ENHANCED PRODUCTION OF DIHAPLOID LINES VIA ANTHER CULTURE OF TETRAPLOID POTATO (*SOLANUM TUBEROSUM* L. SSP. *TUBEROSUM*) CLONES

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Abstract

A total of 1000 anther-derived plants was regenerated from tetraploid potato (*Solanum tuberosum* L.) genotypes. Capacity to undergo androgenesis was analysed in 41 potato cultivars and 7 clones grown either in the greenhouse or in the field. Of the 48 genotypes, 33 produced embryos and 23 regenerated shoots from embryos. Anther-derived plantlet production was determined in genotypes 86110, Agria, Calgary, Escort, Helios, Idole, JO0982, JO1432, Kainuun Musta, Kardal, KE48, Matilda, Nicola, Petra, Pito, Rustica, Stirling, Torridon, Ute, Van Gogh, Vebeca, Vento and White Lady. The highest number of shoots (24 shoots/100 anthers) was obtained from cv. Calgary, when anthers were isolated from field-grown donor plants. Incubating anthers at 28 C, rather than at 20 C or 24 C, enhanced embryo production in four genotypes tested. However, shoot production was improved only in cv. Pito cultured at 28 C. When anthers of cv. Petra were cultured at 28 C for four weeks, followed by reduction of culture temperature to 24 C, a high rate of shoot production was recorded (14 shoots/100 anthers). The ratio between dihaploids and tetraploids varied among the anther-derived plants of the different genotypes. The number of dihaploids was highest in potato clone JO1432 (100%) and in cv. Calgary (93%) and lowest in cvs. Pito (21%) and Torridon (6%).

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Introduction

Anther culture of potato (*Solanum tuberosum* L. ssp. *tuberosum*) is potentially useful for producing dihaploid (2n = 2x = 24) lines from tetraploid (2n = 4x = 48) cultivars. Potato breeding at the diploid level has become attractive to simplify both breeding programs and genetic analyses of breeding material (12). Conventional potato breeding at the tetraploid level demands an evaluation of a large number of seedlings and is consequently very time consuming (21).

Successful production of dihaploids is the first step in analytical breeding of potato at the diploid level (3). In most cases dihaploids have been obtained through pollination of potato with selected clones of *Solanum phureja* Juz. & Buk. (10). In this method dihaploids develop from unfertilized ovules by parthenogenesis (9), or from fertilized triploid zygotes by chromosome elimination (4). However, the exact mechanism of production of dihaploids with *S. phureja* remains unclear (4). Often this method produces aneusomatic dihaploids, which contain *S. phureja* DNA (30) and may have variable chromosome numbers (4). Thus, sporadic appearance of undesirable *S. phureja* characteristics is possible in the potentially useful dihaploid breeding lines.

An alternative way to produce dihaploids is through androgenesis. Although progress has been made in anther culture of dihaploid *S. tuberosum* lines (7, 27) and in wild or primitive cultivated potato species (11, 19), tetraploid potato cultivars have not, however, responded well to anther culture and the response has been restricted to only a few genotypes (31). Dunwell and Sunderland (6) were the first to report plantlet regeneration from anther culture of *S. tuberosum* cv. Pentland Crown, and Mix (16) obtained calli and a few dihaploid shoots from tetraploid potato genotypes. Johansson (13) tested the androgenic capacity of 11 cultivars, five of which produced dihaploids. Uhrig and Salamini (28) obtained many anther-derived plants from selected embryogenic potato clones but commercial cultivars and most of the tetraploid breeding lines were non-embryogenic. Tiainen (25) reported androgenic response and plantlet formation of five *S. tuberosum* cultivars.

To enhance success in anther culture, both physical and chemical factors must be optimized. The effect of incubation conditions of potato anthers in culture has been studied by Calleberg and Johansson (1). They reported that potato genotypes had different androgenic capacity at different incubation temperatures, but direct regeneration of shoots was greatest at 20 C. Calleberg *et al.* (2) reported also that the highest embryo yield in most of the genotypes tested was obtained at 25 C, rather than at 20 C.

