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Use of 2n pollen-producing triploid hybrids to introduce tetraploid Mexican wild species germ plasm to cultivated tetraploid potato gene pool*

K.D. Adiwilaga and C.R. Brown**

U.S. Department of Agriculture, Agricultural Research Service, Irrigated Agricultural Research and Extension Center, RR 2 Box 2953A, Prosser, WA 99350-9687, USA

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Summary. Tetraploid (2n = 4x = 48) 2EBN Mexican wild species in the series Longipedicellata, which consists of *Solanum fendleri*, *S. hjertingii*, *S. papita*, *S. polytrichon*, and *S. stoloniferum*, were crossed with two 2EBN cultivated diploid (2n = 2x = 24) clones. The resulting triploid hybrids (2n = 3x = 36) produced 2n pollen (triplandroids) by the mechanism of parallel orientation of anaphase II spindles. The percentage of stainable pollen in 520 triploids ranged between 0 and 23.5%, with a mean of 2.7%. Triploids producing between 13.0 and 23.5% stainable pollen were crossed as staminate parents to the tetraploid cultivars, resulting in abundant pentaploid (2n = 5x = 60) and near-pentaploid hybrid progeny. Crosses of triploids with lower percentage of stainable pollen as pollen parent to the tetraploid cultivars did not yield fruit, unless rescue pollen from a tetraploid cultivar was added 2 days later. Pentaploid hybrids were selected among selfed tetraploid progenies using morphological and isozyme markers transmitted from their cultivated diploid parents. These pentaploid hybrids were vigorous and had uniformly sterile pollen. They were female fertile and were crossed with tetraploid cultivars, yielding an average of 19 seeds per fruit. Triplandroids provide the opportunity of transferring 2EBN tetraploid Mexican wild species in the series Longipedicellata germ plasm into the 4EBN cultivated potatoes.

Key words: Germ plasm enhancement – Interspecific hybrids – Rescue pollen – *Solanum* – Triplandroids

Introduction

Wild potato species have long been used in breeding programs as sources of valuable traits for improvement and to enrich the genetic base of the cultivated potato (Hawkes 1958; Glendinning 1979; Ross 1986). Resistances to potato virus Y, potato leaf roll virus, and frost were introduced from *Solanum etuberosum* (Hermsen 1980; Brown 1984a; Chavez et al. 1988a, b), late blight resistance from *S. verrucosum* (Abdalla and Hermsen 1973), and potato virus X resistance from *S. acaule* (Von Wangenheim 1954). Wild potato species in the series Longipedicellata, which consist of the tetraploid species (2n = 4x = 48) *Solanum fendleri*, *S. hjertingii*, *S. polytrichon*, *S. papita*, and *S. stoloniferum*, are potential sources of valuable traits for potato breeding. *Solanum fendleri*, *S. hjertingii*, *S. polytrichon*, and *S. stoloniferum* harbor resistance to latte blight (Magoon and Cooper 1959; Hawkes 1958; Ross 1958; Ramanna and Abdalla 1970; Van Soest 1985). *Solanum stoloniferum* provides potato virus Y immunity (Magoon and Cooper 1959; Brown 1984a; Ross 1958). *Solanum hjertingii* has been found to lack enzymatic browning of tubers (Woodwards and Jackson 1985).

Valuable characters present in wild species are useful for potato breeding only when they can be expeditiously transferred to potato cultivars. The first step in conventional introgression of wild species genes requires production of F_1 hybrids. Thereafter, brackcrossing is employed to insert valuable wild genes into the cultivated gene pool while accumulating desirable cultivated traits. The type of backcrossing employed in introgression breeding of potato typically utilizes a group of unrelated breeding clones with advanced horticultural traits as a recurrent parent pool (Plaisted 1980).

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** Corresponding author

Crosses between the wild species in the series Longipedicellata and the tetraploid cultivars have not been successful (Magoon and Cooper 1959; Woodwards and Jackson 1985; Van Soest 1985). The tetraploid Mexican wild species were assigned an Endosperm Balanced Number (EBN) of 2, whereas cultivated tetraploid potato (*S. tuberosum* ssp. *tuberosum* 2n = 4x = 48) was assigned an EBN of 4 (Johnston and Hanneman 1982). According to the EBN hypothesis (Johnston et al. 1980), only species with identical EBN can be readily crossed. Therefore, it is expected that 2EBN cultivated diploids, such as *S. phureja* and *S. tuberosum* haploids, would cross with members of the series Longipedicellata resulting in viable triploid F_1 hybrids (Ramanna and Abdalla 1970; Van Soest 1985). The triploid hybrids produced aborted pollen and were sterile, a barrier to further use in sexual breeding programs.

