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

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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 C_3 crop species. A different response is expected in C_4 plants because they possess specialized anatomy and biochemistry which concentrates CO_2 at the site of fixation.
- To test this hypothesis, genetic strategies were investigated to reduce stomatal density in the C_4 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 C_4 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.

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.

Significance/Impacts

This study provides support for prediction from modelling studies that engineering to reduce stomatal conductance can improveiWUE and act to lower plant water use without reducing biomass accumulation of a model C_4 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 C_4 grasses.

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