

# Investigating genotype-dependency in barley transformation



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*Agrobacterium*-mediated transformation is one of the most efficient ways to analyse gene function in plants. However, in cereal crops - wheat and barley, it is currently genotype-dependent with most commercially important cultivars remaining recalcitrant. We performed a transformation pilot study using a subset from a recombinant inbred line (RIL) population of 160 lines (GPMx) which we derived from a cross between 2-rowed *Golden Promise* (highly transformable) and 6-rowed *Morex* (recalcitrant) cultivars (Fig.1). The F<sub>10</sub> population has been genotyped with 1536 gene-based markers, followed by assembling a genetic linkage map that is suitable for QTL mapping. Our aim is to identify genes underlying barley transformability, with the objective of enhancing the transformability of a wide range of barley cultivars.



Figure 1: Inflorescence from Morex (left), GPMx012 (centre) and Golden Promise (right) barley cultivars.

## Factors underlying genotype-dependency

There are three recognised factors underlying genotype-dependency: 1) susceptibility to *Agrobacterium* 2) callus inducibility and 3) regeneration of callus into plantlets.

### Agrobacterium infection

Embryos from recalcitrant cultivars such as Morex often develop tissue necrosis, or browning, after *Agrobacterium* infection. In some species this can be overcome by altering the tissue culture conditions e.g. adding antioxidants; or by manipulating genes that are induced or repressed by *Agrobacterium*.



Figure 2: Golden Promise (left) and Morex (right) immature embryos in tissue culture one week after *Agrobacterium* infection.

### Callus inducibility

We have studied callus inducibility in a range of cultivars from Golden Promise (highly inducible) to Morex (poor). In tissue culture several different types of callus develop, most of which are not embryogenic. We have found some correlation between callus induction and subsequent shoot regeneration but it is thought that they are independently controlled.

