Haploid lines produced by gametic embryogenesis from genetically modified (GM) lines of potato cultivar 'Pito'

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Introduction

Haploid lines from cultivated potato (*Solanum tuberosum*) and other related *Solanum* species can be produced via parthenogenesis using prickle pollination with *S. phureja* IVP clones or alternatively through gametic embryogenesis using anther/microspore culture techniques. Anther culture is a technique which can now be utilised for production of haploid lines in a relatively diverse range of *Solanum* species. The haploid plants have subsequently been used in breeding programmes and genetic studies.

In the present study, genetically modified (GM) potatoes were tested for their ability to respond to anther culture.

Materials and Methods

The plant material in the present study consisted of genetically modified (GM) tetraploid lines of the Finnish cultivar 'Pito'. The cv. Pito was transformed with the P1 gene of Potato virus Y (PVY) using *Agrobacterium*. The transgenic potatoes expressed the P1 gene in sense or antisense orientation and the plants were either resistant or susceptible to PVY.

Anther culture of the transgenic potatoes was carried out by following the standard procedure (Rokka 2003).

Figure 5.
Two anther-derived dihaploid lines from cv. Pito; with a regular growth type and dwarf habit. Photo by J. Middlefell-Williams.

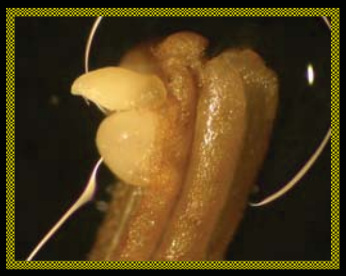

Figure 1.
Emerged microspore-derived embryo structures from cv. Pito after three weeks culture. Photo by J. Middlefell-Williams.

Figure 2.
Further developed embryos at the torpedo-stage. Photo by J. Middlefell-Williams.

Figure 3.
Green shoots regenerated from potato anthers. Photo by V-M Rokka.

Figure 4.
A dihaploid line derived from cv. Pito. Photo by J. Middlefell-Williams

Results and Discussion

Nine transgenic tetraploid lines with the P1 gene and the non-transgenic cv. Pito showed a high response to anther culture through gametic embryogenesis. Almost 28,000 anthers were cultured, and approximately 10% produced microspore-derived embryo structures (Figures 1 and 2). In total, 1,368 green plants were regenerated (Figures 3 and 4).

Flow cytometric analyses indicated that 363 (30%) of the regenerated green plants were dihaploids (2n=2x), while most the remaining plants were tetraploids (2n=4x). The plants developed were very vigourous in the greenhouse (Figure 4), but a few had a dwarf growth habit with smaller and darker leaves and a compact bushy appearance which was also typical of certain non-transgenic dihaploids (Valkonen et al. 1999) (Figure 5). This character was due to a recessive gene and linked to low endogenous gibberellin production levels in the dwarf lines.

The next aim is to study the dihaploids in terms of segregation between the transgenic virus-derived resistance character and the 'natural' PVY resistance to gain a greater understanding of the instability/silencing of virus resistance in transgenic lines.

References:

Rokka, V-M. 2003. Anther culture through direct embryogenesis in a genetically diverse range of potato (*Solanum*) species and their interspecific hybrids. In: Doubled Haploid Production in Crop Improvement. A Manual. (ed. by Maluszynski, M. et al.) Kulwer Acad. Publ. pp. 235-240.

Valkonen, J.P.T., Moritz, T., Watanabe, K.N. & Rokka, V-M. 1999. Dwarf (di)haploid *pito* mutants obtained from a tetraploid potato cultivar (*Solanum tuberosum* subsp. *tuberosum*) via anther culture are defective in gibberellin biosynthesis. Plant Science 149: 51-57.