Evaluation and Licensing Opportunities

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Patent Literature

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The University Of Sheffield.

EPF Modification

Modifying stomatal density for improved yield through protection from abiotic and biotic stress

Demonstrated in rice, wheat and barley

NOW with results for gene-edited maize (see page 5)

Stomata play a crucial role in regulating both CO_2 uptake for photosynthesis and water loss. This regulation is determined both in terms of the density of stomata on plant leaves and through the control of guard cells regulating changes in stomatal aperture. Stomata therefore have direct effects on photosynthesis and influence many other aspects of plant physiology and performance. The amount of CO_2 fixed by photosynthesis relative to the amount of water vapour lost to the atmosphere is termed water-use efficiency (WUE) and stomata are the primary control point determining WUE.

Making crops more tolerant to conditions of water shortage and improving their WUE without compromising yield will have an immense agronomic benefit. In terms of environmental impact, cultivation of cereal crops accounts for nearly a third of global water consumption and many cultivation areas have increasing issues with water scarcity.

Now Professor Julie Gray and co-workers at the University of Sheffield have demonstrated that through gene editing (or genetic modification) of the endogenous peptide factors encoded by the EPIDERMAL PATTERNING FACTOR (EPF) gene family stomatal density (SD) can be altered. EPFs have a role in stomatal patterning and differentiation, since overexpression of EPF1- and EPF2-like genes or downregulation of the EPF9-like gene leads decreased stomatal density and plants with **improved WUE and drought tolerance**.

The EPF pathway is conserved across plant species, with **rice**, **barley**, **wheat** and **maize** all possessing Arabidopsis EPF orthologs.

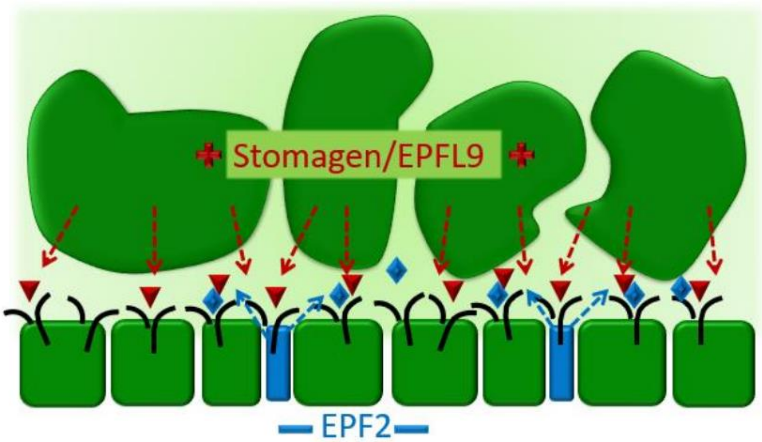

Figure 1: Epidermal Patterning Factors (EPFs) control stomatal development by regulating entry of plant cells into the stomatal lineage. Positive and negative acting EPF peptides increase or decrease stomatal density (SD). Plants have two types of EPFs that regulate stomatal density. EPF1 and EPF2 inhibit cells from entering the stomatal lineage, while EPFL9 (aka Stomagen) is a competitive inhibitor of the same receptor and block EPF1/2 activity, stimulating increased stomatal numbers. **Thus, plants with fewer stomata can be produced by either over-expressing EPF1/2 (e.g. transgenically) or downregulating EPFL9 by gene editing or mutagenesis approaches**.

The inventors have edited EPFL9 homologs in rice and maize. In addition the team has also performed overexpression experiments of EPF1 in rice, wheat and barley. Below is a summary of these results (further details are in the referenced scientific literature). The team has demonstrated that a moderate reduction in stomatal density results in **reduced crop water use**, improved **drought tolerance** with no decrease in yield under normal conditions, and with **increased yield under stressed conditions**.

