Optimizing *Agrobacterium*-mediated transformation of potato

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Introduction

Agrobacterium-mediated transformation is one of the most efficient methods to analyze gene function in plants. Unfortunately, in potato it is genotype-dependent with most cultivars being recalcitrant. There are many variables that can be optimized to improve the efficiency of transformation (Fig. 1).

We are systematically testing each of these variables in a range of cultivars – Stirling, Mayan Gold, Russet Burbank and Andigena, alongside the responsive cultivar Desiree.

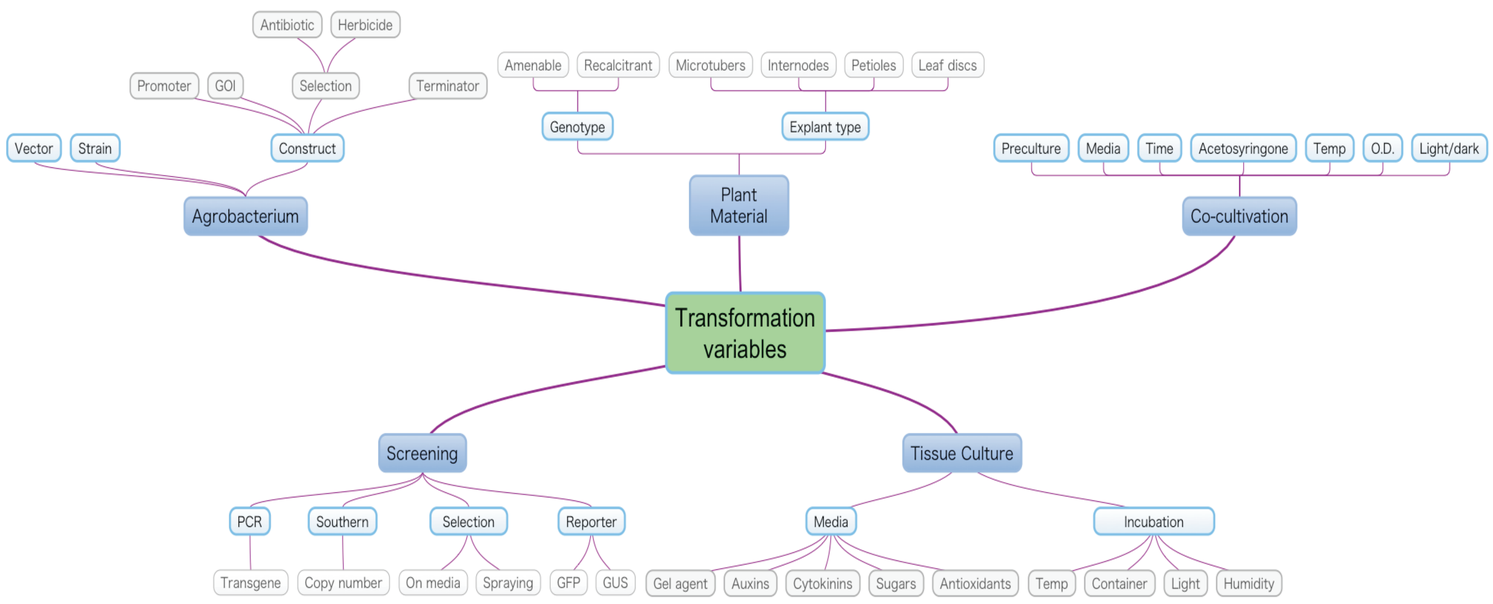

Figure 1: Some of the many variables that can affect the efficiency of transformation experiments

Explant material

Most explant material from Desiree is suitable for transformation (Fig. 2a-c). We have tested internodes, petioles, roots, leaf discs and microtuber discs. All have been effective. We are currently testing the efficiency of regeneration following transformation of microtuber discs without a callus phase (Fig. 2d-f).

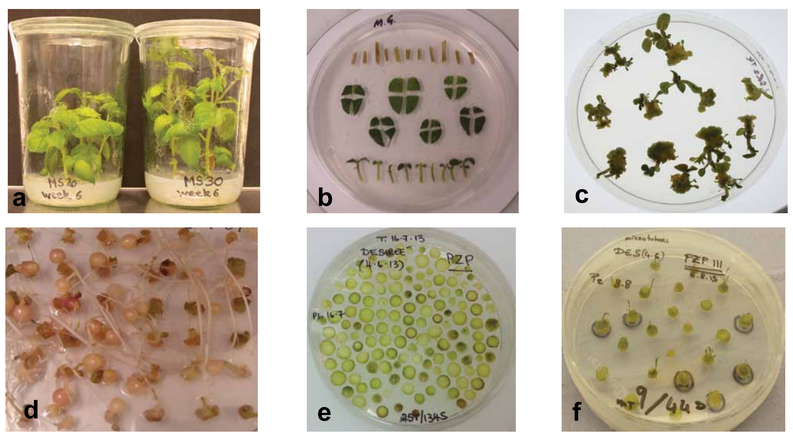

Figure 2: Different types of potato explants used for transformation a. stock plants in tissue culture, b. explant material, c. regenerating shoots on callus, d. microtubers, e. microtuber discs, f. regenerating shoots on microtuber discs.