The discovery of triploid hybrids between *Solanum stoloniferum* and cultivated diploids, which produced fertile 2n pollen by means of parallel orientation of spindles in anaphase II (Brown 1988), lent credence to a breeding scheme utilizing crosses between 4EBN cultivated tetraploid and 2n pollen-producing 2EBN triploids. Subsequently, it was reported that triploid interspecific hybrids of *S. acaule*, a 4x-2EBN wild species, crossed with a 2n pollen-producing cultivated diploid, produced highly stainable large pollen (Brown and Adiwilaga 1990). These triploids were crossable to cultivated tetraploids through the functioning of 2n = 3x pollen (triplandroids). The frequency of occurrence of such triploids involving wild species in the series Longipedicellata and their crossability to tetraploid cultivars to produce BC_1 is the subject of this report. Production of BC_2 by crossing BC_1 to cultivated tetraploids is also reported. This is the first example of use of triplandroids to introgress genomes of species from Longipedicellata into the cultivated gene pool.

Materials and methods

Parental materials used in this study are the tetraploid Mexican wild species *Solanum fendleri*, *S. hjertingii*, *S. polytrichon*, *S. papita*, and *S. stoloniferum*, 2n pollen-producing, cultivated diploid clones IVPB2 and DM56-4, and tetraploid cultivars 'Lemhi Russet,' 'HiLite Russet,' and 'Katahdin' (Table 1). The wild species were obtained from the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay/WI. Clone IVPB2 was selected out of a seedling progeny of seedspot marker haploid inducers, originally developed by Dr. J. G. Th. Hermsen, Institute of Plant Breeding (IvP), Agricultural University, Wageningen, The Netherlands (Hermsen and Verdenius 1973), and maintained and distributed in the United States by Dr. J. Bamberg at IR-1. Clone DM56-4 was selected from a *S. phureja*–*S. stenotomum* population and supplied by Dr. F. Haynes, formerly at the Department of Horticulture, North Carolina State University, Raleigh/NC. Clone DM56-4 harbors unique isozymes of phosphoglucoisomerase (PGI).

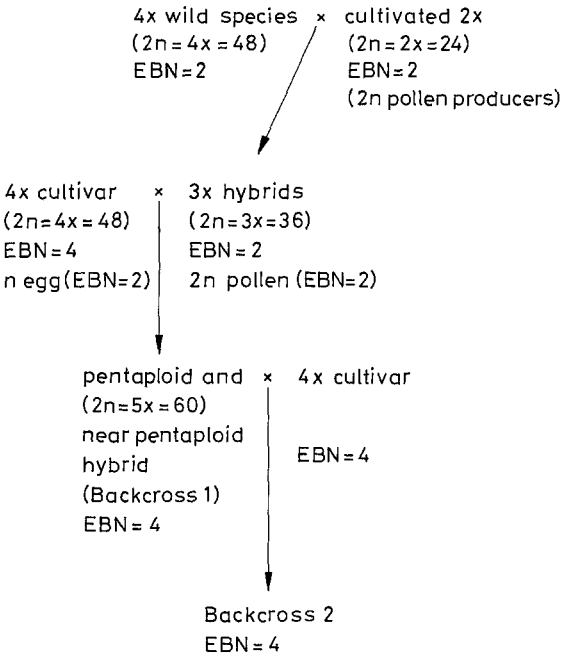

Fig. 1. Scheme of crosses for germ plasm introgression from 2EBN tetraploid species in the series Longipedicellata into 4EBN cultivated potato gene pool

Table 1. *Solanum* species and clones used in germ plasm transfer from wild species in the series Longipedicellata to cultivated potato

Wild species in the series Longipedicellata (2n = 4x = 48, EBN = 2)				
S. fendleri	*S. hjertingii*	*S. papita*	*S. polytrichon*	*S. stoloniferum*
PI 275156	PI 186559	PI 283102	PI 184773	PI 160224
PI 275161	PI 251065	PI 498031	PI 186547	PI 195164
PI 275165	PI 251067	PI 251740	PI 255522	PI 205510
PI 458418	PI 283103	PI 262895	PI 275240	PI 230477
PI 497994	PI 498019	PI 498027	PI 275241	PI 275248

Cultivated (2n = 2x = 24, EBN = 2)	
IVPB2	*tuberosum* haploid × *phureja* hybrid
DM56-4	*phureja* × *stenotomum* hybrid