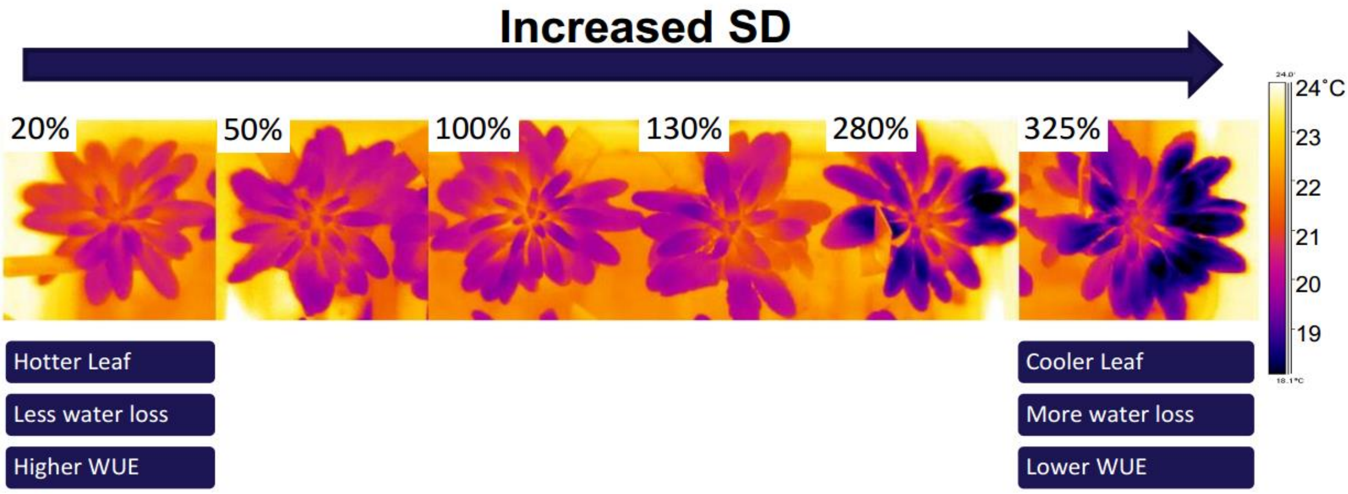

Figure 2: Initial experiments with reduced (down to 20%) or increased (up to 325%) stomatal density (SD) as shown above demonstrated that the SD has a profound effect on leaf temperature and water loss, pointing to the possibility that a decrease in SD will positively impact the plants WUE.

