Theor Appl Genet (1984) 68: 155–161

The use of Endosperm Balance Number and 2n gametes to transfer exotic germplasm in potato

M. K. Ehlenfeldt[1] and R. E. Hanneman, Jr[2]

Department of Horticulture[1]; USDA-ARS-NCR, Department of Horticulture[2]; University of Wisconsin, Madison, WI 53706, USA

Received December 31, 1983; Accepted January 17, 1984
Communicated by H. F. Linskens

Summary. A triploid hybrid ($2n=3x=36$) between a colchicine-induced 4x(2EBN) *Solanum brevidens* (a non-tuber-bearing species) and 2x(2EBN) *S. chacoense* (a tuber-bearing species) was used as a vehicle for germplasm transfer to *S. tuberosum* Group Tuberosum. The use of 2n gametes from the triploid allowed the unique opportunity for transferring exotic germplasm from Series Etuberosa to Gp. Tuberosum material. The triploid hybrid used had a pollen stainability of less than 0.1%. Observations of microsporogenesis revealed that metaphase I pairing configurations were primarily 12 bivalents and 12 univalents with occasional trivalents. Anaphase I separations were irregular, often with lagging univalents. Meiotic observations and pollen morphology suggest that the stainable pollen produced by the hybrid was $2n=3x=36$. A single pentaploid hybrid ($2n=5x=60$) was produced by the fertilization of a rare 2n egg from the triploid with a normal male gamete from the clone 'Wis AG 231' ($2n=4x=48$). Limited crosses to other 1, 2 and 4EBN species and cultivars were unsuccessful. The pentaploid hybrid had a more regular meiosis than the triploid and dramatically improved pollen stainability (37% stainable pollen). Stylar blocks prevented estimates of male fertility in crosses. Female fertility in 47 crosses with nine cultivars averaged 19 seeds per fruit. Although *S. brevidens* is non-tuber-bearing, and the triploid produced only stolons, the pentaploid hybrid tuberized well under field conditions, despite being very late. Results suggest that the tuberization response is a dosage and/or threshold effect. This approach to the incorporation of 1EBN germplasm indicates the utility of the EBN concept coupled with 2n gametes. Further, it demonstrates a means for the introgression of 1EBN species genes into Gp. Tuberosum material.

Key words: *Solanum* – Potato – Endosperm Balance Number – 2n gametes – Series Etuberosa

Introduction

The ease of germplasm transfer within the tuber-bearing Solanums is well recognized (Hawkes and Hjerting 1969). Hybridization between most South American diploid species can be achieved readily and the resultant offspring are usually highly fertile (Marks 1965). Genomic differentiation, even between distantly related species, appears to be minor with only small structural chromosomal differences noted (Ramanna and Hermsen 1979b).

Until recently most Mexican diploid and tetraploid species and the non-tuber-bearing diploid species of Series Etuberosa were considered to be strongly isolated from cultivated material due to crossability barriers (Hawkes and Hjerting 1969; Pandey 1962). This material represents a relatively untapped reservoir of germplasm of potential value for breeding for disease resistance, quality and environmental adaptation (Hermsen 1980; Toxopeus 1964). The species of Series Etuberosa, especially, are recognized as having virus Y, leafroll, and frost resistance (Hermsen 1980; Jones 1979; Ross and Rowe 1965).

Hermsen and Taylor (1979) reported the first fully documented hybrids of a Series Etuberosa species, *S. etuberosum*, with a tuber-bearing species, *S. pinnatisectum*, of the Mexican series, Pinnatisecta. In contrast to most other interspecific *Solanum* hybrids, these hybrids had limited meiotic pairing, averaging 3.64 bivalents per cell, and virtually complete male and female sterility (Ramanna and Hermsen 1979a). Fertility was restored by allopolyploid production (Hermsen et al. 1981), and crossing with *S. verrucosum* was possible, but questions remained regarding the compatibility of the combined *S. etuberosum* – *S. pinnatisectum* genomes with *S. tuberosum* Gp. Tuberosum material.

Johnston et al. (1980) proposed the concept of Endosperm Balance Number (EBN) as an explanation for the success or failure of both interspecific and interploidy crosses. The EBN concept expands upon work by Lin (1975) regarding genomic dosage in the endosperm, and proposes that species can be assigned "effective ploidies" (EBN numbers) which must be in a 2:1 maternal to paternal ratio in the endosperm. This ratio is necessary for both successful endosperm development and the subsequent development of hybrid embryos. These "effective ploidies" can be manipulated by chromosome doubling or reduction.