The ploidy of anther-derived potato has been reported to vary extensively. Regenerated plants have either been haploid, diploid, mixoploid or polyploid (13, 15, 25).

The objective of this study was to extend the applicability of producing dihaploid lines by anther culture to several *S. tuberosum* cultivars. The effect of temperature on anther-derived embryo and shoot production, as well as on the ploidy distribution of the anther-derived plants, was also studied.

Materials and Methods

Plant Material

Twenty-five tubers of each genotype (Table 1) (provided from Boreal Plant Breeding, Jokioinen, Finland) were sown in 21 cm diam. pots in the greenhouse in each experiment. The experiments were carried out during 1992-1994. The anther donor material was cultured under a photoperiod of 18 h (high pressure sodium lamps Sylvania SHP-T-400) at temperatures ranging from min 14 C (night) to max 26 C (day). In summer the photoperiod was not controlled. Altogether 40 tetraploid potato clones (Table 1), of which 34 were commercial cultivars, were tested for androgenic response. In the summer of 1994, seventeen clones (Table 2) (including nine genotypes not previously tested in the greenhouse) were planted in the field for use as anther donors.

Anther Culture

Flower buds, 4-7 mm in length, were harvested and surface-sterilized as previously reported by Tiainen (26). Ten anthers were plated per 60 mm diam. Petri dish that contained Murashige and Skoog (MS) basal medium (17) solidified with 6% (w/v) wheat starch (Sigma Co.) and supplemented with 6 % (w/v) sucrose, 0.5 % (w/v) activated charcoal, 3 mg l^{-1} BA (6-benzylaminopurine), 50 mg l^{-1} l-cysteine-HCl and 200 mg l^{-1} ascorbic acid according to Tiainen (26). Ascorbic acid was filter sterilized and added to autoclaved medium. The cultures were incubated under illumination of 25-50 µmol m^{-2} s^{-1} and a photoperiod of 16 h.

After eight weeks of culture, the numbers of embryos and shoots were assessed. The embryos, which did not develop shoots in the anther culture medium, were transferred to regeneration medium according to Wenzel and Uhrig (32). The final shoot number was recorded after 4-8 weeks.

The effect of incubation temperatures was studied over an eight week period on anther culture of 86110, Pito, Van Gogh and White Lady. Equal numbers of anthers per day were randomly distributed to each incubation temperature (20, 24 and 28 C) under a photoperiod of 16 h. Anther culture response of 86110, Calgary, Nicola, Petra, Pito and Van Gogh was tested by a four-week-incubation at 28 C, after which the cultures were transferred to 24 C. The embryos which had not developed shoots, were transferred to regeneration medium (32). Twenty anthers per plate were incubated in these experiments. The numbers of embryos were assessed after eight weeks, and the final shoot number was recorded 4-8 weeks after the transfer to

