on reproductive development and photosynthetic capacity.

Significance/Impacts

This study provides support for prediction from modelling studies that engineering to reduce stomatal conductance can improve WUE and act to lower plant water use without reducing biomass accumulation of a model C₄ crop. However, it also highlights: (1) the potential for pleiotropic effects on a range of developmental when a mobile signaling peptide is expressed ubiquitously; and (2) the currently limited understanding of the genetic basis for stomatal development in C₄ grasses.

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A side-by-side comparison of wildtype sorghum and the improved variety with fewer stomata shows how the engineered plants can maintain plant productivity with less water.