Johnston and Hanneman (1982) reported that *S. fernandezianum* (Series Etuberosa) and the diploid Mexican species *S. cardiophyllum* are 1EBN, whereas the species cross-compatible with cultivated diploids are 2EBN. Other Series Etuberosa species as well as most other Mexican diploid species have also been shown to 1EBN (Hanneman 1983).

Johnston and Hanneman (1982) produced a 3x hybrid between 4x(2EBN) *S. brevidens* and a weedy South American species, 2x(2EBN) *S. chacoense*, utilizing the knowledge of EBN. This report concerns the fertility and meiosis of this unique hybrid, the methods for its utilization, and the fertility of a subsequent hybrid with a Gp. Tuberosum cultivar.

Materials and methods

Stocks and clones used are presented in Table 1. The plant introductions *S. brevidens* PI 218228, *S. chacoense* PI 230582, 4x *S. chacoense* PI 230582 and *S. cardiophyllum* ssp. *cardiophyllum* PI 283062 were obtained from the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin. The breeding lines 'Wis AG 231', 'W726', and 'W744' represent advanced selections provided by Dr. S. J. Peloquin, Department of Horticulture, Madison, Wisconsin.

Crosses of the 3x *S. brevidens–S. chacoense* (BC) hybrid were performed on potted plants in a screenhouse at the Potato Introduction Station, Peninsular Branch Experiment Station, Sturgeon Bay, Wisconsin. All flowers were treated with a 4 ppm solution of 2,4-D one day post-pollination to assure fruit retention. Fruits were allowed to develop to maturity on the plant and after harvesting, were ripened another four weeks prior to seed extraction. Upon extraction, seeds were immediately treated with a 1,500 ppm solution of gibberellic acid for 24 h then rinsed and germinated on moistened filter paper in petri dishes. Seedlings were transferred to a potting mix after germination.

Crosses with the 5x *S. brevidens–S. chacoense*–Gp. Tuberosum (BCT) hybrid were performed in the greenhouse and field using either a cut-stem technique (Peloquin and Hougas 1959) or direct pollination on potted plants. No 2,4-D was used in these crosses. Fruit and seed were treated as described previously except that seed was dried after extraction and stored.

For meiotic analyses, buds were fixed for 48 h in a 6:3:2 solution of methanol, chloroform and 45% propionic acid (saturated with iron acetate), respectively. After fixation buds were stored in 90% ethanol or examined immediately. Macerated anther tissue was stained with a 1% acetocarmine solution and prepared for examination using the squash technique.

Pollen stainability was estimated by staining with 1% acetocarmine. A minimum of 200 grains were counted for each determination, except in cases of stainability levels less than 1%. Estimates were made for these samples.

Results

Male fertility and meiosis of the triploid S. brevidens–S. chacoense hybrid

The pollen stainability of the triploid *S. brevidens–S. chacoense* (BC) hybrid was exceptionally low (less than 0.1%). The occasional pollen grains which stained deeply with acetocarmine had diameters which aver-

Table 1. *Solanum* species and clones used in germplasm transfer between *S. brevidens* and *S. tuberosum* Gp. Tuberosum

Species or cultivar	Ploidy	Abbreviation	Identity
S. brevidens	2x	brd	PI 218228
S. chacoense	2x	chc	PI 230582
S. chacoense	4x	chc	PI 230582
S. cardiophyllum spp. *cardiophyllum*	2x	cph	PI 283062
S. tuberosum Gp. Tuberosum			
'Atlantic'	4x	tbr	cultivar
'Butte'	4x	tbr	cultivar
'Kennebec'	4x	tbr	cultivar
'Merrimack'	4x	tbr	cultivar
'Monona'	4x	tbr	cultivar
'Norgold Russet'	4x	tbr	cultivar
'Norland'	4x	tbr	cultivar
'Superior'	4x	tbr	cultivar
'Wis AG 231'	4x	tbr	cultivar
'W726'	4x	tbr	cultivar
'W744'	4x	tbr	cultivar
S. brevidens (4x) × *S. chacoense* (2x) hybrid	3x	BC	PI 218228 × PI 209411
BC × Wis AG 231	5x	BCT	parents described above