Cultivated tetraploid (2n = 4x = 48, EBN = 4)
cv 'Katahdin'
cv 'Lemhi Russet'
cv 'HiLite Russet'

The scheme of the crosses is described in Fig. 1. Initial crosses involving the wild species were done with inflorescences on intact plants. More advanced crosses involving hybrid plants were done using cut stems (Peloquin and Hougas 1959) in a greenhouse maintained at 26 °C. The crossing techniques followed were described by Plaisted (1980). In some crosses between tetraploid cultivars and triploid hybrids, successful fruit set development was obtained by pollinating tetraploid flowers, first with selected triploids and then 2 days later with tetraploid

cultivar pollen. Triploid hybrids derived from crossing tetraploid Mexican wild species with the cultivated diploids were screened for the presence of 2n pollen. This was done by staining the pollen with a mixture of 1:1 2% acetocarmine w/v in 43% v/v acetic acid:glycerol for 24 h. Pollen grains were observed and counted under 400× magnification. Approximately 300 grains from each slide were counted. Grains that were uniformly stained and roughly circular in outline were scored as stained. Meiosis of pollen mother cells was observed by fixing flower buds in a mixture of 3:1,95% ethanol:glacial acetic acid v/v saturated with ferric acetate for 24 h. Fixed buds were washed and stored in 70% ethanol at 4 °C. Single anthers were macerated in a drop of 2% acetocarmine in 45% v/v acetic acid. Debris was removed, a coverslip was applied, and the slide was gently heated over an alcohol flame. Light pressure was applied and the slide was observed under 1,000× magnification.

Fluorescent microscopic observation of pollen tubes in the styles was done by fixing the pollinated stigmas and styles in FAA (1:8:1 formalin:80% ethanol:glacial acetic acid) 48 h after pollination was performed. Following fixation, they were rinsed with tap water, treated with 8 *N* sodium hydroxide aqueous for 24 h, rinsed with tap water, and stained with 0.1% aniline blue dye in 0.1 *N* K_3PO_4. Styles were then mounted in a drop of dye solution, covered, squashed gently, and observed at 100× magnification with a fluorescent microscope (Martin 1958).

The chromosome number of hybrids was counted in root-tip preparations. Root tips taken from rooted cuttings grown in coarse vermiculite were immersed in water in an ice bath for 24 h. They were fixed in 3:1,95% ethanol:glacial acetic acid for 24 h and stored in 70% ethanol at 4 °C. Root tips were hydrolyzed in 1 *N* HCl in 55 °C and then squashed in a drop of 2% acetocarmine in 45% v/v acetic acid. Chromosomes were counted under 1,000× magnification. Isozyme analyses were performed following methods described elsewhere (Quiros 1981).

Results

Solanum fendleri (fen) and *S. papita* (pta) were easily crossed with the cultivated diploids, as evidenced by a 42.6–50.3% rate of fruit set (Table 2). However, *S. hjertingii* (hjt), *S. polytrichon* (plt), and *S. stoloniferum* (sto) were more difficult to cross, with fruit set ranging from 10.9 to 28.5%. The fruits of *S. polytrichon* abscised and were shed 1–2 weeks after pollinations. However, viable seeds from all combinations were obtained.

Crosses with fen, hjt, plt, pta, and sto produced a mean seed per fruit of 25.4, 9.2, 16.8, 21.1, and 15.8, respectively. Hybrid seeds from crosses involving fen, hjt, plt, and sto were normal with fully developed embryos, while approximately 80% of seeds derived from pta was shrivelled and did not germinate. All hybrid plants derived from 4x–2x crosses had triploid (2n=3x=36) chromosome numbers.

Some triploid hybrids produced large pollen, with the size approximately twice that of normal diploid pollen and stained deeply with acetocarmine (Fig. 2). This suggested that the pollen had 2n or near 2n chromosome number and was viable. The range of percentages of stained pollen production in the triploids involving fen, hjt, plt, pta, and sto was 0–23.5, 0–6.6, 07.7, 0–7.8, and 0–13.0, respectively (Table 3).

The distribution of percentages of the stained pollen composited over all hybrid progeny is shown in Table 3. Out of 520 hybrid progeny, about 35% produced less than 1% stainable pollen. Triploids with less than 1% stainable pollen were considered as non-2n pollen producers. In addition, 29% had between 1 and 3%, 35% had between 3 and 9%, about 2% had between 9 and 12% pollen stainabilities, and 0.8% had greater than 12% pollen stainability. The overall mean of pollen stainability was 2.7%.

