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Interspecific hybridization between the cultivated potato *Solanum tuberosum* subspecies *tuberosum* L. and the wild species *S. circaeifolium* subsp. *circaeifolium* Bitter exhibiting resistance to *Phytophthora infestans* (Mont.) de Bary and *Globodera pallida* (Stone) Behrens

2. Sexual hybrids

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Summary. Crossability between the diploid species *S. circaeifolium* subsp. *circaeifolium* (*crc*) and other diploid species, primarily diploid *S. tuberosum* subsp. *tuberosum* (*tbr*-2x), was studied. Forty-seven hybrids were obtained from crosses between *crc* as female parent and *tbr*-2x and some other species from series *Tuberosa* as male parents. Of these hybrids 17% were diploids; the other 83% were triploids, probably carrying two genomes of *crc*. Female fertility was sufficient to obtain offspring from backcrosses with the cultivated parent. Pollen stainability of the F_1 varied, and micro-pollen as well as unreduced pollen occurred. During meiosis of the diploids and triploids a rather high proportion of univalents was found, and in the triploids on average two or three trivalents per cell were found. All hybrids were resistant to *Globodera pallida* pathotypes 2 and 3, and 75% of the tested genotypes were highly resistant to *Phytophthora infestans*. Solanidine, tomatidine, tomatidenol, and demissidine glycosides were found in tubers of the hybrids. Comparisons with somatic hybrids between *crc* and *tbr*-2x are made. It is concluded that *crc* is a valuable *Solanum* species that can and should be included in potato breeding programs.

Key words: *Solanum circaeifolium* subsp. *circaeifolium* Bitter – Diploid *Solanum tuberosum* subsp. *tuberosum* L. – Interspecific hybridization – Cytogenetics – Disease resistances

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Introduction

Solanum circaeifolium subsp. *circaeifolium* (*crc*) belongs to the series *Circaeifolia*, which is endemic to Bolivia and consists of three diploid species, all occurring in the high eastern Andean rain forest zone (Hawkes and Hjerting 1989). In contrast to other Bolivian diploid wild species, the species from series *Circaeifolia* do not, or only rarely, intercross with species from other series. On the basis of this and additional plant morphology and protein electrophoresis data, Hawkes and Hjerting (1989) suggested that *Circaeifolia* is a rather primitive isolated series, probably with affinities to the Mexican series *Pinnatisecta*. Van Soest et al. (1983) did not succeed in interspecific hybridization using the species of series *Circaeifolia*. Interspecific hybridization between the species *S. capsicibaccatum* (*cap*) from series *Circaeifolia* and the species *S. lignicaule* (*lgl*) from the series *Tuberosa* has been reported by Chavez et al. (1988a). Louwes and Hoekstra (1989) obtained seeds from crosses between *S. circaeifolium* subsp. *quimense* (*qum*) and diploids of *S. tuberosum* subsp. *tuberosum* (*tbr*-2x), *lgl*, *S. chomatophilum* (*chm*), and *S. polyadenium* (*pld*). Only the progeny of *qum* × *lgl* proved to consist of true hybrids (unpublished results). Chavez et al. (1988b) could intercross *cap* with *S. commersonii* (*cmm*). Chavez et al. (1988a) suggested that *cap* and *lgl* have an Endosperm Balance Number (EBN) of 1, according to the EBN system of Johnston et al. (1980), while *cmm* is already known to have 1 EBN. Following the EBN hypothesis normal endosperm development only occurs when male and female gametes of both species have the same EBN. On the basis of crossing experiments

at the International Potato Center (1987) it was concluded that *S. circaeifolium* (no subspecies defined) also has 1 EBN. Most diploid species from South America have 2 EBN, including *tbr*-2x. The EBN hypothesis appears to predict the outcome of interspecific crosses quite accurately. However, Ehlenfeldt and Hanneman (1988) reported a clear exception to the EBN hypothesis when they obtained not only triploids but also 24% diploid hybrids from a cross between the two diploid species *cmm* (1 EBN) and *S. chacoense* (*chc*) (2 EBN). The outcome of difficult interspecific crosses also appears to depend largely on the number of attempts made and whether many different environments and many genotypes, representing broad genetic variation of both species, have been used (Hermsen 1979).