Fig. 1. Large stainable pollen grain in triploid *S. brevidens–S. chacoense* (BC) hybrid (675×)

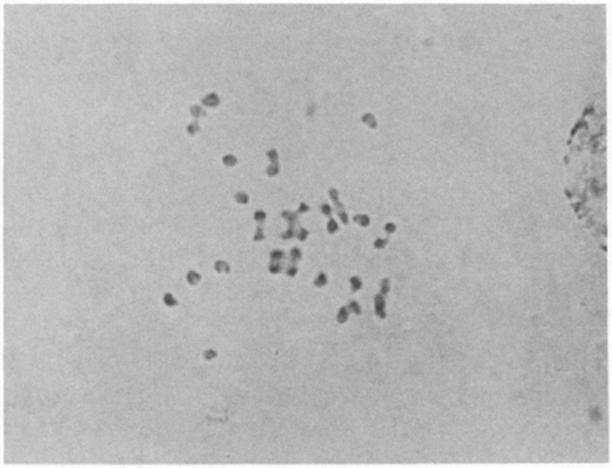

Fig. 2. Metaphase I in *S. brevidens–S. chacoense* (BC) hybrid, with trivalent and quadrivalent-like figure (1,500×)

aged 25% larger than those of typical 'n' gametes in diploids (Fig. 1). This size difference suggests that the pollen grains were of a higher chromosome number or fully 2n. The relative scarcity of these stainable grains precluded the practical testing of this plant as a male in test crosses.

Meiosis in the BC hybrid was observed to determine the basis for the exceptionally low male fertility as well as to evaluate the degree to which pairing and gene exchange might occur between non-homologous genomes. Metaphase I cells were examined for pairing configurations. The most frequent configuration observed among fully resolvable cells was 12 bivalents and 12 univalents (10 of 13 cells). In the remaining cells, trivalent or quadrivalent-like configurations could be seen (Fig. 2). Among partially resolvable cells (those having 5 or more chromosome groups resolvable) a single trivalent was observed in approximately half of the cells (13 of 28). In all cases trivalents were seen as "Y" shaped figures. Univalents occurring at metaphase I often failed to reach the metaphase plate. Those reaching the plate appeared to lack proper spindle attachments and were presumably the source of laggards seen in anaphase I.

Second division proceeded normally with the univalents remaining from the first division dividing independently and producing high numbers of micronuclei during cytokinesis. Seventy-eight percent (78%) of all pollen tetrads had micronuclei. Parallel spindle orientations occurred 35% of the time at metaphase II; however, they did not give rise to the expected levels of 2n pollen, since the overall frequency of any kind of stainable pollen was estimated to be less than 0.1%.

Female fertility of the 3x S. brevidens–S. chacoense hybrid

Endosperm Balance Number (EBN) theory requires that each female gametophyte (egg and central cell) be fertilized by a male gamete with a complementary EBN (the same number as the egg) in order for normal seed development to occur. Female fertility testing in the triploid was designed to test for the frequency of three respective EBN gamete types. *S. brevidens* and *S. cardiophyllum* served as 1EBN testers (producing ½ EBN gametes), *S. chacoense* as a 2EBN tester (1EBN gametes), and 4x *S. chacoense* and the Gp. Tuberosum cultivars 'Superior' and 'Wis AG 231' as 4EBN testers (2EBN gametes).

Only two crosses were successful (Table 2). Crosses of BC with *S. chacoense* produced three plump seeds, however none germinated. The cross with the 4x clone 'Wis AG 231' produced a single plump seed which germinated to produce a pentaploid hybrid ($2n=5x=60$). This plant presumably arose from the fertilization of a 3x(2EBN) egg by a 2x(2EBN) pollen grain.

Table 2. Results of crosses between the 3× *S. brevidens – S. chacoense* (BC) hybrid and 1EBN, 2EBN and 4EBN testers

Cross	Pollinations	Fruit	Seed
BC × brd (1EBN)	43	13	0
BC × cph (1EBN)	51	23	0
BC × chc (2EBN)	40	18	3
BC × 4x chc (4EBN)	18	0	0
BC × 'Superior' (4EBN)	34	13	0
BC × 'Wis AG 231' (4EBN)	20	13	1

Note: brd = *S. brevidens*; cph = *S. cardiophyllum;* chc = *S. chacoense*

Table 3. Results of crosses between 5× *S. brevidens* – *S. chacoense*-Gp. Tuberosum (BCT) hybrid and 4x (4EBN) Gp. Tuberosum cultivars