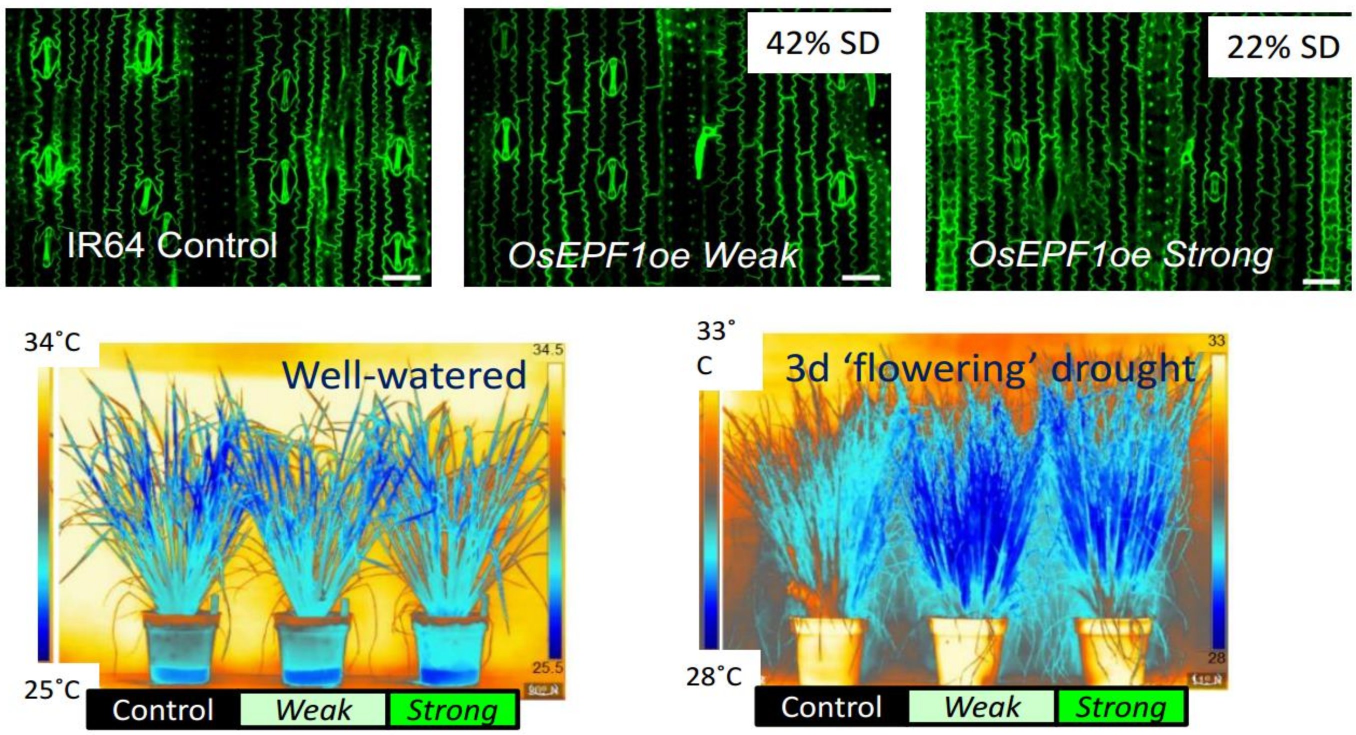

Figure 3: Rice plants with EPF1 overexpression (with either 42% SD "weak" expression or 22% SD "strong" expression compared to wt control) were tested in well-watered or drought conditions. The above images show SD in the top row and plant temperature in the two pictures in the bottom row. During drought stress rice plants with less SD have a higher leaf temperature and lose less water.

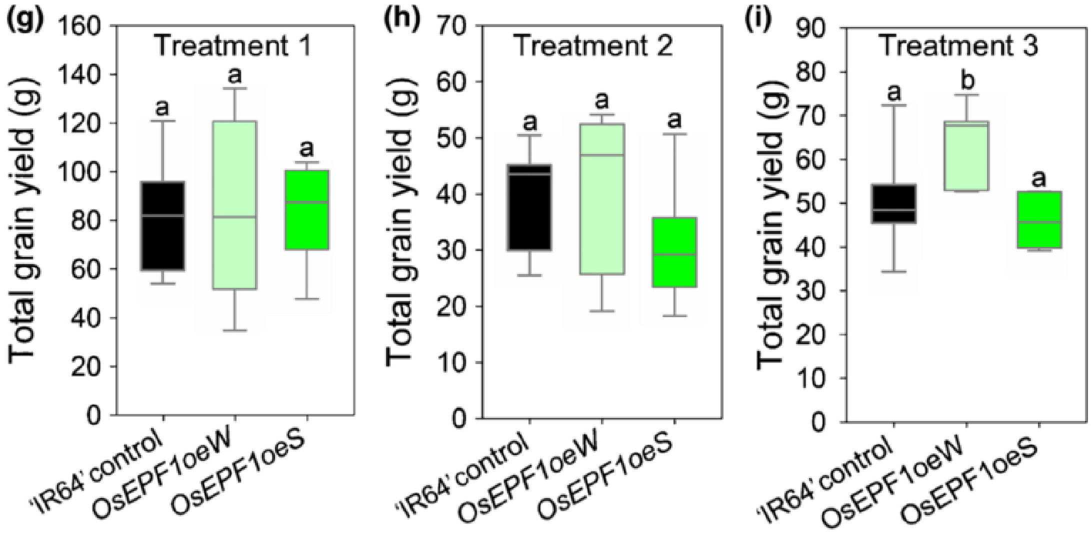

Figure 4: The rice plants with weak and strong EPF1 overexpression: Changes in SD affects water loss and temperature, and enhances yield following flowering drought. Shown above are the total grain yields of (g) well-watered, (h) vegetative drought and (i) flowering drought plants: Rice plants with moderately reduced SD show an improved yield under drought conditions compared to wt.