In species from series *Circaeifolia* (*cap*, *crc*, and *qum*) van Soest et al. (1983) found high levels of race nonspecific resistance to *Phytophthora infestans* (Mont.) de Bary. Chavez et al. (1988b) reported resistance to *Globodera pallida* Stone pathotypes 2 and 3 (Pa2 and Pa3) in *cap*.

There are only a few *crc* accessions available in gene banks. For two of these accessions, some evaluation data have been published (Hoekstra and Seidewitz 1987). For example, resistance has been reported against Pa2, Pa3, *P. infestans* and *Erwinia carotovora* (Jones) Bergey et al. Many other important characters, however, such as the level and composition of steroidal glycoalkaloids, have not been described.

In this paper attempts to hybridize *crc* with some wild diploid species having 1 EBN and some wild and cultivated diploid species (2 EBN) from series *Tuberosa* are described, and the hybrids obtained are studied for several characters. The results are compared with those of Mattheij et al. (1992), who obtained somatic hybrids between *crc* and *tbr*-2x.

Materials and methods

Crossing experiments

Genotypes. True seeds of *crc* accession BGRC 27058 were sown in 1988, and crosses were made using 11 genotypes, of which 8 were maintained in vitro during subsequent years. A range of genotypes of other species was used in the same crosses, both as male and female parents. In 1988 two diploid Mexican species, *S. polyadenium* (*pld*) accessions PI 175444, PI 230480, PI 275238, PI 320342, and PI 347768 and *S. pinnatisectum* (*pnt*) accession PI 275230, were available. Furthermore, in 1988, 1989, and 1990 the following genotypes from our own institute were used: 6 diploid genotypes of *S. tuberosum* (*tbr*-2x), of which SH76-128-1865 (referred to as *tbr*1) was most frequently used; 2 diploid genotypes, KW84-19-2471 and KW84-27-2587, derived from crosses between *tbr*-2x and *S. goniocalyx* (*gon*) and referred to as (*gon* × *tbr*)-1 and -2 respectively; *S. phureja* (*phu*) genotype P81-1868-435; and two *S. berthaultii* (*ber*) genotypes, *ber*-1 and -9.

Crosses. In 1988 crosses between *crc* and either *pld, pnt, tbr*-2x, *phu*, or *ber* were made reciprocally. In 1989 *crc* was pollinated with *tbr*-2x and (*gon* × *tbr*). In 1989 and 1990 hybrids obtained from crosses made in 1988 and 1989 were intercrossed and backcrossed using *tbr*-2x and *crc*. The crosses were made outdoors in an aphid-free screenhouse, in a controlled environmental chamber (19 °C/15 °C day/night, 16-h photoperiod) and in a greenhouse. The plants were grown in pots (18–25 cm), and flowers were emasculated 1 or 2 days before anthesis. Plants were pollinated twice with fresh pollen or sometimes with viable pollen that had been stored in a freezer. Fruits were collected after 6–8 weeks.

True seeds and seedlings. After disinfection of the fruits in 96% alcohol plump seeds were removed, treated with gibberellic acid (GA_3, 50–500 ppm for 2 h), and placed on MS medium (Murashige and Skoog 1962) supplemented with 0.5% sucrose, 0.8% agar, pH 5.8, (MS5) or MS10 (MS supplemented with 1% sucrose) in petri dishes or glass culture tubes in vitro. Germinated seeds were transferred to MS30 (MS supplemented with 3% sucrose) and propagated.