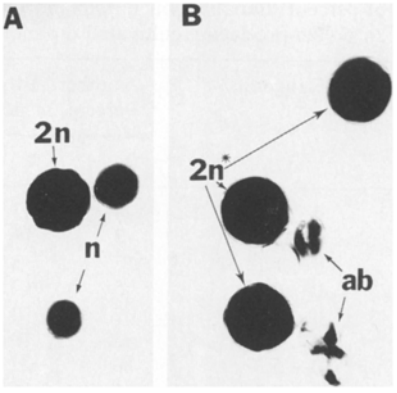

Fig. 2. **A** Stainable pollen including n=1x=12 pollen (n) and 2n=2x=24 pollen (2n) produced by the diploid parent DM56-4. **B** Stainable 2n=3x=36 (2n*) pollen from interspecific triploid hybrid *Solanum stoloniferum* × DM56-4. Aborted pollen is unstained and shrivelled (ab). The diameters of n=1x, 2n=2x, and 2n=3x types of pollen averaged 19, 26, and 29 μm, respectively. 400×

Table 2. Result of crosses between 4x wild species in the series Longipedicellata with two cultivated diploid parents, clone IVPB2 and DM56-4

Crosses	No. of pollin.	No. of fruit	Percent success	No. of seed	Seed/ fruit
fen × IVPB2	332	167	50.3	4,303	25.7
fen × DM56-4	290	136	46.9	3,413	25.1
hjt × IVPB2	685	132	19.3	848	6.4
hjt × DM56-4	550	157	28.5	1,884	12.0
plt × IVPB2	156	23	14.7	423	18.4
plt × DM56-4	331	46	13.9	700	15.2
pta × IVPB2	89	44	49.4	522*	11.9
pta × DM56-4	108	46	42.6	1,395*	30.0
sto × IVPB2	588	92	16.5	1,102	12.0
sto × DM56-4	523	57	10.9	1,119	19.6
Mean	365	90	29.3	1,571	17.7

* Many aborted seeds
fen = *S. fenderli*; hjt = *S. hjertingii*; plt = *S. polytrichon*; pta = *S. papita*; sto = *S. stoloniferum*

Table 3. Distribution of percent stainable pollen (triplandroids) produced by the triploid hybrids between wild species in the series Longipedicellata and 2n pollen-producing cultivated diploids, IVPB2 and DM56-4

Crosses	Range in %	Number of hybrid progeny Percent 2n pollen							Total
		0–1	1–3	3–6	6–9	9–12	12–15	>15	
fen × IVPB2	0–15.7	4	43	24	3	2	1	1	78
fen × DM56-4	0–23.5	7	14	31	7	1		1	61
hjt × IVPB2	0– 1.1	17	3						20
hjt × DM56-4	0– 6.6	5	12	1					18
plt × IVPB2	0– 7.7	2	10	17	6				35
plt × DM56-4	0– 4.5	3	10	6					19
pta × IVPB2	0– 5.3	60	22	5					87
pta × DM56-4	0– 7.8	61	16	30	5				112
sto × IVPB2	0– 7.3	18	10	5	2				35
sto × DM56-4	0–13.0	5	10	30	9		1		55
Total		182	150	149	32	3	2	2	520
(%) of triploids		35.0	28.8	28.65	6.15	0.6	0.4	0.4	100

Table 4. Success of pollination, number of seeds, and number of hybrids from crosses between 4x cultivars and 3x hybrids (from 4x species in the series Longipedicellata × 2x cultivated species), followed by addition of rescue 4x cultivar pollen

Crosses	No. of pollin.	No. of fruit	No. of seed	No. of hybrid
4x[c] × [(fen × IVPB2) + 4x[d]]	105	78	6,336	16[a]
4x × [(fen × DM56-4) + 4x]	194	47	6,429	3[b]
4x × [(hjt × IVPB2) + 4x]	129	12	481	0[a]
4x × [(hjt × DM56-4) + 4x]	5	2	36	3[b]
4x × [(plt × IVPB2) + 4x]	1,079	182	12,154	8[a]
4x × [(plt × DM56-4) + 4x]	431	80	3,067	13[b]
4x × [(pta × IVPB2) + 4x]	17	–	–	–
4x × [(pta × DM56-4) + 4x]	457	86	3,517	4[b]
4x × [(sto × IVPB2) + 4x]	253	52	3,184	2[a]
4x × [(sto × DM56-4) + 4x]	186	60	3,280	1[b]

[a] Hybridity was confirmed by the presence of embryo spot marker

[b] Hybridity was confirmed by the presence of unique phosphoglucoisomerase isozyme marker

[c] "4x" represents composite of crosses involving 'Katahdin,' 'Lemhi Russet,' and 'HiLite Russet' as pistillate parents

[d] The symbol "+4x" denotes use of rescue pollination

Triploids with more than 3% stainable pollen were crossed as pollen parents to tetraploid cultivars. With this method of crossing, three triploids, which also had high percentages of stainable pollen (greater than 13%), resulted in seed production. The rest of the tetraploid-triploid crosses did not result in berry development.

