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Brachypodium SPEECHLESS2 Promoter Drives Expression of a Synthetic EPF to Reduce Stomatal Density in Sugarcane Without Pleiotropic Effects

Background/Objective

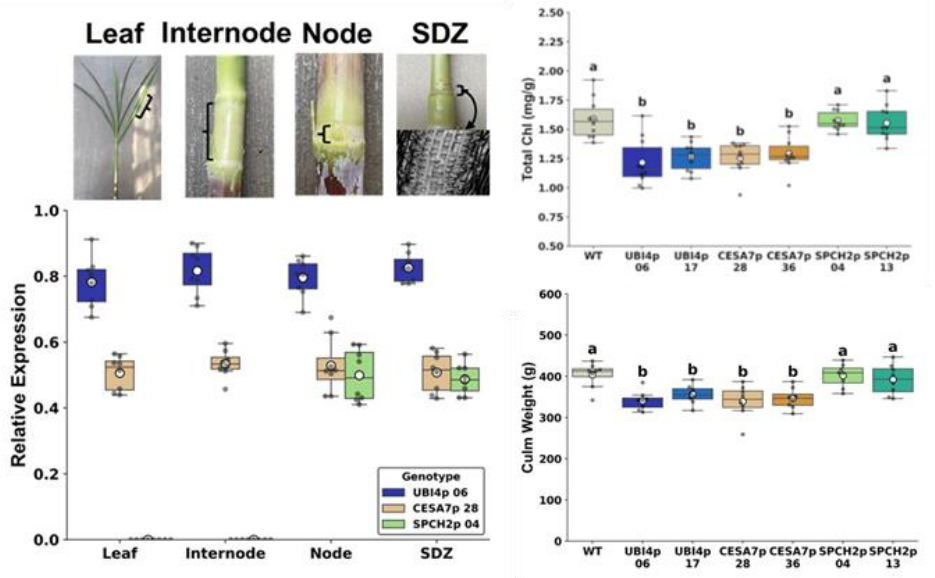
Stomata are microscopic pores that regulate the exchange of CO_2 and water vapour, making them a major target for engineering plants with improved intrinsic water use efficiency (iWUE). Proof-of-concept studies have demonstrated the potential to increase iWUE by reducing stomatal density (SD) and stomatal conductance (g_{sw}) by ubiquitously expressing EPIDERMAL PATTERNING FACTOR (EPF) family genes. However, unwanted effects on leaf, stem, and reproductive traits are often observed when EPFs are misexpressed in this fashion. We sought to test if these effects result from pleiotropy and to identify a targeted promoter that can circumvent the side effects while retaining the desired reduction in SD.

Approach

A previously reported synthetic EPF (EPF_{syn}) was expressed in sugarcane (*Saccharum* spp.) using two putatively tissue-specific promoters from *Brachypodium distachyon* (BdCESA7p and BdSPCH2p) and a ubiquitous control from *Zea mays* (ZmUBI4p).

Results

BdSPCH2p control reduced SD to statistically equivalent levels as ZmUBI4p on the abaxial (23%) and adaxial (23%) leaf surfaces. Transgenic lines UBI4p and CESA7p showed EPF expression in tissue types often associated with pleiotropic effects in EPF-expressing low SD plants. Transgenic plants carrying either the BdCESA7p or ZmUBI4p EPF_{syn} cassettes displayed leaf chlorosis, reduced leaf nitrogen and chlorophyll content, and altered stem architecture. However, transgenic SPCH2p restricted EPF_{syn} expression to the stomatal development zone and leaf nodal tissues by utilizing the BdSPCH2p promoter and produced transgenic plants without the associated pleiotropic effects.



Expression pattern and pleiotropic effects of transgenic lines UBI4p (blue), CESA7p (orange), and SPCH2p (green).

Significance/Impacts

These findings advance our understanding of how expression of EPF family members affects iWUE in sugarcane and highlight that using tissue-specific promoters can help mitigate unintended effects and enhance the application of this technology. These results represent an important step toward engineering low-SD crops since they show that targeted gene expression can reduce stomatal density without impairing agronomically important traits.

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