TABLE 1.—*Anther culture capacity of 40 tetraploid potato clones.*

Clone (origin *)	Anthers plated	Total no. of embryos formed	Embryos/ 100 anthers	Total no. of shoots regenerated	Shoots/ 100 anthers
86110 (HUN)	240	2	1.0	1	0.4
Agria (D)	1370	3	0.2	1	0.1
Ailsa (GB)	150	0	0.0	0	0.0
Amati (NL)	160	0	0.0	0	0.0
Arinda (F)	240	0	0.0	0	0.0
Asterix (NL)	630	0	0.0	0	0.0
Aurora (NL)	350	5	1.4	0	0.0
Binella (NL)	630	0	0.0	0	0.0
Bintje (NL)	290	0	0.0	0	0.0
Brodick (GB)	210	1	0.5	0	0.0
Calgary (NL)	620	279	45.0	33	5.3
Concorde (NL)	500	1	0.2	0	0.0
Element (NL)	440	3	0.7	0	0.0
Escort (NL)	1120	15	1.3	2	0.2
Fambo (NL)	700	0	0.0	0	0.0
Fresco (NL)	390	1	0.3	0	0.0
Hertha (NL)	1290	0	0.0	0	0.0
Idole (NL)	340	1	0.3	0	0.0
Jaakko (FIN)	290	0	0.0	0	0.0
JO0982 (FIN)	310	1	0.3	1	0.3
JO1112 (FIN)	630	0	0.0	0	0.0
JO1405 (FIN)	1140	2	0.2	0	0.0
JO1432 (FIN)	720	43	6.0	12	1.7
JO1460 (FIN)	500	0	0.0	0	0.0
Kardal (NL)	690	31	4.5	10	1.4
Lady Rosetta (NL)	1490	1	0.1	0	0.0
Matilda (SWE)	710	9	2.0	2	0.3
Nicola (D)	950	19	2.0	12	1.3
Osmo (FIN)	210	2	1.0	0	0.0
Petra (D)	160	19	11.9	4	2.5
Pito (FIN)	5090	181	3.6	87	1.7
Provita (NL)	130	0	0.0	0	0.0
Puikula (FIN)	220	3	1.4	0	0.0
Saturna (NL)	400	1	0.3	0	0.0
Torridon (GB)	2340	87	3.7	50	2.1
Ute (D)	210	3	2.7	1	0.5
Van Gogh (NL)	1740	295	17.0	89	5.1
Vebeca (NL)	1030	9	0.9	1	0.1
Vento (NL)	160	3	1.9	1	0.6
White Lady (HUN)	430	34	7.9	16	3.7
total	29220	1054	3.6 (mean)	323	1.1 (mean)

*Boreal Plant Breeding, Jokioinen, Finland. Potato catalogues: NIAB 1994, EAPR 1985, Carlsson, H. Potatissorter i svensk produktion 1991, SAC Potato Varieties 1989, UPOV.

TABLE 2.—*Anther culture capacity of potato clones grown in the field.*

Clone (origin *)	Anthers plated	Total no. of embryos formed	Embryos/ 100 anthers	Total no. of shoots regenerated	Shoots/ 100 anthers
Brodie (GB)	100	0	0.0	0	0.0
Calgary (NL)	153	105	68.6	37	24.2
Darwina (NL)	180	0	0.0	0	0.0
Element (D)	100	4	4.0	0	0.0
Helios (D)	100	8	8.0	4	4.0
Idole (NL)	100	5	5.0	2	2.0
JO1432 (FIN)	160	0	0.0	0	0.0
Kainuun Musta (FIN)	195	6	6.0	2	2.0
KE48 (HUN)	100	14	14.0	2	2.0
Matilda (SWE)	100	0	0.0	0	0.0
Pito (FIN)	420	0	0.0	0	0.0
Pompadour (F)	160	0	0.0	0	0.0
Puikula (FIN)	100	5	5.0	0	0.0
Rustica (D)	100	21	21.0	4	4.0
Stirling (GB)	160	6	3.8	1	1.0
Van Gogh (NL)	180	34	18.9	6	6.0
White Lady (HUN)	120	44	36.7	5	4.2
total	2528	252	10.0 (mean)	63	2.5 (mean)

*Boreal Plant Breeding, Jokioinen, Finland. Potato catalogues: NIAB 1994, EAPR 1985, Carlsson, H. Potatissorter i svensk produktion 1991, SAC Potato Varieties 1989, UPOV.

regeneration medium. The means and standard errors of the means were calculated.

Cytology

Chromosome counts were made from mitotic cells of root tips produced in MS medium supplemented with 2% (w/v) sucrose, 0.8% (w/v) agar and 0.1 mg^{-1} NAA (α-naphthaleneacetic acid). Chromosome numbers were examined and counted according to Tiainen (26).