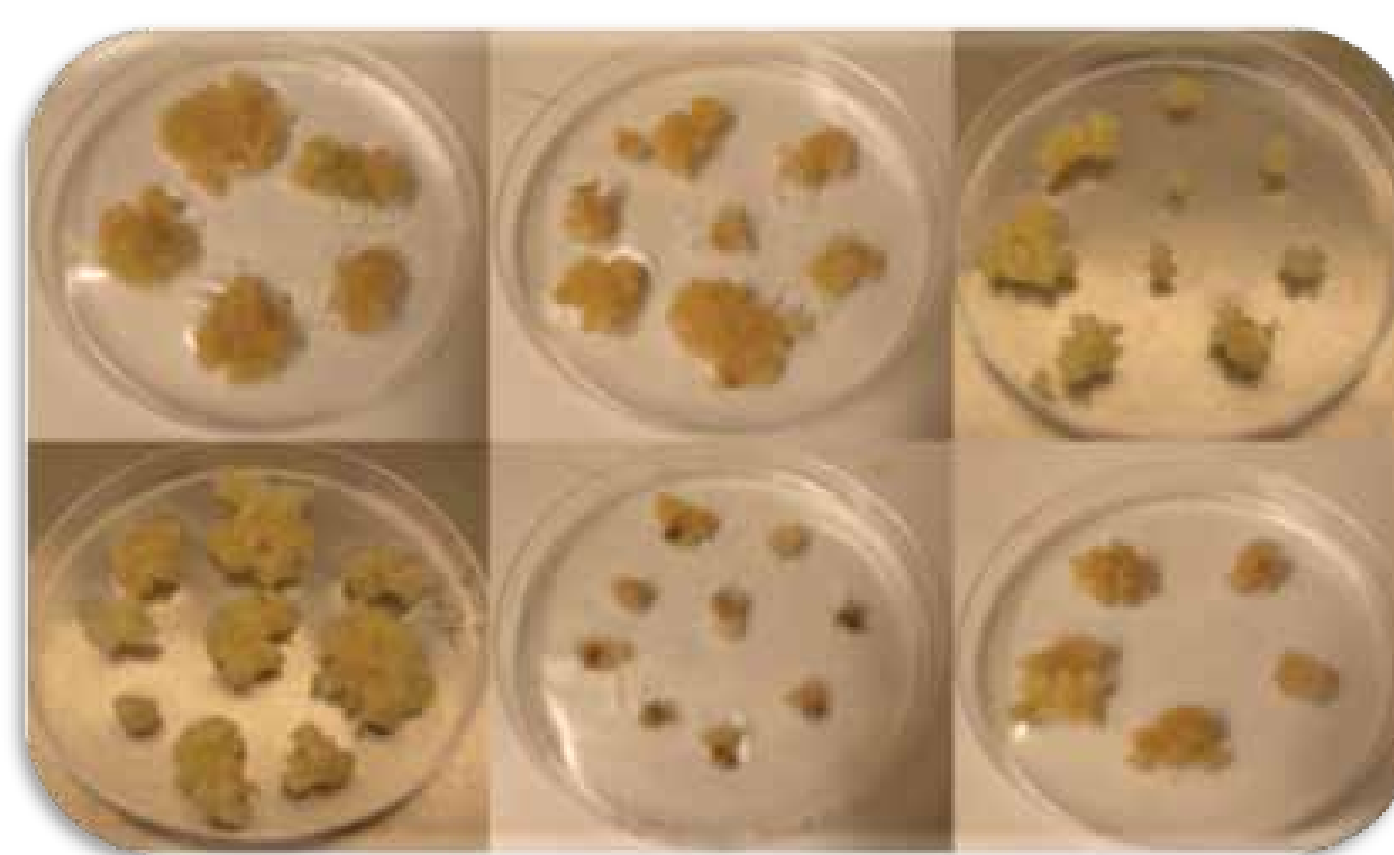


Figure 3: Callus development in a range of cultivars: Golden Promise, Clipper, Derkado (top, L-R), Triumph, Morex, Optic (bottom, L-R).

### Shoot regeneration

The ability of plants to regenerate from callus is genotype dependent. Various growth regulators have been used in tissue culture media with varying success, but there is no universal media that promotes shoot regeneration in the majority of cultivars.

