Project number: 6808
Funding source: Science Foundation Ireland
Date: May 2018
Project dates: Jan 2016 – June 2017

Evaluating the potential of EMT to overcome genotype dependency in potato

Key external stakeholders:

Ag-biotech sector, public research and breeding organisations and seed breeding companies

Practical implications for stakeholders:

Agrobacterium-mediated transformation (AMT) is the favoured tool for engineering novel crop varieties for the ag-biotechnology industry. Although efficient, the applicability of AMT remains limited by its inability to transform a broader range of genotypes (i.e. genotype dependency, GD) in major crop species. Consequently, this can challenge the improvement of elite breeding material with additional traits of economic importance (e.g. disease resistance). Ensifer-mediated transformation (EMT) is a viable alternative to AMT that has proven effective on single varieties of monocot and dicot crop species. While EMT has broad licensing appeal across the ag-biotechnology sector, its propensity for genotype dependency within individual crop species remains undetermined. To investigate this further, this project has examined the presence/absence of EMT-related genotype dependency in potato; a crop for which AMT is highly genotype dependent.

The outcome/technology or information/recommendation is that:

- EMT has significantly less GD when applied to potato than AMT

Main results:

- EMT technology has the potential to be a technology that can transform a broader range of potato varieties than its current market equivalent, AMT

Opportunity / Benefit:

In contrast to AMT where the prevalence of GD for users is a constant technical limitation that impedes the genetic enhancement of a broad range of varieties, output from this project indicates that EMT is not impeded by GD to the same extent as AMT. The impact of this research is significant as GD remains the primary disadvantage of AMT.

Collaborating Institutions:

n/a

Teagasc project team: Dr. Manuel LopezVernaza (PD), Dr. Manfred Klaas (PD)
Dr. Ewen Mullins (PI)

1. Project background:

The motivation behind this proposal is driven by the conclusions of an independent 'Go To Market' assessment recently completed on Ensifer Mediated Transformation (EMT). Based on one-to-one interviews with market stakeholders, this study concluded that EMT has the potential to be broadly licensed across the

Contact Ewen Mullins Email: ewen.mullins@teagasc.ie.

ag-biotechnology sector. To facilitate this however, it was determined that the level of genotype dependency (if any) with EMT relative to that of AMT, the market competitor, must first be quantified. AMT has confirmed genotype dependency across crop species, which implies in the case of potato for example that AMT efficiencies can vary from 40% down to a negligible 0.2% dependent on the variety chosen. With AMT, this leads to breeding companies employing conventional techniques to transfer traits from an engineered variety into elite commercial lines; as opposed to being able to simply engineer the elite line in the first place. The Go To Market strategy identified the phenomenon of GD as a key driver for any alternative platform to possess, in order to effectively challenge AMT's market share. Hence, the goal of this project was to comparatively assess the potential for genotype dependency with EMT v. the established levels from AMT on selections of elite varieties.

2. Questions addressed by the project:

- Complete a comparative assessment of the potential for GD between AMT and EMT on a range of elite potato varieties

3. The experimental studies:

The generation of *in vitro* potato tissue culture plantlets is required to commence both the AMT and EMT process of genetic transformation. To achieve this 2 – 3 cm long sprouts were taken from potato tubers, surface sterilized and cultured on propagation medium at 24 ˚C under a 16 h/8 h light/dark cycle. Plantlets that originate from these segments are generally established for 5 – 6 weeks before use in transformation experiments. A total of 10 potato varieties were examined, including Homeguard, Orla, Setanta, Cara, Estima, Shannon, British Queen, Desiree, Bikini, Sarpo Mira.

Transformation was performed on 6 week old aseptic plants by cutting internodal (0.3 – 0.5 cm long) segments longitudinally and incubating them in appropriate incubation medium overnight at 24 ˚C in dark. On the day of infection, single colony derived cultures of *E. adhaerens* OV14 and *A. tumefaciens* each supplemented with the pC5105 vector (E5105 and A5105 respectively) were prepared for infection. For transfection, explants were inoculated with a bacterial suspension for 45 min with gentle shaking in co-cultivation medium, blot dried on sterile filter paper and transferred to callus induction supplemented with acetosyringone which stimulates the induction of the requisite virulence genes. Post co-cultivation (3 days for AMT, 5 days for EMT), explants were washed in media containing the antibiotic cefotaxime by gentle shaking for 45 min, and dried on sterile filter paper.

To determine the propensity of transient transformation, the activity of β-glucuronidase in putative transgenic plants was visualised by treating tissues in GUS solution (50 mM sodium phosphate buffer pH 7.0, 1 mM EDTA, 0.1 mM of ferri- and ferro-cyanide, 0.1 % SDS, 40 mM X-Gluc) and incubating at 37 ˚C for at least 24 h. To remove the masking effect of chlorophyll material was trimmed before washing in 90 % (v/v) ethanol. If the tissue is amenable to EMT/AMT transformation, GUS activity will be recorded in the transformed tissues indicating the successful transfer of the GUS gene into the plant cells. Transient transformation was recorded as the number of treated explants showing signs of GUS activity relative to the total number of explants treated per each potato genotype x bacteria treatment (A5105, AMT v. E5105, EMT).