Fluorescent microscopic observation of tetraploid styles 2 days after pollinations by triploids with less than 13.0% stainable pollen revealed that only a few pollen tubes penetrated the entire length of styles in comparison to pollinations by cultivated tetraploid pollen. It was found, however, that when tetraploid cultivar pollen was added 2 days after tetraploid × triploid crosses, fruits were obtained. The fruits contained a mixture of tetraploid × tetraploid and tetraploid × triploid hybrid seeds. This technique of pollinating with tetraploid cultivar pollen after the desired "difficult" cross was made is similar in objective, but different in means, to embryo rescue executed through tissue culture. Tissue culture of developing embryos has been used to preserve embryos that otherwise would not have developed into viable seeds, due to developmental barriers intrinsic to the genotypes involved or due to interrupted fruit development. Based on this similarity in objective the term "rescue pollination" is introduced here.

Rescue pollination leads to mixtures of the desired tetraploid-triploid (4x-3x) interspecific hybrid and the tetraploid-tetraploid (4x-4x) noninterspecific hybrid progeny. Genetic markers were used to separate interspecific hybrids from noninterspecific hybrids. Cultivated diploid clone IVPB2 contains a dominant embryo spot marker, which was present in the triploids and transmitted to the 4x-3x hybrids. Therefore, seeds from 4x-3x crosses that had IVPB2 parentage were separated from tetraploid seeds by the presence of embryo spot marker and then planted as backcross 1 (BC_1). Clone DM56-4 has phosphoglucoisomerase (PGI) bands that differ from both the wild species and tetraploid cultivars (Fig. 3). These diagnostic PGI bands were passed to the triploids and to the 4x-3x hybrids. Seed mixtures of 4x-3x crosses that had DM56-4 in the triploid parentage and were rescue-pollinated with additional tetraploid pollen were grown in the greenhouse. Seedling mixtures were screened for the presence of this PGI marker to separate 4x-3x interspecific hybrids that had DM56-4 parentage from noninterspecific 4x-4x hybrids. The results of those

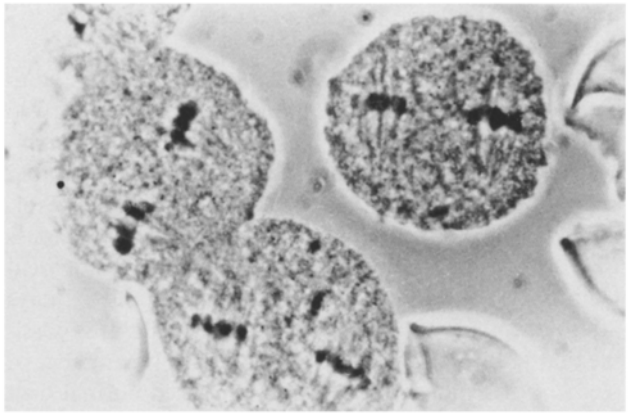

Fig. 3. Parallel orientation of anaphase II spindles in *Solanum stoloniferum* × DM56-4 triploid hybrid. 1,000 ×

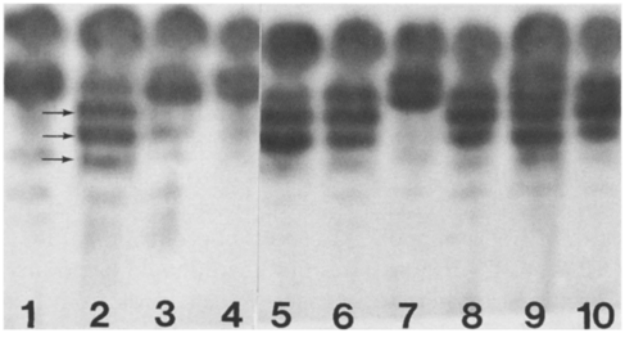

Fig. 4. Use of unique PGI phenotype derived from clone DM56-4 to select 4x–3x hybrids. *Lane 1* – 'Lemhi Russet' (4x cultivar); *lane 2* – diploid parent DM56-4, *arrows* indicate unique bands; *lane 3* – diploid parent IVPB2; *lane 4* – *Solanum stoloniferum; lanes 5* and *6* – triploid progeny from *S. stoloniferum* × DM56-4 cross; *lanes 7* through *10* – progeny from 'Lemhi Russet' × (*S. stoloniferum* × DM56-4) cross. The progeny were selected from seed from berries that were subsequently crossed with 'Katahdin' pollen to effect "rescue pollination." *Lane 7* is a nonhybrid, while *lanes 8* through *10* show the unique isozyme phenotype of DM56-4 and thus were confirmed 4x–3x hybrids