Cytological and fertility studies

Pollen. Pollen was stained with lactophenol-acid fuchsine, and the percentages of normally stained pollen, unstained or poorly stained pollen, 2n-pollen, and micro-pollen were determined with at least 200 pollen grains. The term micro-pollen denotes pollen that is less than one-third of the size of normal pollen (diameter: 17.5–26.5 μm), while 2n-pollen has four germination pores and a diameter greater than 22.5 μm. In 1988 pollen germination and pollen-tube growth in vivo in each cross combination was studied by fluorescence microscopy (356 nm). Approximately 48 h after pollination, four pistils were fixed for 1 h in a 3:1 mixture of alcohol and acetic acid, macerated in 1 *N* NaOH in water at 60 °C for 30–60 min, the duration depending on the volume of the pistil, and stained in a solution of 0.1% aqueous aniline blue for at least 2 h.

Mitosis. Root-tip squashes were used to determine the exact number of chromosomes. Root tips were pretreated with 8-hydroxy-quinoline (4–5 h), fixed in a 3:1 alcohol:acetic acid mixture, hydrolyzed in 1 *N* HCl in water (at 60 °C for 8 min), stained for 2 h with Feulgen reagent, and squashed in a haematoxylin solution. The nucleolar chromosomes were also studied in all of the genotypes; these chromosomes carry a satellite that results from a distinct secondary constriction in the short arm.

Meiosis. Microsporogenesis was studied in metaphase I (M I) and/or anaphase I and II (A I, A II); the tetrad stage was also studied. Anthers were fixed, stored, and squashed following the method described in Mattheij et al. (1992).

Agronomically important characters

Potato cyst nematode resistance. Resistance to *G. pallida* pathotypes 2 and 3 (Pa2, Pa3) was determined following Dellaert et al. (1988). For each pathotype, three replicates per tested genotype were grown in small pots. When the plants were sufficiently rooted, each was inoculated with 25–30 cysts. Newly formed cysts visible on the rootball were counted.

Resistance to Phytophthora infestans. Resistance to *P. infestans* was tested in a laboratory experiment following the method described in Mattheij et al. (1992).

Steroidal glycoalkaloids. The presence and levels of solanidine, solasodine, tomatidenol, tomatidine, and demissidine glycoside were determined on a tuber sample of at least five tubers, following the method of van Gelder et al. (1989). Data are presented on a 0–5 scale, where 0 = absent, 1 = 0.1–50, 2 = 51–100, 3 = 101–150, 4 = 151–200, and 5 = more than 200 mg/kg fresh tuber weight.

Table 1. Results of interspecific crosses with *S. circaeifolium* subsp. *circaeifolium*

Cross	Number[a]			Percentage[b]		Number of hybrids		Pollen tubes observed in:[c]
	p	f	s	g	v	2x	3x	
crc × *tbr*-2x						2	27	
Summer 1988	400	74	49	69	94			Ovary
Spring 1989	49	7	3	0	–			
Summer 1989	57	0	–					
Autumn 1989	250	74	28	42	50			
tbr-2x × *crc*	300	0	–					Lower style, ovary
crc × (*gon* × *tbr*)	151	92	52	41	67	6	8	
crc × *phu*	10	5	2	50	100	0	1	Ovary
phu × *crc*	20	0	–					Ovary
crc × *ber*	16	5	6	50	100	0	3	Ovary
ber × *crc*	20	0	–					Upper style
crc × *pnt*	30	0	–					Upper style
pnt × *crc*	30	0	–					Upper style
crc × *pld*	30	0	–					Stigma
pld × *crc*	750	140	1					Ovary

[a] Number of pollinations (p), of fruits (f), and of seeds (s)
[b] Percentage of germinated seeds (g) and of viable plants of the germinated seeds (v)
[c] Maximum pollen-tube ingrowth in stigma, style, or ovary in vivo

Table 2. Analysis of meiotic M I of 1 diploid and 3 triploid hybrids of the cross *S. circaeifolium* subsp. *circaeifolium* × *S. tuberosum* subsp. *tuberosum*-diploids (*crc* × *tbr*-2x)