Cross	Pollinations	Fruit	Seed	Seed/fruit
'Kennebec' × BCT	10	0	–	–
'Norgold Russet' × BCT	13	0	–	–
'Butte' × BCT	14	0	–	–
Total	37	0	–	–
BCT × 'Atlantic'	1	1	12	12
BCT × 'Kennebec'	7	3	17	6
BCT × 'Merrimack'	10	6	67	11
BCT × 'Monona'	1	1	27	27
BCT × 'Norland'	8	4	51	13
BCT × 'Superior'	10	5	31	6
BCT × 'W726'	4	3	65	22
BCT × 'W744'	6	6	284	47
BCT Total	47	29	554	19
BCT selfed	38	23	108	5

Male fertility and meiosis of the 5x S. brevidens – S. chacoense – Gp. Tuberosum hybrid

The *S. brevidens*–*S. chacoense*–Gp. Tuberosum (BCT) hybrid was a vigorous plant strongly resembling Gp. Tuberosum although possessing a more open canopy and minor differences in inflorescence structure. The male fertility of this plant was greatly improved over the triploid hybrid. Pollen shed was comparable to normal cultivars and pollen stainability averaged 37%. Limited test-crosses, as a male, to three GP. Tuberosum cultivars resulted in no fruit (Table 3). Subsequent examination of styles by fluorescent microscopy indicated that most pollen tube growth stopped in the upper third of the style with very few tubes penetrating the entire length of the style.

In meiosis, both multivalents and univalents were observed although pairing was primarily bivalent. Estimates of multivalent and univalent frequency were not possible because of the polyploid nature of the hybrid; however, univalents were observed in all cells examined. Univalents not reaching the poles following metaphase I, underwent division at metaphase II resulting in micronuclei in 57% of the tetrads observed at telophase II. Occasional bridges were seen at metaphase I.

In this hybrid as in the triploid, moderately high frequencies (33%) of parallel spindle or parallel spindle-like orientations were seen at metaphase II (Fig. 3), however virtually no 2n pollen was formed.

Female fertility of the 5x S. brevidens – S. chacoense – Gp. Tuberosum hybrid

Crosses involving the pentaploid hybrid as a female were accomplished readily (Table 3). Seed set ranged from a low of six seeds per fruit with the cultivar 'Kennebec' to a high of 47 seeds per fruit with a Wisconsin breeding line, 'W744'. Seed set averaged 19 seeds per fruit with the cultivars and 5 seeds per fruit on selfing. Seed development ranged from seeds fully normal in size and appearance to those significantly reduced in size, plumpness or both. Preliminary tests indicated satisfactory germination of these seeds, although germination varied with seed quality.

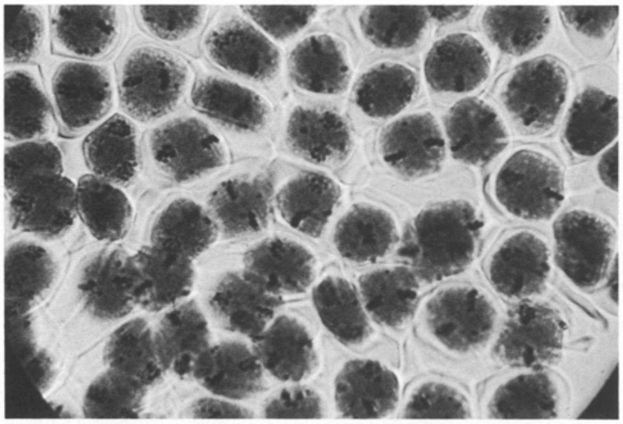

Fig. 3. Parallel spindle orientations at metaphase II in *S. brevidens*–*S. chacoense*-Gp. Tuberosum (BCT) hybrid (675×)

Tuberization of S. brevidens hybrids

The species of Series Etuberosa are strictly non-tuber-bearing, failing to produce even stolons. The BC hybrid with a two to one ratio of non-tuber-bearing to tuber-bearing genomes produced stolons readily although tubers never formed regardless of plant maturity or daylength regime. In contrast, the BCT hybrid having an increased dosage of tuber-bearing genomes (2 non-tuber-bearing to 3 tuber-bearing) tuberized under both greenhouse and field conditions. Tuberization of field grown cuttings was quite late, with tuberization not occurring until mid- to late-September.

