PLANT REGENERATION FROM MESOPHYLL PROTOPLASTS OF DIHAPLOID AND TETRAPLOID POTATO LINES (*SOLANUM TUBEROSUM* L.)

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ABSTRACT

Regeneration of plants from potato (*Solanum tuberosum* L.) mesophyll protoplasts of a tetraploid cultivar Desirée and a dihaploid line 687061/1 is reported. *In vitro* grown plants were used for protoplast isolation. Two different culture protocols were applied for the mesophyll protoplasts of potato cv. Desirée using W-S-S media. The number of plants regenerated was rather low i.e. 15 plants for the first and 60 plants for the second protocol and the time needed to reach entire plants quite long for both protocols (16 and 14 months respectively). The efficiency of plant regeneration was good for the dihaploid line 687061/1 using the same media. A number of 628 plants were regenerated after 12 months in culture.

Key words: potato, mesophyll protoplasts, tetraploid cultivar, dihaploid line.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important species in the genus *Solanum* and the world's fifth important crop (Zuba and Binding, 1989). The tubers are used not only as a source of starch but also as a valuable source of proteins, rich in some essential aminoacids not found in other crops (Ross, 1986), lipids and salts. The classical breeding of potato is difficult because of its vegetative propagation. A new cultivar can be obtained only in 8-10 years after a thoughtful selection of a great number of plants (MacKay, 1987).

The new strategies of plant genetic manipulation are to be considered as a very promising alternative for potato breeding scheme. Potato somatic hybridization with wild *Solanum* species has made possible the transfer into potato germplasm of very important characters, like: resistance to viruses (*S. tuberosum* + *S. brevidens*) (Austin et al., 1985; Fish et al., 1987, 1988; Gibson et al., 1988; Pehu et al., 1990), resistance to *Phytophtora infestans* and *Globodera palida* (*S. tuberosum* + *S. circaeifolium*) (Mattheij et al., 1992) or more recently resistance to Colorado potato beetle (*S. tuberosum* + *S. chacoense*) (Cheng et al., 1995). Resistance to *Phytophtora infestans* and Colorado potato beetle are particularly very difficult, if not impossible to introduce into potato germplasm through conventional breeding. Many other techniques, like somaclonal variation, and *Agrobacterium* mediated or direct gene transfer have been also successfully used for potato genetic improvement (Ferreira and Zelcer, 1989; Rákosy-Tican and Dinu, in press).

As a first step for potato genetic manipulation through inter- or intraspecific somatic hybridization, direct gene transfer or protoclonal variation, efficient methods for protoplast isolation and culture have to be developed. Potato protoplasts have been cultured successfully for many tetraploid or dihaploid lines and for more than 30 wild species (Ferreira and Zelcer, 1989).

We report here plant regeneration from isolated mesophyll protoplasts of tetraploid potato cultivar Desirée and dihaploid line 687061/1. The dihaploid line was derived through parthenogenesis after crossing the cultivar We 38.7/49 with *Solanum phureja*. The dihaploid line was kindly provided by Dr. Traian Gorea from Potato Research Breeding Institute, Brașov. Plant regeneration from protoplasts of this line has not been previously reported.

MATERIALS AND METHODS

Mesophyll protoplasts were isolated from *in vitro* grown plants. *S. tuberosum* cv. Desirée (2n = 4x) was micropropagated on RMB_5 media (Menczel et al., 1981). The dihaploid line 687061/1 was cultured starting from tubers. Plants were grown in perlite in laboratory and nodal fragments were used to initiate *in vitro* cultures. The shoots were sterilized with 70% ethanol for 3 min., and 10% (w/v) sodium hypochlorite for 10 min. Shoot were then washed 3 times with sterile

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water. The medium MS (Murashige and Skoog, 1962) with 20 g/l sucrose, 2.5 mg/l gibberellic acid (GA_3) and 0.8% agar (pH = 5.6) was used to initiate *in vitro* cultures. *In vitro* plants were further micropropagated on MS media with 10 g/l sucrose and 0.8% agar (pH = 5.8). Potato *in vitro* cultures were maintained in a growth chamber with a photoperiod of 16 h, 25°C/23°C temperature and 4000 luxes light intensity. 3-4 weeks old *in vitro* plants were used for protoplast isolation for both tetraploid and dihaploid line.

For protoplast isolation the leaves were harvested and cut in small slices. The enzyme mixtures for protoplast isolation were in the case of cv. Desirée: 1% (w/v) Cellulase R-10, 0.25 (w/v) Macerozyme R-10 in W-S-S isolation medium (Sidorov et al., 1987), pH = 5.8, and in the case of dihaploid line: 1.5% Cellulase R-10, 0.3% Macerozyme in W-S-S isolation medium, pH = 5.5. The enzyme mixtures were filter sterilized. The incubation in the enzyme solutions was made for 18 h at room temperature for cv. Desirée, and 4 h, obscure light on a shaker at 8-10 rpm, for the dihaploid line. After isolation, the protoplast suspensions were filtered through a nylon mesh (100 μm ø) and protoplasts were floated by centrifugation at 1500 rpm, 5 min. Collected mesophyll protoplasts were washed twice with 0.5 M sorbitol or W_5 solution (Menczel et al., 1981) at 800 rpm for 3 min.