Genotype	Number of cell	Configurations per cell[a] (range)			
		IV	III	II	I
Diploid					
(*crc*2 × *tbr*1)-1	9			8.33 (6–11)	7.33 (2–12)
Triploid					
(*crc*6 × *tbr*1)-2	24	0.13 (0–1)	3.04 (0–7)	9.75 (4–14)	6.79 (3–11)
(*crc*6 × *tbr*1)-3	15		1.80 (1–4)	10.13 (7–13)	10.33 (7–15)
(*crc*5 × *tbr*4)-1	9		2.50 (1–4)	10.75 (8–14)	7.00 (5–10)

[a] IV, Quadrivalent: III, trivalent; II, bivalent; I, univalent

Results

F_1 hybrids

Out of 933 pollinations using *crc* as a female parent and *tbr*-2x, (*gon* × *tbr*), *phu*, and *ber* as males, 47 hybrid genotypes were obtained (Table 1), whereas other cross combinations were not successful. Hybrids were obtained in three different crossing periods: in the summer of 1988 (*crc* × *tbr*, *crc* × *phu*, and *crc* × *ber*), in the spring of 1989 [*crc* × (*gon* × *tbr*)], and in the autumn of 1989 [*crc* × *tbr* and *crc* × (*gon* × *tbr*)]. The climatic conditions in these three periods resembled more or less the natural conditions at sites in Bolivia where *crc* occurs: high relative humidity, temperatures below 25 °C, and shade or dimmed sunshine. In the summer of 1988 and autumn of 1989, these climatic conditions occurred naturally, and successful hybridizations were carried out between plants growing in the screenhouse. In the early spring of 1989, interspecific hybrids were obtained from plants grown in an unheated greenhouse. No hybrids were obtained when the climatic conditions were hot and bright, and humidity was low as was the case in the summer of 1989. Initially 11 genotypes of *crc* were used for crossing, and on 9 of these, hybrids were obtained. One *crc* genotype produced in total 24 hybrids, another one produced 8 hybrids, and the remaining 7 between 1 and 4 hybrids. True seeds produced in the autumn of 1989 had poor quality (Table 1), probably because the mother plants died before the fruits had ripened due to the low temperature. In the spring of 1989 the 2 genotypes of (*gon* × *tbr*) where the most successful as pollinators, whereas in the other crossing periods the *tbr*-2x genotype SH76-128-1865 was better. The in vitro sown seeds took between several days and more than a year to germinate. However, most seeds germinated in several weeks.

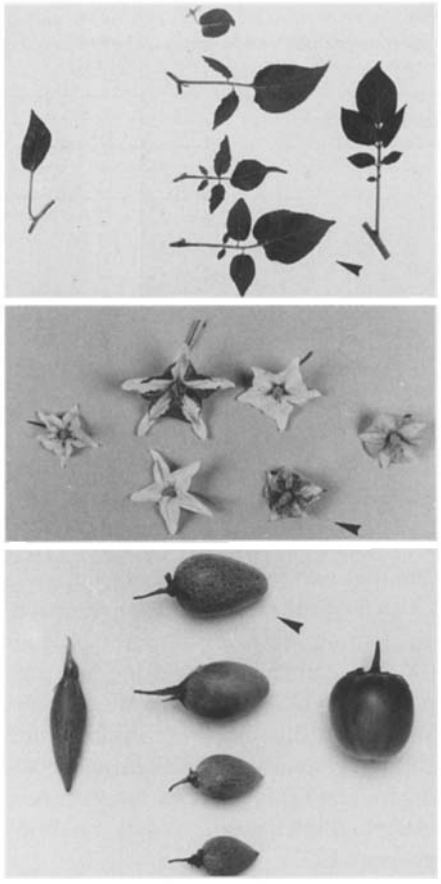

Fig. 1. Morphology of leaves, flowers, and fruits of *S. circaeifolium* subsp. *circaeifolium* (*left*), a *S. tuberosum* subsp. *tuberosum* diploid (*right*), and one diploid and three triploid interspecific hybrids (*middle*, diploid is indicated by *arrow*)