Tuber morphology of the BCT plants differed significantly from that of typical cultivars. The plants produced thickened stolons which often further thickened, with no discernible demarcation, to form club-shaped tubers with considerable second growth. The tubers had shallow eyes with prominent eyebrows and were covered with numerous lenticles. Both skin and flesh were cream colored, although exposure to light caused the skin to darken to a purple color.

A singularly novel aspect of this plant was the appearance of subterranean flowers on the tubers. These flowers appeared to be fully normal in size, shape and color and were identical to aerial flowers except that they were borne upon greatly reduced and

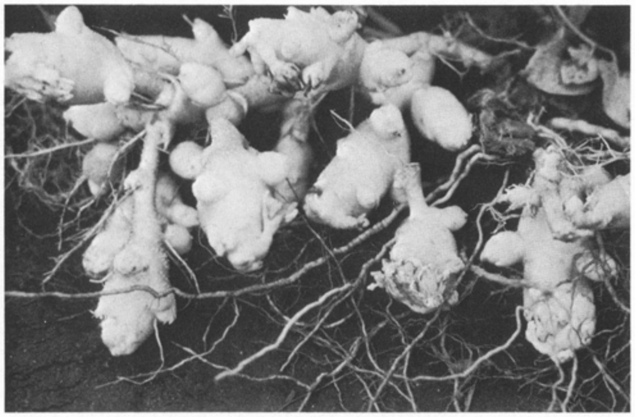

Fig. 4. Tubers of *S. brevidens–S. chacoense*-Gp. Tuberosum (BCT) hybrid. Note flowers on tubers

thickened peduncles and were closely appressed to the tubers (Fig. 4). Flowers appeared fully functional; however, their fertility was not tested.

Discussion

Interspecific hybrids involving Series Etuberosa species appear to have inherent problems involving the ability of their genomes to pair with the genomes of the tuber-bearing species in initial hybrids, resulting in high levels of male and female sterility. These problems have been noted in diploid progeny of *S. etuberosum* × *S. pinnatisectum* (Ramanna and Hermsen 1979a) and *S. fernandezianum* × *S. cardiophyllum* (Johnston 1980). Whether these pairing difficulties are the result of discrete gene action or are more genomic in nature, they present obvious problems to the direct utilization of such materials.

In the triploid BC hybrid, high levels of sterility were found. The pairing in this hybrid was found to be primarily 12 bivalents and 12 univalents which presumably is the result of homologous pairing of *S. brevidens* chromosomes and the exclusion from pairing of the *S. chacoense* chromosomes. Comparisons of pairing in diploid and allotetraploid hybrids of *S. etuberosum* and *S. pinnatisectum* by Ramanna and Hermsen (1979) suggest that autosyndesis is the general rule in polyploids such as these. The occasional pairing of *S. chacoense* chromosomes to form trivalents, however, raises the possibility that some *S. brevidens* and *S. chacoense* chromosomes may regularly pair as bivalents with the remaining *S. brevidens* homolog behaving as a univalent. This, as well as the trivalent pairing, would allow gene exchange between *S. brevidens* and other genomes, if balanced gametes can be formed.

In addition to pairing problems, EBN dosage can be an important factor both in terms of fertility evaluation and in further utilization of hybrids. The evaluation of female fertility, in lieu of stainability observations, is complex in an inter-EBN hybrid since the hybrid contains two entirely different genomes with respect to EBN. The BC hybrid contains two *S. brevidens* genomes each of which may be considered to have an EBN value of ½ and a single *S. chacoense* genome which may be considered to have an EBN value of 1. The EBN value of hybrids is by all indications the summation of genomic EBN values. In this context, the BC hybrid is 2EBN (= ½ + ½ + 1). If EBN is considered to be a single discrete factor which can segregate randomly, four types of gametes may be produced with respect to EBN:

½ EBN = ½(brd)

1EBN = ½(brd) + ½(brd) or 1(chc)

1½ EBN = ½(brd) + 1(chc)

2EBN = ½(brd) + ½(brd) + 1(chc).

Ideally each of these types of gametes should be tested for in a hybrid such as this, by pollinating with complementary (identical EBN) male gametes. In this case pure species were used to test for ½, 1 and 2 EBN gametes, but it was not possible to test for 1½ EBN gametes. From a utilization point of view it is probably most desirable to attempt crosses which provide gametes which have compatible EBN values and are numerically balanced. The production of the 5x BCT hybrid supports this point of view. Similarly the 5x hybrid which is 4EBN (= ½ + ½ + 1 + 1 + 1) is most compatible with 4x(4EBN) species and cultivars.