Results

Androgenic Capacity of the Potato Cultivars

Twenty-eight of the 40 greenhouse-grown potato clones produced embryos in anther culture. The highest embryo yields (embryos/100 anthers) were obtained from clones Calgary (45.0), Van Gogh (17.0), Petra (11.9), White Lady (7.9) and JO1432 (6.0). From 29,220 isolated anthers 1,054 embryos were obtained. The overall mean embryo yield/100 anthers of the potato clones was 3.6 (Table 1). Seventeen genotypes (86110, Agria,

Calgary, Escort, JO0982, JO1432, Kardal, Matilda, Nicola, Petra, Pito, Torridon, Ute, Van Gogh, Vebeca, Vento and White Lady) regenerated shoots derived from embryos and a total of 323 shoots formed. Thirty-one percent of the embryos regenerated shoots, however, the genotypes differed greatly in their capacity for shoot regeneration. Most of the embryos formed shoots directly on the anther culture medium. The highest shoot yields (shoots/100 anthers isolated) were observed in cvs. Calgary (5.3), Van Gogh (5.1), White Lady (3.7), Petra (2.5) and Torridon (2.1) (Table 1).

Eleven of the 17 genotypes grown in the field, produced embryos, of which 9 clones (Calgary, Helios, Idole, Kainuun Musta, KE48, Rustica, Stirling, Van Gogh and White Lady) regenerated shoots (Table 2). JO1432, Matilda and Pito, which had been classified as responsive genotypes from greenhouse studies (Table 1) did not form embryos from anthers isolated from field-grown plants. Moreover, there was no difference in the anther culture response of field and greenhouse grown-plants of cvs. Matilda, Pito and Van Gogh (data not shown) cultured at the same time.

Influence of the Incubation Temperature

Embryo production was enhanced in each of the four potato genotypes (86110, Pito, Van Gogh, White Lady), when anthers were incubated at 28 C. The lowest embryo yields were obtained at 20 C. The mean numbers of embryos per anther were: 0.9 embryos/100 anthers at 20 C, 3.3 embryos/100 anthers at 24 C and 11.9 embryos/100 anthers at 28 C (Table 3).

Shoot formation was not affected as much as embryo production by the incubation temperature. Only for cv. Pito, shoot formation was ten times greater at 28 C than at 20 C. Overall, the highest percentage of shoot formation per embryo occurred at 20 C (56%) and the lowest at 28 C (25%). Some brown undeveloped embryos were obtained at 28 C and the high temperature had a negative effect on shoot regeneration (Table 3).

The best shoot regeneration of cv. Petra was obtained when the anthers were incubated for four weeks at 28 C followed by additional four weeks at 24 C. The other four potato genotypes tested under this temperature regime did not give a better embryo or shoot yield than was previously obtained at 24 C or 28 C (Table 4).

Cytology

Chromosome counts of the anther-derived plants were determined for 931 regenerants: 516 (55.4%) plants were diploid, 408 (43.8%) were tetraploid and the remainder (0.8%) were either triploid, hexaploid, octoploid or mixoploid. The number of dihaploids obtained was genotype-depen-

TABLE 3.—*Effect of incubation temperature on anther culture response in tetraploid potato clones.*