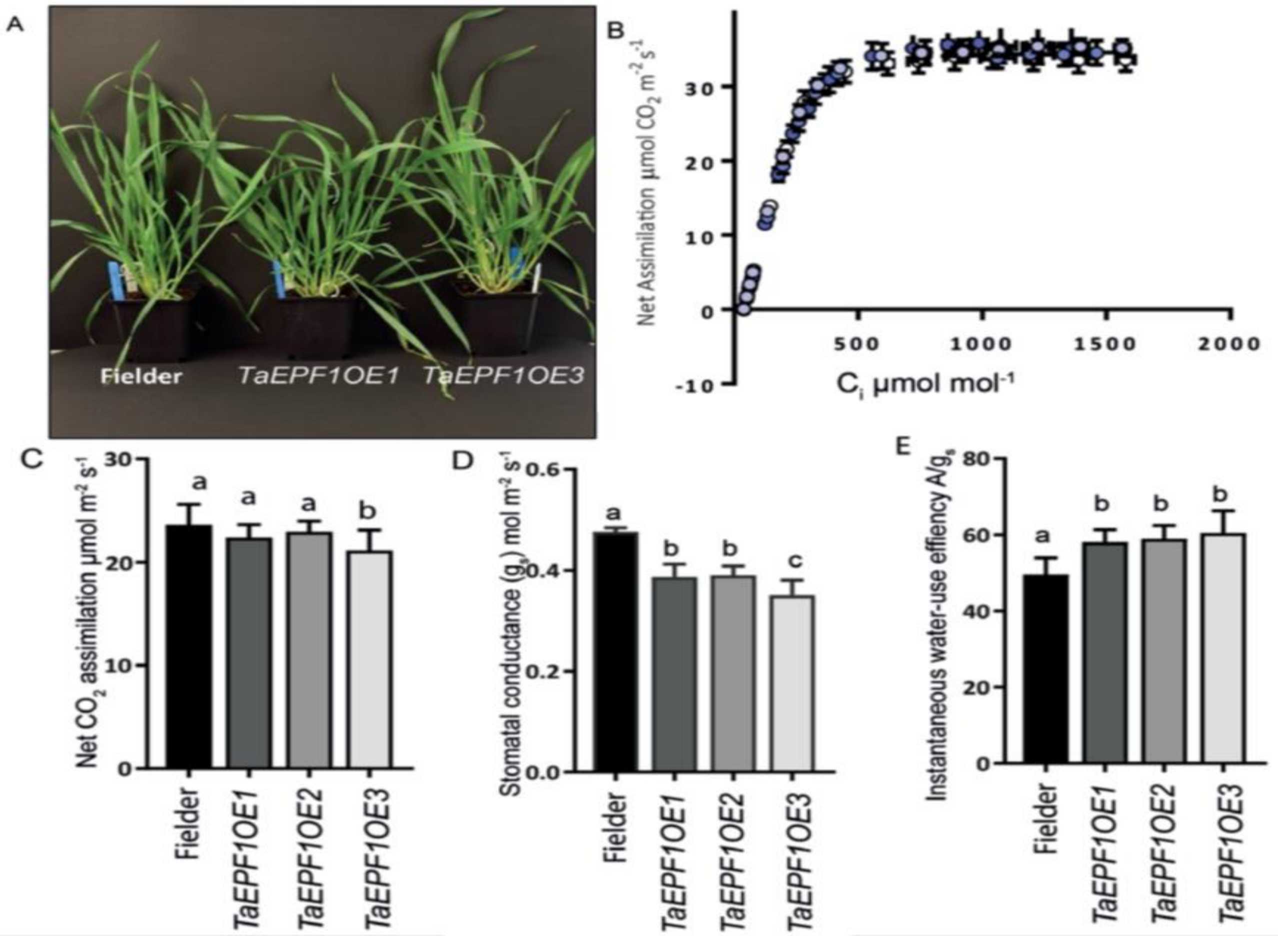

Figure 5: Wheat plants overexpressing TaEPF1 grow normally and are more water-use efficient. (A) Wheat transgenic lines TaEPF1-OE1 (moderate decrease in SD) and TaEPF1-OE3 (more severe decrease in SD) are not visibly distinguishable from wt (Fielder) plants. (B) & (C) Assimilation rates are similar, indicating that biochemical limitations on photosynthesis do not differ between the lines. (D) Stomatal conductance is decreased at lower SD. (E) WUE is higher in the TaEPF1 overexpression lines compared with Fielder (wt).