The fertilization of a rare 2n egg from the BC triploid with a nomal gamete from the 4x(4EBN) clone 'Wis AG 231' resulted in a pentaploid hybrid with improved pairing and dramatically improved male and female fertility. As in the triploid, it was not possible to determine if pairing was autosyndetic or allosyndetic. The improved fertility may be due entirely to improved numerical segregations, and a higher tolerance of aneuploidy in the gametes rather than improved pairing itself. The improved fertility, however, allowed crossing to 4x(4EBN) cultivars with little effort.

The production of the pentaploid hybrid represents an important step in the utilization of Series Etuberosa germplasm. Previously it has not been clear whether Series Etuberosa material inhibited tuber production. This plant suggests that Series Etuberosa germplasm does not inhibit tuberization per se, but rather that tuberization may be controlled by a dosage or threshold effect. Hermsen (1980) reported formation of small tuber-like organs on 4x *S. etuberosum* × *S. pinnatisectum* hybrids which had a 2 : 2 balance of non-tuber-bearing to tuber-bearing genomes although none had been reported on the diploid hybrids. The BCT hybrid having a 2 : 3 ratio of non-tuber-bearing to tuber-bearing genomes tuberized readily.

The production of this plant and its subsequent crosses to Gp. Tuberosum cultivars has also demonstrated the compatibility of the *S. brevidens* genome with cultivated materials. The 5x hybrid, although slow growing initially, was vigorous and fertile once past the seedling stage. The flowering tubers produced are indicative of both potentially new traits which may arise in exotic hybrids as well as the physiological upsets inherent in such hybrids.

Utility of the EBN concept

The germplasm of Series Etuberosa suffers four potential problems with respect to its ultimate incorporation into cultivated materials: 1) crossing barriers due to endosperm imbalance; 2) the sterility of the initial F_1 hybrids, although this may be overcome through somatic doubling of the hybrids to produce fertile allotetraploids; 3) potential chromosome loss due to pairing failures in each cross prior to incorporation, although recent evidence suggests that diminution of Etuberosa dosage in secondary hybrids may increase homoeologous pairing (Ramanna and Hermsen 1982) and 4) decreased frequency of quantitative genes within the population in each cross subsequent to the initial cross.

The method presented here provides the means to largely overcome the potential problems of incorporating this germplasm into useful forms. The basis for this method resides in the utilization of a 2n gamete approach coupled with a knowledge of the EBNs of all species involved. Formation of the triploid hybrid was accomplished by ploidy-EBN manipulation. The use of a 2n gamete from the 3x(2EBN) BC hybrid was used subsequently to overcome the basic infertility of the initial hybrid, and knowing the EBN value of the potential gametes permitted the correct selection of the other parent with respect to EBN. The 2n gamete from the BC hybrid also served to efficiently transfer the *S. brevidens* germplasm to the secondary hybrid, BCT. Overall, this method of crossing permits this germplasm to be transferred relatively intact from *S. brevidens* to a cultivar-compatible form with only modest effort. Limited observations of the progeny of 5x BCT × 4x Gp. Tuberosum cultivar crosses suggest that traits have been transferred to these advanced hybrids and that this single additional cross to Gp. Tuberosum largely restores normal tuber type (Fig. 5).

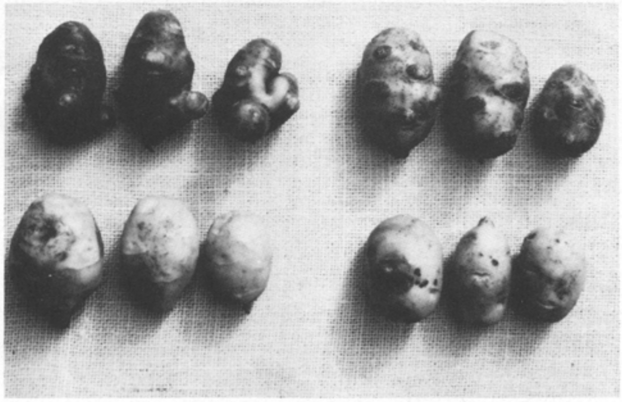

Fig. 5. Tubers of *S. brevidens–S. chacoense*-Gp. Tuberosum (BCT) × Gp. Tuberosum crosses. *Top row:* two clones of BCT × 'W744'; *bottom row:* two clones of BCT × 'Monona'