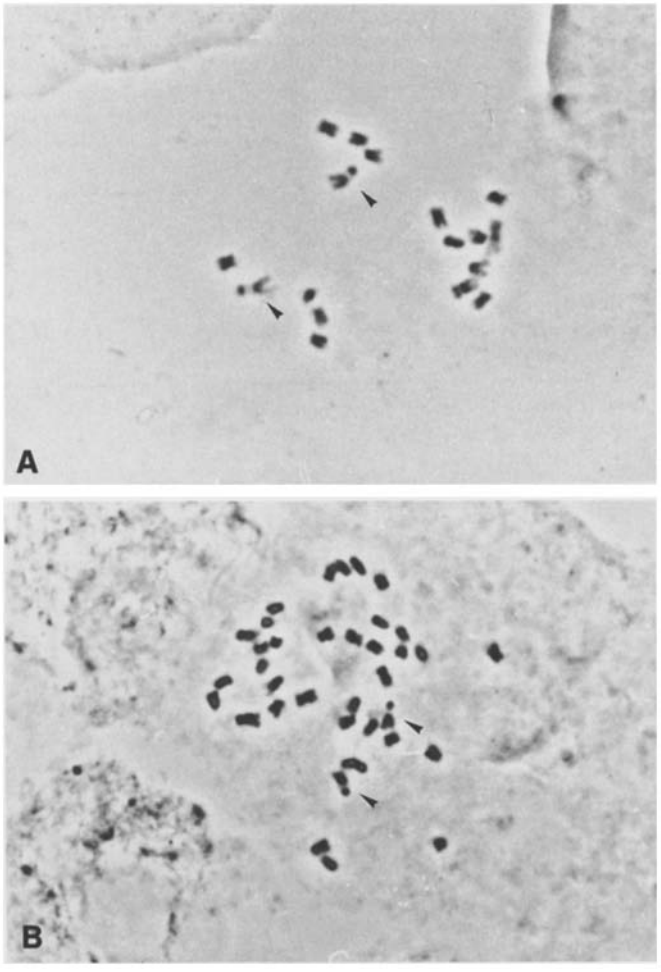

Fig. 2 A, B. Somatic metaphase. **A** Incomplete cell of a genotype of *S. circaeifolium* subsp. *circaeifolium* (*crc*) with two nucleolar chromosomes with large, clearly stained satellites (*arrows*). **B** Triploid hybrid of the cross *crc* × *S. tuberosum* subsp. *tuberosum* diploid with two *crc*-like nucleolar chromosomes (*arrows*)

The hybrid character of the obtained seedlings was established by examining morphological markers and nucleolar chromosomes (see cytology and fertility). *Crc*, the pistillate parent, has several morphological characteristics that are different to those of *tbr* (Mattheij et al. 1992). The hybrids were found to be intermediate between the parents for shape of leaf, flower, and fruit (Fig. 1), and for pubescence of the leaves. For flower color, various shades and patterns were observed in the hybrids, as illustrated in Fig. 1.

Table 1 also lists cross-combinations that did not result in hybrids. No successful combinations were obtained using *crc* as the pollinator. Furthermore, crosses with the two Mexican wild species *pld* and *pnt* were unsuccessful. *Pld* is a self-compatible species, and the direct inhibition of its pollen on the stigma of species belonging to other series is a common reaction (Abdalla 1970). The cross *pld* × *crc* yielded many fruits, but the only plump seed obtained was found not to be a hybrid. Many flat, very small, and probably aborted seeds were observed in the *pld* × *crc* fruits.

Cytology and fertility

Mitosis of F_1 hybrids. The ploidy level of the hybrids is listed in Table 1. Eight hybrids were found to be diploid and 39 triploid, with 1 triploid hybrid being aneuploid, having 37 chromosomes. Most diploid hybrids were obtained using (*gon* × *tbr*) as pollinators.

Root-tip analysis of *crc* and *tbr*-2x indicated a clear difference between the nucleolar chromosomes of both species. The satellite of *crc* was relatively big and clearly stainable (Fig. 2 A), whereas in the *tbr*-2x parents and in the (*gon* × *tbr*) genotypes, the satellite was very small and blurred. In the diploid hybrids only one *crc*-like nucleolar chromosome was found while in the triploid hybrids two *crc*-like nucleolar chromosomes (Fig. 2 B) were present, indicating that *crc* contributed the unreduced gamete.