Table 5. Chromosome counts of hybrids derived from 4x cultivar × 3x interspecific hybrids, i.e., hybrids between wild species in the series Longipedicellata and 2x cultivated species

	Chromosome number			
	57	58	59	60
4x × 3x fen				10
4x × 3x hjt		1		2
4x × 3x plt	1		2	1
4x × 3x pta		1	1	2
4x × 3x sto			2	3
Total	1	2	5	18

Table 6. Success of pollination, number of seed, and number of hybrids recovered from crosses between 4x cultivars and 3x hybrids (from 4x species in the series Longipedicellata × 2x cultivated species) which produced high stainable pollen

Crosses	No. of pollin.	No. of fruit	No. of seeds	Seeds per fruit
4x × (fen × IVPB2)	271	26	119	4.6
4x × (fen × DM56-4)	223	43	210	4.9
4x × (sto × DM56-4)	42	2	14	7.0
Total	536	71	343	4.8

"4x" represents composite of crosses involving 'Katahdin,' 'Lemhi Russet,' and 'HiLite Russet' as pistillate parents

crosses employing rescue pollen are shown in Table 4. Fifty hybrids were obtained, a rate of 0.083 hybrid seeds per fruit. Chromosome number among the BC_1 plants analyzed ranged from 57 to 60 (Table 5). Out of 26 putative pentaploid plants counted, 70% had 60 chromosomes.

Four triploid progeny had high percentages of stainable pollen: 12.0, 13.0, 15.7, and 23.5%. These were derived from hybrids involving sto × DM56-4,

Table 7. Success of pollination, number of seeds, and seeds per fruits of crosses between pentaploid and near-pentaploid hybrid plants (backcross 1) with 4x cultivars

Crosses	No. of pollination	No. of fruit	Percent success	No. of seed	Seed/fruit
[4x × (fen × IVPB2)] × 4x[a]	27	4	14.8	34	8.5
[4x × (fen × DM56-4)] × 4x[a]	226	43	19.0	1,273	29.6
[4x × (hjt × DM56-4)] × 4x[a]	33	6	18.2	39	6.5
[4x × (plt × DM56-4)] × 4x[a]	24	11	45.8	415	37.7
[4x × (pta × DM56-4)] × 4x[a]	72	5	6.9	127	25.4
[4x × (sto × DM56-4)] × 4x[a]	47	39	0.8	168	3.9
Total	429	108	25.2	2,056	19.0

fen = *S. fendleri*; plt = *S. polytrichon*; pta = *S. papita*; hjt = *S. hjertingii*; sto = *S. stoloniferum*
The symbol "4x" represents a composite of crosses involving 'Katahdin,' 'Lemhi Russet,' and 'HiLite Russet' as pistillate parents
4x[a] = 'Katahdin'

fen × IVPB2, fen × IVPB2, and fen × DM56-4, respectively. The results of crosses between tetraploid cultivars and three of the triploid hybrids with highly stainable pollen are shown in Table 6. A total of 343 hybrid seeds was recovered, an average of 4.8 seed per fruit. This was a much higher level of recovery of hybrids than with triploid parents with lower pollen stainabilities, where rescue pollination was used.

Meiosis in three triploids that had 13.0, 15.7, and 23.5% was observed to determine the cytological mechanism of 2n pollen formation. Parallel orientation of the anaphase II spindles was observed in all clones (Fig. 4). Lagging chromosomes were frequently found in the second meiotic metaphase plates.

Backcross 1 plants were vigorous and uniformly male sterile with no stainable pollen. Those that flowered were pollinated with 'Katahdin' pollen, which was readily available in large quantities (Table 7). Crosses of backcross 1 involving sto and plt pentaploids crossed with 'Katahdin' were the most fecund, with success of pollination at 83.0 and 45.8%, respectively. On the other hand, crosses of backcross 1 pentaploids containing pta germ plasm with 'Katahdin' were 6.9% successful. Overall, seed were produced at a rate of 19.0 per berry.