Brown rust in Barley

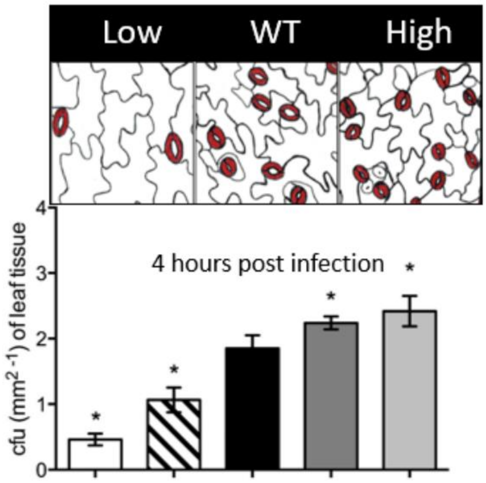

Figure 6: In addition to the benefit of improving the WUE of plants with moderately reduced SD, these plants have also been shown to have improved disease resistance because pathogen entry points have been effectively limited. The picture and graph above show a control wild type barley plant (Golden Promise) and two barley lines with reduced SD. Visually it is clear that infection is markedly reduced in the modified plants and this is summarised in the graph. On the right hand side is an experiment with Arabidopsis plants infected with *P. syringae*. The two lines with low SD (white and striped bars in the graph) had markedly reduced infected leaf areas compared to wt (solid black bar in the graph) with plants with higher SD showing even higher infection rates (two grey bars).

In summary the inventors have shown that a moderate reduction in stomatal density shows:

Drought and heat tolerance resulting in **yield protection** in:

- Arabidopsis
- Barley
- Rice
- Wheat

Pathogen resistance for:

- *P. syringae* in Arabidopsis
- Brown rust (*P. triticina*) in wheat and barley

Genome edited maize has been created and the T2 homozygous lines are currently undergoing testing with very promising early results.

References:

Hepworth *et al.* (2018) Stomatal development: focusing on the grasses *Current Opinion in Plant Biology* **41**, pp 1-7

Caine *et al.* (2019) Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions *New Phytologist* **221, 1** pp 371-384

Dunn *et al*. (2019) Reduced stomatal density in bread wheat leads to increased water-use efficiency *Journal of Experimental Botany*, **Vol. 70, No. 18** pp. 4737–4747

NEW RESULTS:

In the experiments the inventors had guide RNA designed to target both of the EPFL9a-like genes, introducing 1 or 2bp insertion causing premature translation termination. They are testing lines with mutations in gene 1 only, or in both genes; currently growing T2 homozygous plants. The plants look phenotypically normal and initial experiments have shown reduced stomatal density (Figure 7), similar carbon assimilation and improved WUE (Figure 3).

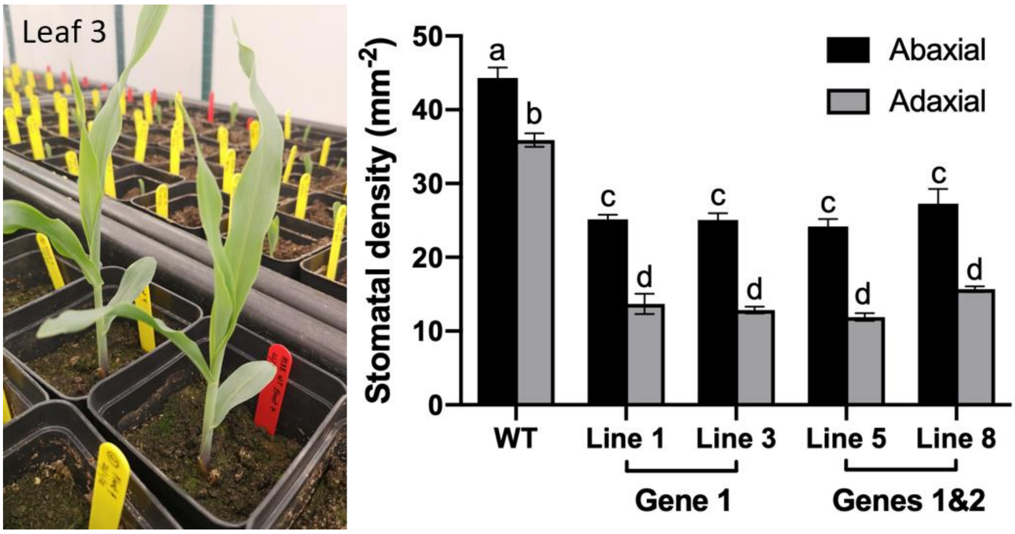

Figure 7: Initial experiments show reduced stomatal density (SD) of GE plants. SD is reduced by ~43% on the abaxial and ~62% on the adaxial leaf surface.