Clone	Incubation temperature	Anthers plated	Total no. of embryos formed	Embryos/ 100 anthers	Embryos/anther mean (±SE)*	Total no. of shoots regenerated	Shoots/ 100 anthers	Shoots/anther mean (±SE)*	(%) Embryos developed into shoots
86110									
	20 C	110	0	0	0.00 (±0.00)	0	0	0.00 (±0.00)	0
	24 C	110	0	0	0.00 (±0.00)	0	0	0.00 (±0.00)	0
	28 C	110	5	4.5	0.05 (±0.08)	1	0.9	0.01 (±0.03)	20
Pito									
	20 C	1260	6	0.5	0.00 (±0.01)	5	0.4	0.00 (±0.01)	83
	24 C	1240	28	2.3	0.02 (±0.02)	10	0.8	0.01 (±0.01)	36
	28 C	1300	89	6.8	0.07 (±0.03)	49	3.8	0.04 (±0.03)	55
Van Gogh									
	20 C	310	10	3.2	0.03 (±0.04)	5	1.6	0.02 (±0.03)	50
	24 C	320	18	5.6	0.06 (±0.09)	3	0.9	0.01 (±0.02)	17
	28 C	240	61	25.4	0.25 (±0.02)	3	1.3	0.01 (±0.02)	5
White Lady									
	20 C	260	2	0.8	0.01 (±0.02)	0	0	0.00 (±0.00)	0
	24 C	240	17	7.1	0.07 (±0.08)	7	2.9	0.03 (±0.05)	41
	28 C	210	67	31.9	0.32 (±0.30)	2	1.0	0.01 (±0.02)	3
TOTAL									
	20 C	1940	18	0.9	0.01 (±0.01)	10	0.5	0.01 (±0.01)	56
	24 C	1910	63	3.3	0.03 (±0.02)	20	1.1	0.01 (±0.01)	32
	28 C	1860	222	11.9	0.12 (±0.05)	55	3.0	0.03 (±0.02)	25

*SE standard error

TABLE 4.—*Anther culture in incubation at 28 C for four weeks followed by a transfer to 24 C.*

Clone	Anthers planted	Total no. of embryos formed	Embryos/ 100 anthers	Total no. of shoots regenerated	Shoots/ 100 anthers
86110	420	11	2.6	1	0.2
Nicola	460	8	1.7	3	0.7
Petra	1360	nd.*	nd.*	186	13.7
Pito	720	24	3.3	11	1.5
Van Gogh	520	42	8.1	6	1.2
total	3480			207	5.9 (mean)

*embryo production in cv. Petra not determined

dent (Fig. 1). The largest proportion of tetraploid plantlets was obtained in cv. Torridon, for which nearly all regenerants (94%) were tetraploid. In contrast, nearly all of the anther-derived plants of JO1432, Calgary, White Lady and Van Gogh were dihaploid. Of the anther-derived plantlets of cv. Petra, which produced the highest number of shoots, 68% were dihaploid and 32% tetraploid.

Discussion

More than 500 dihaploid plantlets were produced by anther culture of 23 tetraploid potato genotypes. This is the first study where androgenic capacity has been extended to many commercially important cultivars. Successful application of anther culture to such a wide range of potato cultivars increases the importance of this technique for potato improvement.

Genotype is one of the most significant factors affecting anther culture efficiency. In potato, Uhrig and Salamini (28) demonstrated that potato cultivars and 4x breeding clones rarely regenerated more than a few embryos through androgenesis. However, some of the 4x clones had superior androgenic efficiency and this potential was transmitted to the F_1 progeny. It was suggested that a dominant gene acted in favor of androgenesis (28). Singsit and Veilleux (22) suggested that androgenic competence and regeneration into plants were under independent genetic control and that the genes for regeneration of embryos act only in the presence of the androgenic genes. Sonnino *et al.* (23) assumed that androgenic ability in potato was controlled by several genes. Manipulation of environmental conditions surrounding the anther donor plants and controlling physical and chemical factors during anther culture can increase the number of geno-