Table 3. Analysis of A I and A II in meiosis of triploid hybrids between *S. circaeifolium* subsp. *circaeifolium* and *S. tuberosum* subsp. *tuberosum*-diploids (*crc* × *tbr*-2x): number of cells with 0, 1, 2, 3, or more lagging chromosomes per cell and mean number of lagging chromosomes and number of poles with 12, 13, etc. chromosomes per pole

Hybrid	n [b]	Lagging chromosomes per cell						Number of chromosomes per pole											
		0	1	2	3	>3	mean	12	13	14	15	16	17	18	19	20	21	22	32–35
A I																			
(*crc*5 × *tbr*4)-1	25	15	9	1			0.44			2	2	4	12	16	9	2	2	1	
(*crc*5 × *tbr*5)-1 [a]	11	7	2	1	1		0.64						9	4	5	4			
A II																			
(*crc*5 × *tbr*4)-1	15	1	4	9	1		1.67			3	2	9	16	14	8	6	1	1	
(*crc*3 × *tbr*1)-1	25	1	4	5	6	9	3.40	1	3	3	11	15	16	11	19	7	1	1	6

[a] Triploid hybrid with 37 chromosomes
[b] Number of cells analyzed

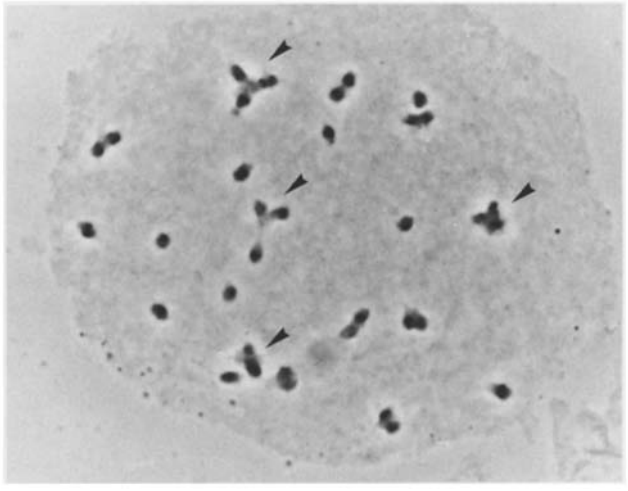

Fig. 3. Chromosome associations at diakinesis in a triploid hybrid, (*crc*6 × *tbr*1)-3, of the cross *S. circaeifolium* subsp. *circaeifolium* × *S.- tuberosum* subsp. *tuberosum*-diploid: 4 III (*arrow*) +7 II+10 I

Meiosis of F_1 hybrids. Chromosome configurations in M I were studied in 1 diploid genotype and 3 triploid genotypes obtained from the cross between *crc* and *tbr*-2x (Table 2). Many univalents were found in the diploid hybrid. The occurrence of univalents in the diploid hybrids is also supported by the observation of one or two micro-cells in 52% of the tetrads of another genotype. Micro-cells can occur when so-called laggards, univalents that do not reach one of the poles during A I and/or A II, group together. Micro-cells result in micro-pollen. The occurrence of micro-pollen was studied in 7 diploid hybrids: 1 had 14% and the others had 2–4% micro-pollen.

The 3 triploid hybrids that were analyzed (Table 2) also had many univalents, a high number of bivalents, and on average 2 or 3 trivalents per cell. Figure 3 shows the chromosome associations at diakinesis of one cell of a triploid hybrid in which 4 trivalents occurred. In 2 triploid hybrids the total number of trivalents and bivalents exceeded 12, which might have been due to autosyndetic pairing, i.e., pairing between non-homologous chromosomes, in *tbr*-2x. Autosyndetic pairing has been reported to occur in triploids (Lange and Wagenvoort 1973) and triploid hybrids (for review see Prakken and Swaminathan 1952). The number of univalents, bivalents, and trivalents found (Table 2) are within the range reported for other triploid interspecific *Solanum* hybrids (Lange and Wagenvoort 1973).