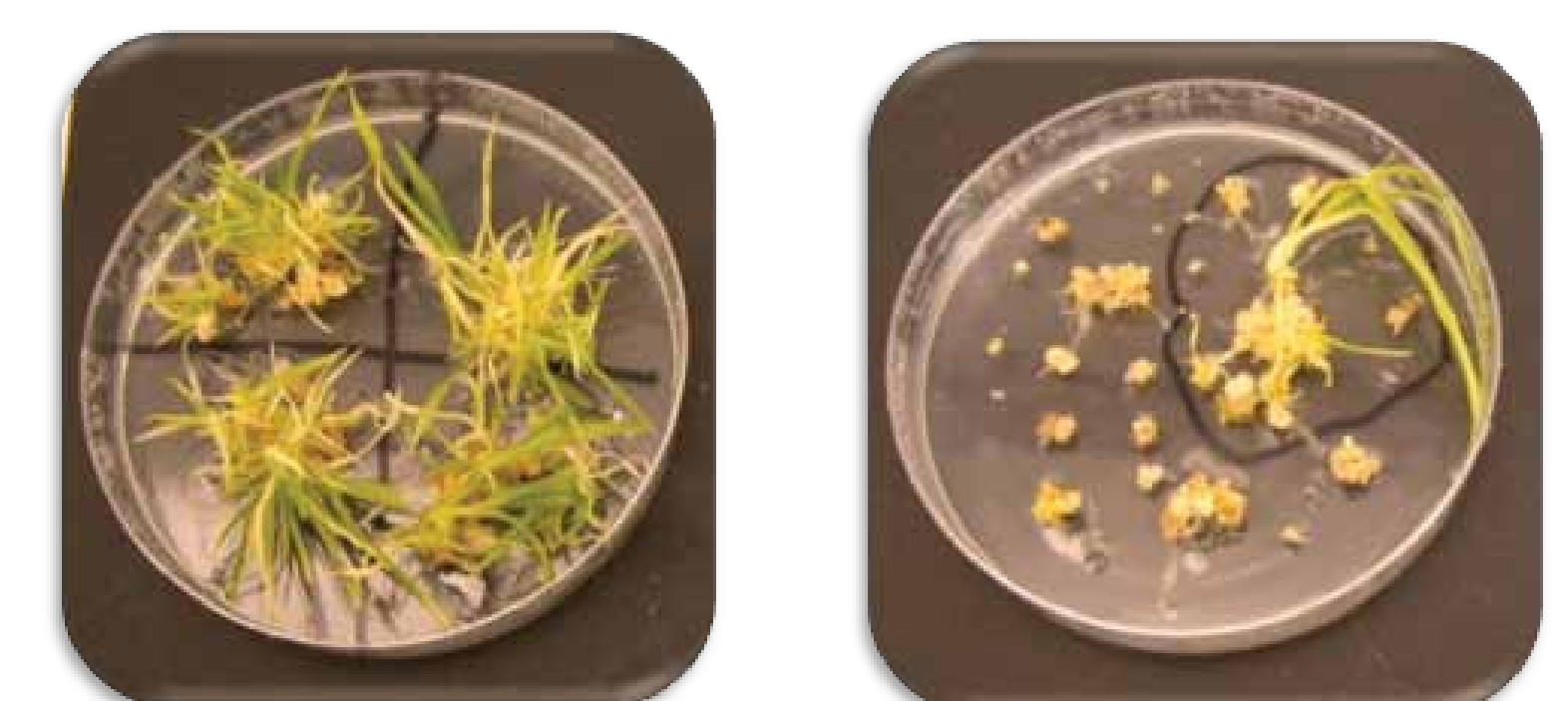


Figure 4: Shoot regeneration in Golden Promise (left) and Optic (right) cultivars. We did not regenerate any Morex shoots.

## Results

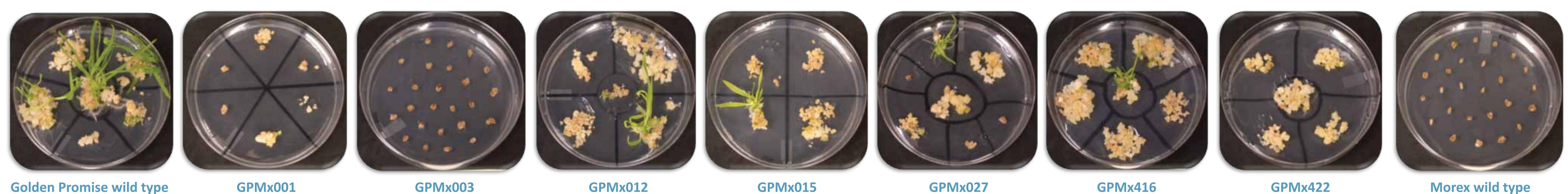


Figure 5: Regenerating shoots in tissue culture following *Agrobacterium*-mediated transformation of GPMx RILs with pBract204 (GUS) vector.