Isolated mesophyll protoplasts were cultured in liquid W-S-S medium (Sidorov et al., 1987). In the case of cv. Desirée, two different culture protocols were used (Table 1). In the first protocol the protoplast density was 2 x 10^4/ml, the first dilution of W-S-S media was made after 16 days of culture at ratio of 1:1, and two other dilutions of medium were performed in the first month of culture. After 6 weeks, the colonies were transferred to ST-1 solid medium (Shepard and Totten, 1977; Shepard, 1980). Calluses were transferred after 10 weeks to fresh medium and after another 3 months to ST-2 medium (Shepard and Totten, 1977; Shepard, 1980). The regenerated shoots were transferred to S-3 medium (Shepard and Totten, 1977; Shepard, 1980) where they developed roots.

The second culture protocol had the following changes: the enzyme solutions were prepared in CPW salts with 0.4 M sucrose (pH = 5.8) The first dilution with W-S-S media (1:1) was done after 10 days of culture, the second dilution after 2 weeks of culture with W-S-S media, containing 0.5 mg/l NAA (α - naphthaleneacetic acid) and 1 mg/l zeatine in 1:1 ratio, and 0.2 ml of the last medium was added at every 2 days for the next 3 weeks of culture. The colonies were transferred to ST-1 medium after 5 weeks of culture.

The mesophyll protoplasts of dihaploid line 687061/1 were cultured in W-S-S liquid medium at a density of 2.8 x 10^5 protoplasts/ml. The first dilution was made after 4 days at a ratio of 1:2 (old : fresh W-S-S). The second dilution with W-S-S was performed after 3 weeks of culture at ratio of 1:1. Cell colonies formed after 7 weeks of culture were transferred to ST-1 medium. The ST-2 medium was supplemented with 1 mg/l zeatine at the first transfer of calluses, and with 0.2 mg/l BA (6 - benzylaminopurine) and 0.5 mg/l GA_3 (gibberellic acid), for the second transfer. The shoots formed roots on S-3 medium.

For all culture protocols tested, protoplast cultures were maintained in diminished light at 27°-28°C. The colonies, on ST-1 medium, were transferred at light (300 luxes, 16 h photoperiod) at 25°/22°C. When secondly transferred to ST-1 medium, the calluses were further kept at 4000 luxes, 16 h photoperiod and 25°/23°C.

RESULTS AND DISCUSSIONS

Potato mesophyll protoplasts isolated from both tetraploid cv Desirée and dihaploid line 687061/1 were induced to regenerate plants. For the tetraploid cv. Desirée better results were obtained by using the second culture protocol. Although the density of the protoplast suspension was the same for both culture protocols tested for this cultivar, the second culture protocol has induced a higher plating efficiency and callus viability (Table 1, Figure 1).

The mean value for the number of plants regenerated per callus was higher in the case

Table 1. The efficiency of plant regeneration from mesophyll protoplasts of potato (*Solanum tuberosum* L.) cv. Desirée and the dihaploid line 687061/1

Cultivar or line	Protoplast density/ml	Planting efficiency (%)	Callus viability (%)	Mean number of plantles/callus	Number of regenerated plants/callus	Total number of plants regenerated
cv. Desirée protocol 1	$2x10^4$	0.18	20.8	1.5	0.1	15
cv. Desirée protocol 2	$2x10^4$	0.49	33.3	1.1	15.5	60
Dihaploid line 687061/1	$2.8x10^5$	7.5	43	5.3	149	628