The results of A I and A II analyses carried out with triploid hybrids between *crc* and *tbr*-2x are summarized in Table 3. From 1 triploid hybrid both stages could be analyzed, and from 2 other triploids only one stage, either A I or A II, could be examined. The frequency of laggards in the genotypes (*crc*5 × *tbr*4)-1 and (*crc*5 × *tbr*5)-1 is similar to that obtained previously for triploid *tbr* (Lange and Wagenvoort 1973), while in the A II of (*crc*3 × *tbr*1)-1 a larger number of lagging chromosomes was observed. The normal distribution of the number of chromosomes found at the poles during A I and A II (Table 3) is in agreement with that reported in the literature (Bleier 1933; Prakken and Swaminathan 1952; Lange en Wagenvoort 1973). The tetrad stage was also studied in the triploid genotypes listed in Table 2. The percentage of cells in which one or two micro-cells were included in the tetrad varied between 32 and 51. Micro-cells in triploid interspecific hybrids have also been reported by Müntzing (1933) and von Olah (1938).

Fertility. One diploid hybrid, (*crc*2 × *tbr*1)-1, was backcrossed with *tbr*-2x and produced on average 1 seed per pollination. Five other diploids, all *crc* × (*gon* × *tbr*), were used in backcrosses with *crc* and intercrossed (Table 4). Only 1 of the 5, [*crc*2 × (*gon* × *tbr*)-2]-3, set fruit in the crosses with *crc*, producing 1 fruit from 66 pollinations, and in the intercross with 1 other diploid hybrid, 3 pollinations resulted in 2 fruits (Table 4).

Table 4. Number of pollinations (p), fruits (f), and seeds (s) from crosses between diploid and triploid hybrids of *S. circaeifolum* subsp. *circaeifolium* × *S. tuberosum* subsp. *tuberosum*-diploid (*crc* × *tbr*-2x) with both parental species and from intercrossing with diploid hybrids

Female parent	Male parent								
crc × *tbr*-2x	*tbr*-2x			*crc*			*crc* × *tbr*-2x		
	p	f	s	p	f	s	p	f	s
Diploids									
1–5 genotypes	253	90	249	271	1	22	145	2	89
Triploids									
8 genotypes	522	93	411	111	1	0	24	0	0

Table 5. Number of chromosomes and of *crc*-like nucleolar chromosomes in offspring of backcrosses (BC_1) of 1 diploid hybrid and 3 triploid hybrids from the cross between *S. circaeifolium* subsp. *circaeifolium* and *S. tuberosum* subsp. *tuberosum*-diploids with the cultivated parent ((*crc* × *tbr*-2x) × *tbr*-2x)

Female parent *crc* × *tbr*-2x	Number of genotypes in BC_1	Number of chromosomes in BC_1	Number of genotypes with number of *crc*-like nucleolar chromosomes of:		
			0	1	2
Diploid					
(*crc*2 × *tbr*1)-1	11	24	7	4	
	1	25		1	
	1	35		1	
	2	36	2		
Triploid					
(*crc*3 × *tbr*1)-1	5	28–32	3	1	1
	1	44			1
	1	50			1
(*crc*6 × *tbr*4)-1	7	30–36	2	3	2
(*crc*15 × *tbr*3)-1	11	26–32	5	4	2

Viable seeds were obtained from all 8 triploids used in the crossing experiments after crosses with *tbr*-2x (Table 4), the success rate being 0.3–5.2 seeds per pollination. No seeds were obtained from backcrosses with the wild species and crosses with diploid hybrids.