4. Main results:

The transient transformation experiments conducted in this study indicated the ability of *E. adhaerens* to infect and transiently transform a larger number of potato genotypes than *A. tumefaciens*. This indicates that EMT has a lower GD potential than AMT, based on the conditions and protocols used in this study and is in effect genotype independent. Specifically, AMT was only recorded on 6 of the treated potato varieties (Homeguard, Orla, British Queen, Desiree and Sarpo Mira), wherein the range of GUS activity ranged from ~5% (British Queen) to >90% (Desiree).

In contrast, for EMT, GUS activity was recorded on all treated varieties, ranging from ~20% (Shannon) up to ~85% for Desiree and Homeguard. For the 4 varieties (Setanta, Cara, Estima and Shannon) that did not record GUS activity following AMT, the range of GUS expression varied from ~20% to ~40% when conducted with EMT, indicating the broader utility of EMT within potato.

Contact Ewen Mullins Email: ewen.mullins@teagasc.ie.

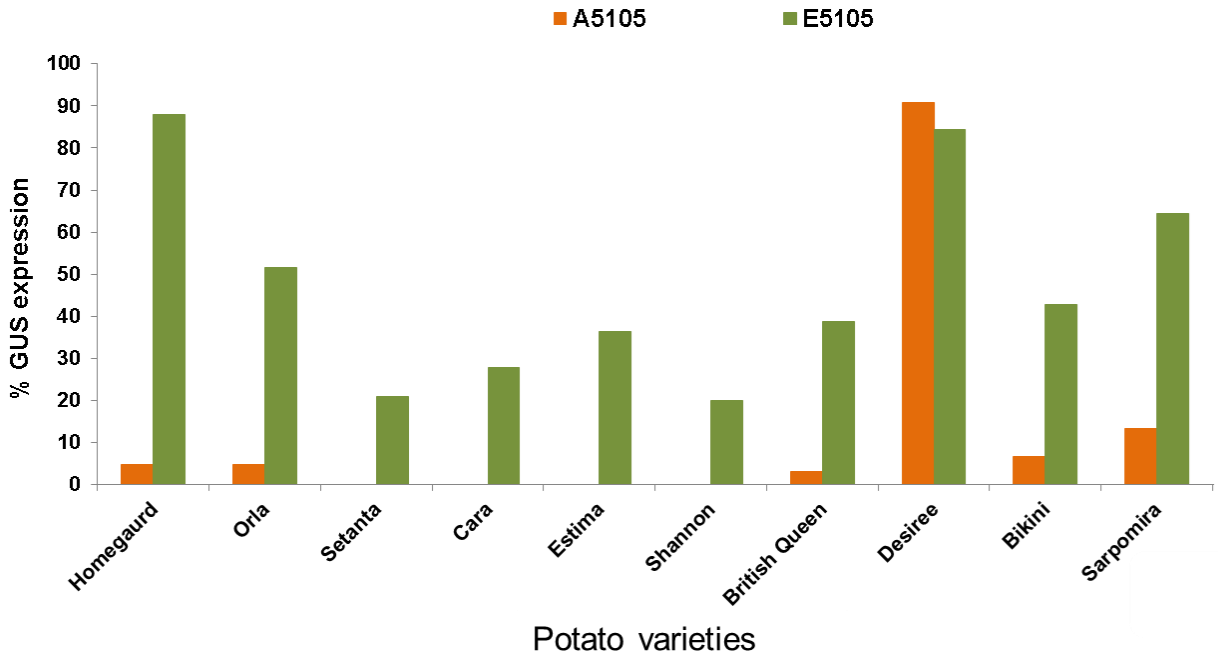

Figure 1: % GUS expression in potato tissue explants following treatment with EMT/AMT. Potato tissues were taken from aseptic cultures of up to 10 potato varieties and EMT / AMT treatments were based on a 3 day co-cultivation for AMT and 5 day co-cultivation for EMT, to ensure optimal transformation for each respective system.

5. Opportunity/Benefit:

The work completed in this project highlighted the potential of EMT to address the issue of genotype dependency within the ag-biotech sector. The current technical limitation of AMT presents an opportunity for EMT to exploit. Based on the results of this preliminary study on one crop, in contrast to AMT, EMT has a greater propensity to transform a broader range of potato varieties. This requires additional focus to determine if the high levels of genotype independency recorded with EMT, based on GUS expression, follow through to inheritance of stably transformed traits. With the ability of EMT to accommodate genome editing, the findings of this study are exciting for the future development and enhancement of potato.

6. Dissemination:

n/a

Main publications:

n/a

Compiled by: Dr. Ewen Mullins

Contact Ewen Mullins Email: ewen.mullins@teagasc.ie.