The "scaling" of ploidies utilized here, moving genes from a 2x(1EBN) species to a 3x(2EBN) hybrid and then to a 5x(4EBN) hybrid, useful in further crossing with 4x(4EBN) cultivars, should prove feasible for all 1EBN *Solanum* materials in which the transfer of germplasm across multiple EBN levels is required. Additionally, it is hoped that this system can serve as a model for the incorporation of formerly inaccessible germplasm in other crops where endosperm development is the barrier to successful hybridization.

Acknowledgements. This work is a cooperative investigation of the U.S. Department of Agriculture, Agricultural Research Service and the Wisconsin Experiment Station. This research was supported in part by the Science and Education Administration of the U.S. Department of Agriculture under Grant No.'s 59-2-2177-1-1-611-0 and 83-CRCR-1-1253 from the Competitive Research Grants Office and by the Graduate School, University of Wisconsin-Madison.

References

Hanneman Jr RE (1983) Assignment of Endosperm Balance Numbers (EBN) to the tuber-bearing *Solanum* species. Am Potato J 60:809–810 (Abstr)

Hawkes JG, Hjerting JP (1969) The potatoes of Argentina, Brazil, Paraguay and Uruguay. A biosystematic study. Ann Bot Mem 3: 525 pp

Hermsen JG Th (1980) Recent progress and future plans for utilizing Mexican wild species for pest and disease resistance. In: Utilization of the genetic resources of the potato III. International Potato Center (CIP), Lima, Peru, pp 129–139

Hermsen JG Th, Ramanna MS, Sawor Z (1981) The effect of chromosome doubling on fertility, meiotic behavior and crossability of *Solanum etuberosum* × *S. pinnatisectum*. Euphytica 30:33–39

Hermsen JG Th, Taylor LM (1979) Successful hybridization of non-tuberous *Solanum etuberosum* Lind. and tuber-bearing *S. pinnatisectum* Dun. Euphytica 28:1–7

Johnston SA (1980) The role and nature of genic balance in endosperm development. PhD Thesis, University of Wisconsin-Madison, 157 pp

Johnston SA, den Nijs TPM, Peloquin SJ, Hanneman Jr RE (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor Appl Genet 57:5–9

Johnston SA, Hanneman Jr RE (1982) Manipulations of Endosperm Balance Number overcome crossing barriers between diploid *Solanum* species. Science 217:446–448

Jones RAC (1979) Resistance to potato leaf roll virus in *Solanum brevidens.* Potato Res 22:149–152

Lin BY (1975) Parental effects on gene expression in maize endosperm development. PhD Thesis, University of Wisconsin-Madison, 190 pp

Marks GE (1965) Cytogenetic studies in tuberous *Solanum* species. 3. Species relationships in some South and Central American species. New Phytol 64:293–306

Pandey KK (1962) Interspecific incompatibility in *Solanum* species. Am J Bot 49:874–882

Peloquin SJ, Hougas RW (1959) Decapitation and genetic markers as related to haploidy in *Solanum tuberosum.* Eur Potato J 2:176–183

Ramanna MS, Hermsen JG Th (1979a) Unique meiotic behavior in F_1 plants from a cross between a non-tuberous and a tuberous *Solanum* species in section *Petota.* Euphytica 28:9–15

Ramanna MS, Hermsen JG Th (1979b) Genome relationships in tuber-bearing Solanums. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the Solanaceae. Academic Press, London New York, pp 647–654

Ramanna MS, Hermsen JG Th (1982) Gene transfer from non-tuberous to tuberous *Solanum* species: evidence from meiosis in hybrids. Euphytica 31:565–572

Ross RW, Rowe PR (1965) Frost resistance among the *Solanum* species in the IR-1 potato collection. Am Potato J 42:177–185

Toxopeus HJ (1964) Treasure-digging for blight resistance in potatoes. Euphytica 13:206–222

Note added in proof

In the 1983 summer season, 103 pollinations of 'Norland' × BCT treated with 2,4-D produced 43 fruit and a total of 40 seeds. These seeds have given rise to advanced hybrids in Group Tuberosum cytoplasm.