Recovery following Agrobacterium infection

Most plant species are susceptible to Programmed Cell Death and necrosis following infection with *Agrobacterium*, particularly recalcitrant cultivars such as Mayan Gold. To combat this we have tested a range of compounds (including cell death inhibitors and antioxidants) to promote recovery and regeneration of shoots following *Agrobacterium* infection. We have found a higher percentage of shoot production following co-cultivation with media containing antioxidant compounds (Fig. 3). These, and other compounds are still being tested.

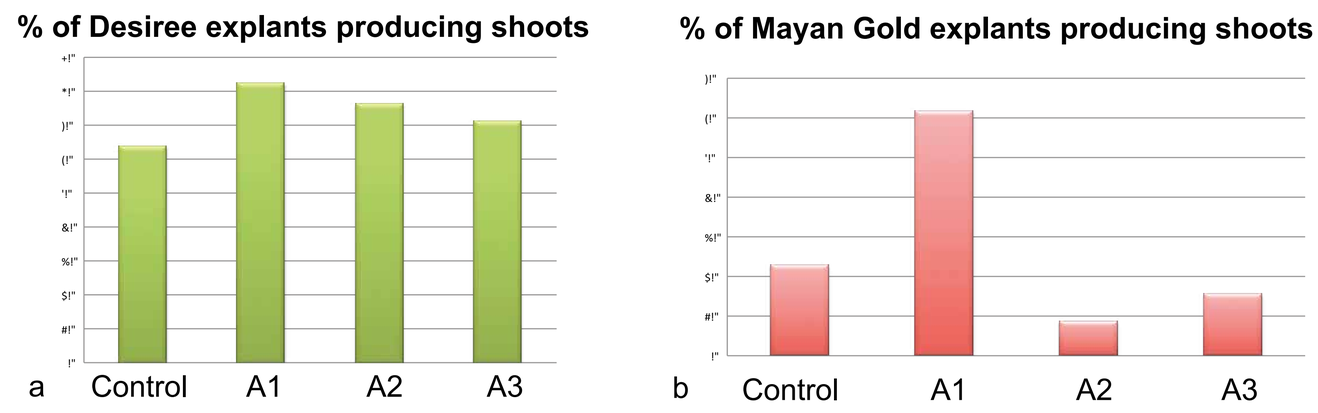

Figure 3: Effect of antioxidants on shoot regeneration. Percentage of a. Desiree, and b. Mayan Gold explants surviving *Agrobacterium* infection to regenerate shoots following co-cultivation with 3 antioxidant compounds (A1-A3). Control with no antioxidant present.

Selection gene

Kanamycin is the standard selection for potato. The relative efficiency of recovering transgenic potato lines with a selectable marker gene can be summarized as:
kanamycin resistance > **hygromycin resistance** > **phosphinothricin resistance** > **phleomycin resistance** > **methotrexate resistance** (ref. Tony Conner).

Agrobacterium strains

Since using AGL1 competent cells for our Desiree transformations we have seen a dramatic increase in efficiency in all our experiments. We are now using AGL1 with all other cultivars.

Confirmation of transgenics

All explants and shoots are maintained on selection media throughout an experiment and tested by polymerase chain reaction (PCR) to confirm the presence of the transgene (Fig. 4 a). Following transformation with our test construct pPZPIII-GUS (Fig 4 b) we can see blue staining in various tissues (Fig. 4 c-e) alongside a negative control (Fig. 4 f).

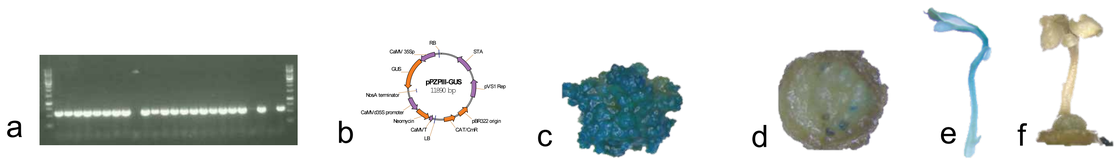

Figure 4: Confirmation of transformation a. PCR for transgene run on gel, b. map of pPZPIII vector, c-e. GUS staining in callus, microtuber disc and shoot, f. non-transformed shoot

Acknowledgements:
Pal Maliga (pPZPIII vector)

Funding: WP 5.2, Seed-corn, FUNGEN

Conclusions

Although *Agrobacterium*-mediated transformation is genotype-dependent there are a number of factors that can be altered to improve efficiency in recalcitrant cultivars. We have made significant improvements in many stages of our protocol and are continuing to optimize variables in all our current cultivars to improve the efficiency of transformation experiments.