Wheat Transformation

Breaking Transformation Barriers

The regulatory networks underlying regeneration capacity of wheat reveal new opportunities for overcoming barriers to highly efficient and genotype independent transformation.

Wendy Harwood

Transformation of many crops requires regeneration of plants in tissue culture as part of the process. Bottlenecks due to poor regeneration are common and have restricted the ability to introduce valuable genes or genome editing components to the crop variety of choice due to so-called 'genotype dependence' of transformation methods. Different strategies to boost transformation in wheat by enhancing regeneration have been employed but without good understanding of the underlying mechanisms involved. In this issue of Nature Plants, Liu *et al*. have been able to shed light on the complex regulatory network responsible for wheat shoot regeneration and in doing so, have uncovered novel genes that increase regeneration in wheat and therefore lead to more efficient transformation [1].

Efficient transformation systems require both highly efficient regeneration and efficient delivery of DNA to the explants used as target tissues. The most common delivery system is the soil bacterium *Agrobacterium tumefaciens* which can transfer target genes or editing components to the plant cell. Barriers to efficient transformation may either be at the Agrobacterium infection stage, or at the regeneration stage, depending on the species and genotype (Fig 1). There has been recent progress to overcoming barriers at the Agrobacterium infection stage by introduction of a *Pseudomonas syringae* type III secretion system to deliver effector proteins [2]. This strategy was able to increase wheat transformation by 400% in the already, highly transformable, genotype Fielder. Inclusion of additional virulence genes has also been reported to help at the Agrobacterium infection stage[3].

Barriers to regeneration often have the most serious impact on transformation and tactics to overcome them in wheat have included the expression of several morphogenic or developmental regulator genes during the regeneration process. These include a *TaGRF4-TaGIF1* chimeric protein that led to high transformation efficiencies in wheat [4]. The same strategy was also used in a transient expression system to enhance regeneration and genome editing efficiencies [5]. Over-expression of the *TaWOX5* gene led to enhanced regeneration and overcame genotype dependency across a range of wheat varieties [6]. The maize genes, *ZmBbm* and *ZmWus2* have also been used in wheat to give efficient and rapid transformation [7]. Despite these advances, the reasons why barriers to transformation differ between varieties remains unclear.

Using a range of 'omics' strategies including RNA-seq, ATAC-seq and CUT&Tag techniques, Liu et al. have greatly increased our understanding of the reasons for different regeneration efficiencies across varieties in wheat. Detailed analysis of transcriptome and epigenetic data, collected at five different time points during shoot regeneration, revealed that over 40,000 genes were differentially expressed during the wheat regeneration process, indicating the massive transcriptome re-programming taking place. By clustering differentially expressed genes into six groups, according to the time point in the tissue culture regeneration process, the authors were able to unpick the regulatory network, and identify 446 transcription factors (TFs) playing key roles in driving the regeneration process.

The initial step in regeneration from immature embryos in wheat involves auxin treatment to drive callus formation. At the earliest time point examined, down-regulation of embryo identity genes was seen, followed by rapid induction of genes in response to the auxin treatment. A clear sequential activation of genes over the different timepoints was observed with genes related to cell division and auxin response followed by those related to root and shoot development. Genes highly expressed after application of auxin were seen to fall into groups, with one group containing *TaBBM* and *TaWOX5,* both previously shown to enhance wheat regeneration and transformation when over-expressed [6,7].

To further unravel the complex regulatory network, the authors extracted networks for *TaBBM* and *TaWOX5*. Their findings suggested that both genes could increase regeneration by regulating genes involved in processes required for regeneration including cell division, meristem initiation and shoot development. The 446 core TFs driving the regeneration process showed a sequential pattern of regulation, with those in an early time-point cluster regulating those in later clusters. By examining these TFs in a highly regenerable variety (Fielder) compared to one with poor regeneration (Jimai22), it was seen that half of the core TFs showed differences in transcription pattern during shoot regeneration, including *TaBBM* and *TaDOF3.4.* Data from a further 12 wheat varieties was examined to support the findings, together with data from an extensive comparison of the regeneration process in Arabidopsis and wheat. Another member of the DOF family, *TaDOF5.6,* was seen to be upregulated during wheat and Arabidopsis regeneration, leading to further study of DOF TF family members.

As *TaDOF5.6* and *TaDOF3.4* were the most up-regulated of the DOF TFs in wheat after auxin treatment and were known to regulate key downstream genes involved in the regeneration process, they were chosen to test whether they could boost regeneration and thus transformation efficiency. In the regenerable variety Fielder, both *TaDOF5.6* and *TaDOF3.4* boosted transformation efficiency from 26% to 50 and 55% respectively. It was also shown that transformation efficiency was increased in varieties with poor regeneration, with 29% efficiency achieved in Jimai22. Plants over-expressing these TFs grew and developed normally and produced fertile seeds.

Although previous reports have described strategies to boost wheat transformation and overcome genotype dependence, such strategies do not work uniformly over different varieties. The study by Liu et al. provides the tantalising possibility of having bespoke strategies for given wheat varieties based on understanding the activity of key transcription factors at specific tissue culture stages. It also opens the door to application of a far greater panel of candidate 'Regeneration Factors' to enhance future wheat transformation and genome editing capability.

References

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Figure legend

Figure 1. Barriers to efficient wheat transformation may be at the Agrobacterium infection stage (Barrier 1) or at the regeneration stage (Barrier 2). Increased susceptibility to Agrobacterium can be visualised by expression of reporter genes such as the *gus* gene. The impact of 'Regeneration Factors' can be seen as greatly increased shoot regeneration which in turn leads to higher transformation efficiencies.