Discussion

In the present research, the first step of germ plasm transfer from the species in the series Longipedicellata into the cultivated potato was effected by the production of triploid hybrids between the wild species and cultivated diploids which produce 2n pollen. Successful production of triploid hybrids for initial introgression of *Solanum fendleri* (Van Soest 1985; Woodwards and Jackson 1985), *S. hjertingii* (Woodwards and Jackson 1985), *S. polytrichon* (Ramanna and Abdalla 1970), and *S. stoloniferum* (Brown 1988) germ plasm has been reported. With the exception of triploids involving sto, these were sterile. The problem posed by sterility due to odd ploidy was solved by selection of interspecific triploid hybrids involving all the wild species in the series Longipedicellata, which produced fertile 2n pollen. Mendiburu and Peloquin (1976) introduced the term "triplandroid" for 2n(=3x) pollen produced by triploids. Triplandroids from 2EBN triploids are microspores having the same chromosome number as the triploid sporophyte and the same EBN level as 1n gametes from 4EBN species. Union between a triplandroid and a 1n (n=2x=24) egg from cultivated tetraploid should result in a viable 4EBN backcross 1 progeny. Therefore, the triplandroid gamete provides a direct channel for germ plasm transfer from 2EBN wild species into 4EBN cultivated potato gene pool. There should be no barrier to further germ plasm transfer from backcross 1 progeny to cultivated tetraploid potato gene pool as predicted by the EBN hypothesis.

In potato, percent pollen stainability has been used to measure pollen fertility. Triploids with 13% (derived from sto), and 15.7 and 23.5% (derived from fen) stainable pollen were directly crossable with 4EBN tetraploid cultivar, resulting in BC_1 generation (Table 6). However, crosses between triploids with pollen stainability less than 13% as pollen parent to the cultivars did not yield fruit, unless tetraploid rescue pollen was applied 2 days after tetraploid × triploid crosses were made. It is possible that tetraploid rescue pollen increased the number of seeds in the fruits, which in turn enhanced normal fruit development and prevented fruit abscission. Therefore, its effect may have been to promote normal fruit development by raising the number of developing ovules above a threshold. The availability of diagnostic markers for hybrid screening such as embryo spot (Hermsen and Verdenius 1973; Peloquin and Hougas 1959) and isozyme markers as used in this experiment is essential in using this technique. By employing rescue pollen, triploid hybrids involving all the wild species in the series Longipedicellata were crossed to the tetraploid cultivars. These results are the first report of the use of triploid-producing triplandroids to introgress these species into the cultivated 4EBN gene pool. The success of production of BC_2 seed (Table 7) demonstrates that there should be no further sexual barriers to introgression of genes from the Longipedicellata. Chromosome counts of plants derived from 4x-3x crosses revealed that 70% of them were pentaploids (2n=5x=60), indicating fertilization of a normal n=2x=24 tetraploid egg by 2n=3x=36 pollen. The remaining BC_1 population had between 57 and 59 chromosomes. This suggests the presence of aneuploid triplandroids, which might have resulted from chromosome loss due to lagging chromosomes in the second meiotic division.

Mok and Peloquin (1975) described three mechanisms of 2n pollen formation: parallel spindles, which is genetically equal to first division restitution, and premature cytokinesis I and II, which are genetically equal to second division restitution. The only restitution mechanism that leads to the formation of balanced gametes in this odd-ploidy case is parallel spindles. In the presence of second division parallel spindles, all chromosomes that were distributed by meiotic nondisjunction in an irregular fashion during the first division will unite at one pole. As a result of parallel spindles, two balanced 2n=3x=36 microspores, with the sporophytic chromosome numbers, would be formed. Tripolar orientation would lead to one balanced 2n microspore. Meiosis in odd-ploidy situations is similar in principle to meiosis in the presence of synaptic mutants as reported by Okwuagwu and Peloquin (1981) and Peloquin (1983), or to a desynaptic mutant as reported by Ramanna (1983). Synaptic or desy-

naptic mutants are characterized by the perturbation of chiasmata formation, which results in the formation of merely univalents during metaphase I. Likewise, meiotic nondisjunction leads to irregular distribution of homoeologues in pairing configurations, while parallel spindles restores balanced sets of homoeologues. Mechanisms involving second division restitution in odd-ploidy result in gametes with unbalanced chromosome numbers, since cytokinesis occurs immediately after the first meiotic division. Taking into account these arguments and the fact that only parallel spindles were observed during microsporogenesis of the triploid hybrids, it is reasonable to assume that parallel spindles is the mechanism leading to the formation of fertile 2n pollen in these triploid hybrids.