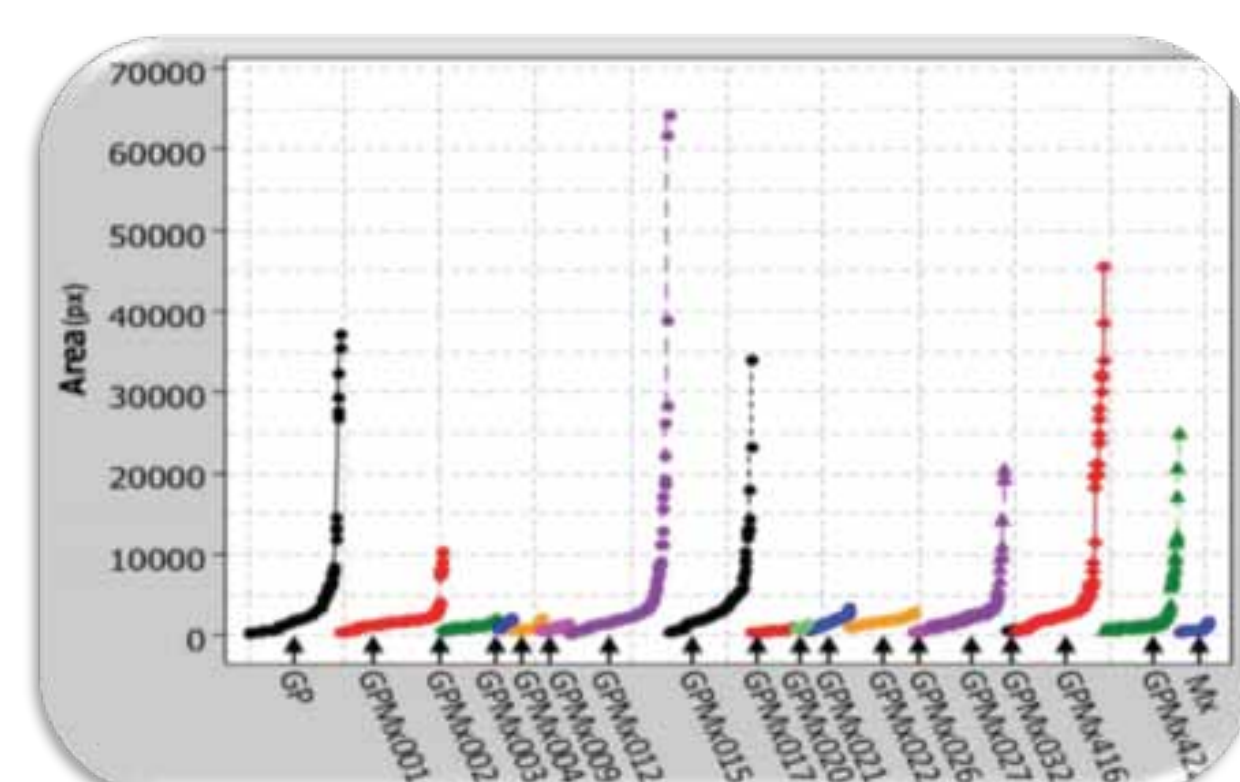
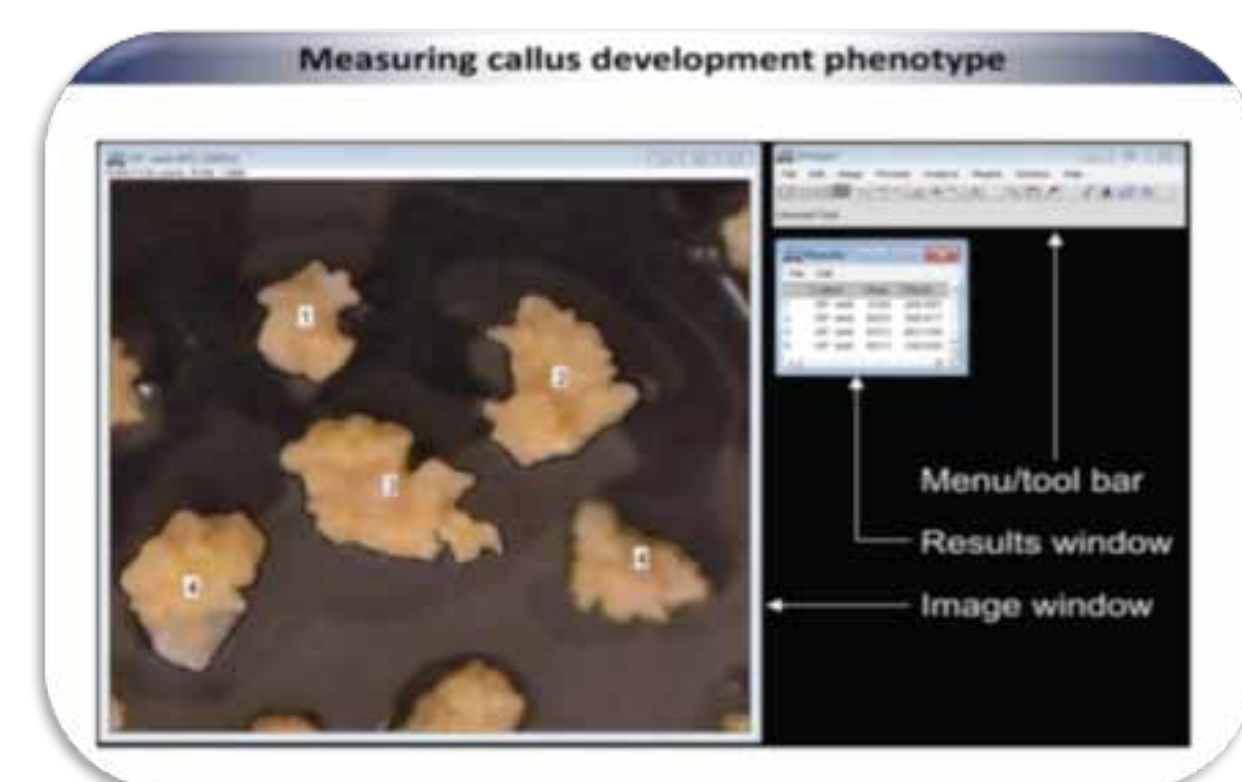


Figure 6: The area of each callus was measured using ImageJ (left), and plotted for each RIL (right).