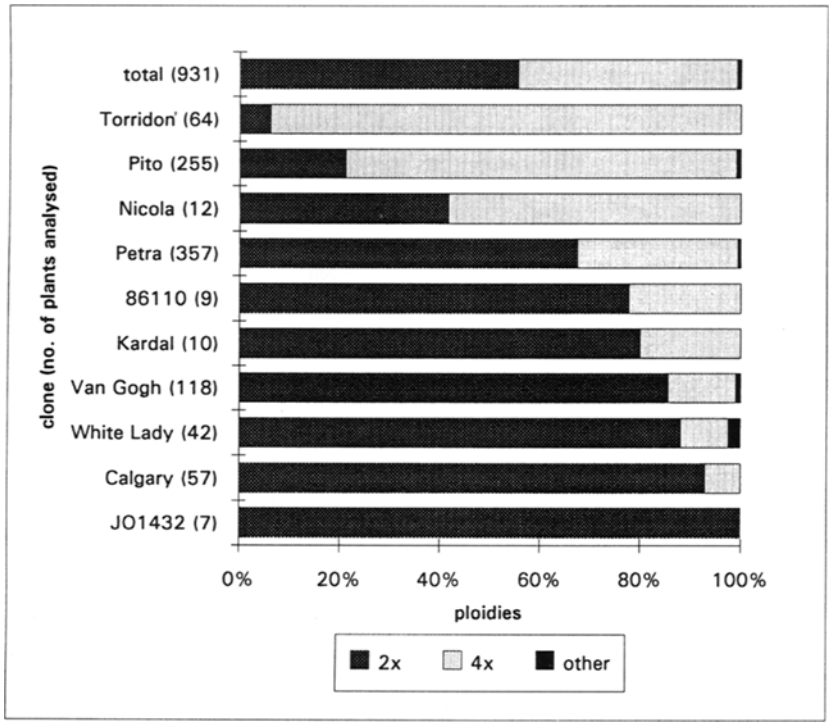

FIG. 1. Frequency of dihaploids, tetraploids and other ploidies (triploid, hexaploid, octoploid or mixoploid) of anther-derived regenerants among ten tetraploid potato clones.

types which respond. Moreover, Johansson (13) argued that in potato some inhibitory metabolites produced during incubation of anthers might prevent embryo formation and methods to diminish their effects might increase embryogenesis.

In anther culture of potato, genotypes have been divided into unresponsive, moderately responsive and highly responsive categories (24). However, the capacity for embryo formation depends on the season (25), physiological conditions of the donor plant tubers, light intensity and photoperiod and changes of temperature during flower bud formation. In our work, for example, we observed that moderately responsive genotypes can become highly responsive in subsequent experiments or vice versa (unpublished results). Some of the unresponsive cultivars tested in this study may have androgenic competence when the conditions are changed. However, the genotypic limitation of embryogenic pollen grain formation can be manipulated only within limits imposed by the genotype itself (8).

Incubation of anthers at high temperatures (28 - 32 C) increases anther culture response in tobacco (5) and rape (14). Also in potato, embryo production was higher in most of the genotypes incubated at 25 C and 30 C, but direct regeneration into shoots was not stimulated (1). In this study the embryo production was enhanced when anthers were incubated at 28 C. However, shoot production was only improved in cv. Pito. The highest number of shoots per embryo was obtained at 20 C, but the total number of shoots per anther cultured was highest at 28 C. In this study, different cultivars responded differently to incubation temperatures as reported by Calleberg and Johansson (1). The prevention of browning of the embryos and the enhancement of shoot regeneration might be solved by lowering the incubation temperature during the culture of anthers, as was shown in cv. Petra in this study.

The ratio between dihaploids and tetraploids varied among the anther-derived plants of the different tetraploid genotypes. Similarly, ploidy of anther-derived plants has been shown to vary among regenerants of dihaploid donor plants (15). In diploid *Solanum* species, unreduced gametes originating from restitution mechanisms are common (29). Dyads have also been found in many potato cultivars (18) and formation of dyads rather than tetrads may be the reason for a high rate of tetraploids derived from cv. Torridon. However, unreduced microspores are not the only reason for the unexpected ploidies. Pijnacker *et al.* (20) demonstrated that nuclear fusion, endoreduplication and endomitosis are also possible during the regeneration phase.

In conclusion, this study reports promising results for the applicability of anther culture to tetraploid potato cultivars. In the future anther culture can be used together with *S. phureja* pollinations for production of dihaploid lines in potato improvement programs.

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