The distribution of percentage of stainable pollen (triplandroids) in the triploid hybrids was observed (Table 3). Assuming that most of the stainable pollen is 2n, it was difficult to separate the 2n pollen producers from nonproducers unambiguously, since the distribution appears to be continuous.

Male sterility in some of the triploids may be predominantly due to imbalanced chromosome partitioning during microsporogenesis. Some of the triploids exhibit tetrad sterility (data not presented). Tetrad sterility has been found in hybrids between *S. verrucosum* and *S. phureja* (Abdalla and Hermsen 1972), *S. polytrichon* and *S. phureja* (Abdalla and Ramanna 1971), and in breeding lines involving *S. stoloniferum* (Brown 1984b). Tetrad sterility was not widespread in our study and therefore is not a barrier in this breeding scheme.

Triploid potatoes that produced functional pollen have been reported previously. Mok et al. (1975) found triploids in *S. commersonii* and *S. chacoense* that produced 2n pollen by the mechanism of parallel spindles formation in anaphase II. Tarn and Hawkes (1986) reported triploid clones of *S. commersonii* that produced stainable pollen. Jackson et al. (1978) commented on potential gene flow from Andean triploid cultivars, suggested by successful seed production from crosses between triploid and tetraploid or diploid cultivars. In other crops, the use of interspecific triploid hybrids for introgression purposes also has been reported. Thomas et al. (1988) used triploid interspecific hybrids between *Lolium multiflorum* and *L. perenne* for germ plasm introgression. Dweikat and Lyrene (1988) found 2n pollen-producing triploids between blueberry cultivars and *Vaccinium elliottii*.

Sterile 2EBN triploids and the incorrect EBN of 4x-2EBN species when crossed with 4EBN tetraploids can be overcome by somatically doubling the triploid hybrids or the wild species. Doubling restores fertility of triploids and provides the correct EBN in both cases to cross with 4EBN cultivars. This was demonstrated in the use of colchicine-doubled triploids involving *Solanum acaule* (2EBN, $2n=4x=48$) by Brown et al. (1984) in crosses with 4EBN cultivated tetraploids. However, triplandroid-producing triploids have merits over colchicine-doubled allohexaploid hybrids (Brown 1988). It is postulated that species in the Longipedicellata have two genomes; one with affinity to the cultivated genome, and the other lacking such affinity (Hawkes 1981). Only pairing between homologous chromosomes is expected to occur in the allohexaploids arising from somatic doubling, while it is possible that pairing between homoeologous chromosomes will occur during microsporogenesis in allotriploids that produce 2n pollen. Homoeologous chromosome pairing should promote gene exchange across distinct genomes. Dvorak (1983) has questioned whether intergenomic pairing is under a predominant genetic influence. Citing evidence that trivalents are found in unexpectedly high numbers in interspecific triploid hybrids involving cultivated diploids as one parent, he contended that the presence of the cultivated genome alters genetic control and promotes heterogenetic pairing. It is apparent, however, that triploid hybrids, involving cultivated diploids, can be promoters of crossing-over between homoeologues, regardless of genome affinities.

Another alternative in introgression is to use 2EBN tetraploids that produce 2n eggs or 2n pollen. If a trait of interest occurs in genotypes that also express 2n gametes, this coincidence could certainly be used without resorting to triploid hybrids. Breeding of 2n gamete producers in species accessions that do not express this at the 4x-2EBN level would require crosses of clones with desired traits with 2n pollen or egg producers. Simultaneous selection of the target trait and 2n gametes could be carried out in the hybrid population over several mating and selection cycles. This type of approach, however, is likely to involve time delays. Therefore, one of the advantages of triplandroids is the easy conversion of 2n pollen nonproducers at the $2n=4x$-2EBN level to 2n pollen producers at the $2n=3x$-2EBN level by crossing with 2n pollen-producing 2EBN diploids. It is clear that the expression of triplandroids at levels above 12% stainable pollen leads to a simpler scheme of introgression than use of rescue pollination, where pollen stainability is expressed at lower levels. However, at low levels of pollen stainability rescue pollination is a viable technique, although it requires the scoring of morphological or isozyme markers to separate interspecific hybrids from mixtures.

The method of gene introgression as reported in this study appears to be a rapid avenue to transfer traits from tetraploid 2EBN wild species to the 4EBN cultivated potatoes, and it is the first report of application of this technique to species of the series Longipedicellata. Further study in our laboratory deals with the quantification of transmission of isozyme markers in the backcross generations.

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