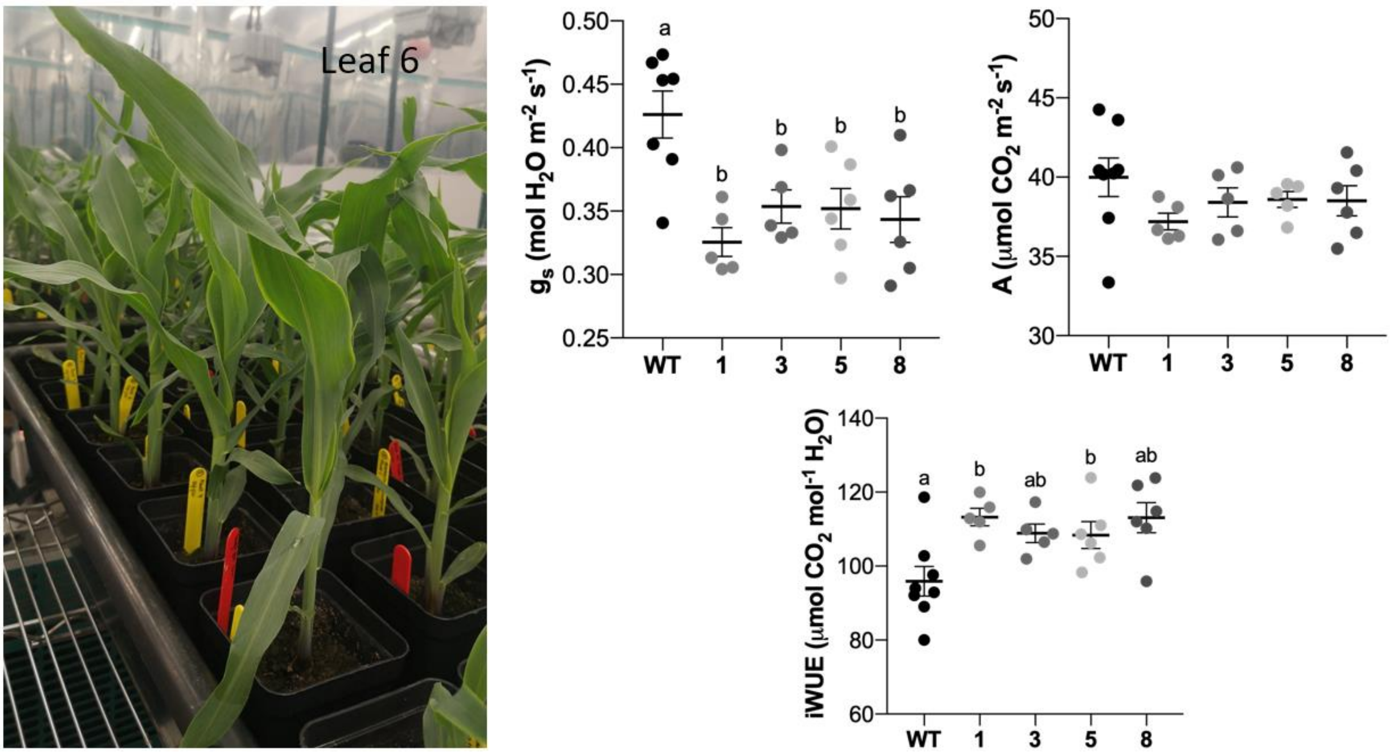

Figure 8: Stomatal conductance is reduced but not carbon assimilation in the GE maize plants under saturating light. WUE is markedly improved in all GE maize lines.