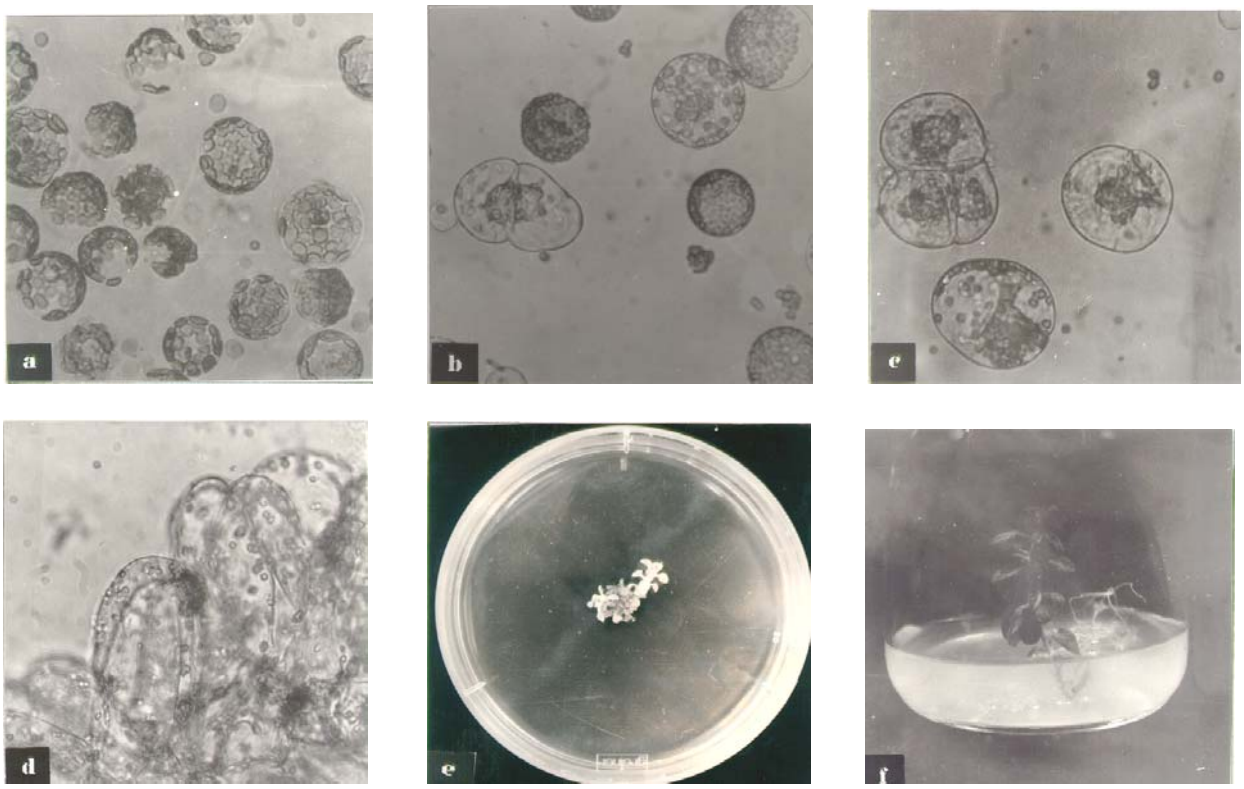

Figure 1. Plant regeneration from mesophyll protoplasts of potato dihaploid line 687061/1; a) fresh isolated mesophyll protoplasts; b) cells undergoing the first division in culture; c) cells performing the second division in culture; d) regenerated cell colonies; e) callus with plantlets; f) regenerated plant

of the first culture protocol but the total number of plants regenerated was higher for the second one (i.e. 60 regenerated plants).

The best results were obtained for regeneration of mesophyll protoplasts of the dihaploid line 687061/1 (Table 1). A total of 628 plants were regenerated, representing a mean of 0.8 plants per 1000 protoplasts originally cultured.

Plant regeneration from protoplasts was previously reported for tetraploid cultivars, including cv. Desirée and dihaploid lines derived through androgenesis or parthenogenesis after crossing with *Solanum phureja* (Ferreira and Zelcer, 1989; Zuba and Binding, 1989; Waara, 1991). For the potato cultivar Desirée, protoplasts isolated from both mesophyll (Tavazza and Ancora, 1986) or cell suspensions (Tavazza et al., 1988) were reported to regenerate entire plants. The culture media used by the authors cited above were based on MS (Marshige and Skoog, 1962) medium, and plants were regenerated during 3-4 months from cell suspension-derived protoplasts and 6 months from mesophyll protoplasts. In our experiments

plant regeneration was achieved only in 16 months for the first and 14 months for the second culture protocol. Since the efficiency of plant regeneration from mesophyll protoplasts of cv. Desirée was rather low (in our experiments) and the time needed for regeneration quite long, additional research for further improvement of the culture protocols would be necessary.

Plant regeneration from protoplasts of the dihaploid line 687061/1 was not previously reported. The efficiency of plant regeneration was very good in this line but it took 12 months from protoplasts to regenerated plants. The plating efficiency may by considered very high and closed to the values reported for other dihaploid lines (Waara, 1991). The efficiency of plant regeneration from calluses was even higher in our experiments than the values reported by Waara (1991) for other dihaploid lines. Plant regeneration from dihaploid lines is a very important step in potato intra- or interspecific somatic hybridization. The fusion of different dihaploid lines might be used in more complex potato breeding schemes as a way of tetraploidization (Wenzel, 1980). Good results in the intraspecific somatic hybridization of potato dihaploid lines were reported by Waara (1991) and Möllers and Wenzel (1991).

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