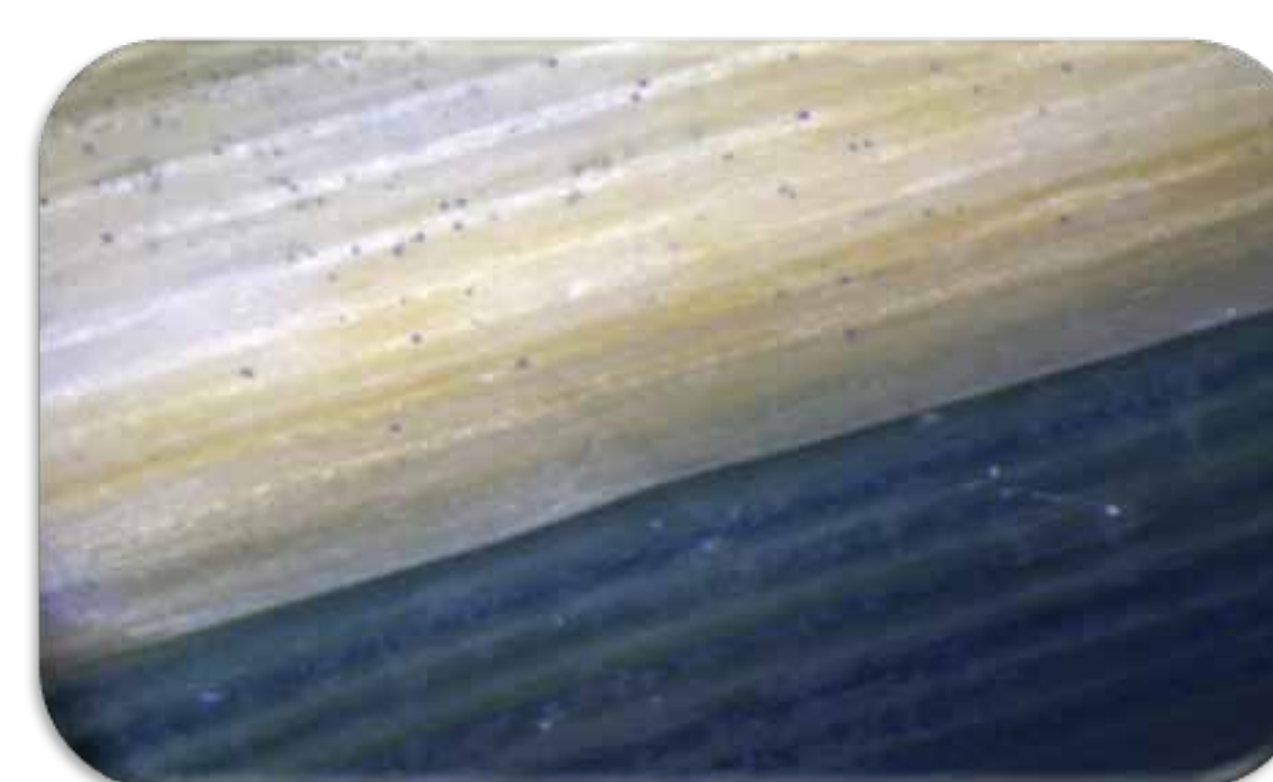


Figure 7: GUS staining in leaves from Golden Promise wild type (top) and a transgenic GPMx line (bottom).



Figure 8: Segregation analysis of seeds from a transgenic GPMx line grown on selective media.

## Conclusions

Our initial study demonstrates that callus inducibility and regenerability traits segregate. This means that these traits are amenable to genetic analysis. The QTLs and genes underlying transformability traits are currently unknown in barley; therefore our project may provide the key to unlocking genotype dependency. Developing a genotype-independent system in barley will provide a valuable tool for the research community enabling high throughput transformation and gene function validation in any cultivar.

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