While pollen fertility (stainability) of the parents was high, for the diploid and triploid hybrids and also for the genotypes obtained from the backcrosses, pollen fertility and the occurrence of micro- and 2n-pollen showed much variation. One diploid (*crc*2 × *tbr*1)-1 had less than 5% stainable pollen and showed many univalents in M I (Table 2). The diploid hybrid [*crc*6 × (*gon* × *tbr*)-1]-1, with the highest frequency of fertile pollen (90%), produced no micro-pollen and was the only functional pollinator in the intercrossing experiment (Table 4). Among the triploids, 60% showed a pollen fertility of more than 20%, and all of them produced micro-pollen.

Cytological analysis of backcross populations. The results of root-tip analyses of four backcross populations, 1 diploid hybrid crossed with *tbr*-2x and 3 triploid hybrids crossed with *tbr*-2x, are presented in Table 5. These data indicate that the normal distribution of chromosomes in male gametes of the triploid hybrids (Table 3) was most likely similar to the variation observed in the number of chromosomes in the female gametes that were functional in backcrossing with *tbr*-2x (Table 5). Four genotypes had a large number of chromosomes (Table 5), probably due to the formation of unreduced gametes. Some diploid *tbr* males produced both n- and 2n-pollen, and since it is likely that in the initial *crc* accession 2n-egg cells occur, the occurrence of unreduced female gametes in the hybrids also seems to be feasible.

Nucleolar chromosomes with the *crc* phenotype were studied for all of the genotypes of the backcross populations (Table 5). The offspring of the backcross on the diploid hybrid was expected to segregate at a 50% without: 50% with ratio of one *crc* nucleolar chromosome. The obtained segregation of 9:6 is not a significant deviation from the expected 1:1 segregation. In the offspring of the triploids, genotypes with 0, 1, and 2 *crc*-like nucleolar chromosomes were found in the ratio 10:8:7, respectively (Table 5). With triploids having a mean configuration of 10 univalents, 10 bivalents, and 2 trivalents (see Table 2) and on the basis of the hypothesis of preferential pairing of *crc* chromosomes, the chance of finding 0, 1, and 2 *crc* nucleolar chromosomes in the offspring would be 1:34:1, respectively. The high frequency of 0 and 2 nucleolar chromosomes in the offspring deviates from this ratio and suggests that at least between the nucleolar chromosomes of the wild and the cultivated species pairing has occurred.

Agronomically important characters

Resistance to G. pallida pathotypes 2 and 3 and to P. infestans. Eight *crc* genotypes were tested for resistance to *G. pallida* pathotypes 2 and 3, and to *G. rostochiensis* pathotype 1. They were all found to be susceptible for *G. rostochiensis*, so that no further testing of resistance to this nematode species was carried out. In a test of *crc* with Pa2 and Pa3, 27 genotypes of *crc* × *tbr* were used of which 22 were triploids, 5 were diploids, and 3 were standard genotypes (standards in Mattheij et al. 1992). All 8 *crc* genotypes had fewer than two cysts per plant, while on all of the hybrids fewer than five cysts were counted for both pathotypes, on 70% of them there was even fewer than three cysts.

Resistance to *P. infestans* of 2 *crc* genotypes, 2 diploids of *tbr*, 8 hybrids, of which 4 were triploids and 4 diploids, and 2 standard cultivars (standards in Mattheij et al. 1992) was tested. Both diploids of *tbr* were highly susceptible, while the *crc* genotypes and 6 hybrids

Table 6. Components and level of steroidal glycoalkaloids of *S. circaeifolium* subsp. *circaeifolium* (*crc*), mean of 8 genotypes, and *S. tuberosum* subsp. *tuberosum*-diploid (*tbr*-2x), mean of 4 genotypes, and 5 diploid and 9 triploid *crc* × *tbr*-2x hybrids

Species/hybrids	Glycoalkaloid content (class 0–5[b])				
	Solanidine glycoside	Solasodine glycoside	Tomatidenol glycoside	Tomatidine glycoside	Demissidine glycoside
crc	0	0	2	5	0
tbr	3	0	0	0	0
crc × *tbr*					
Diploids					
(*crc*2 × *tbr*1)-1	3; 1[a]	1; 0	1; 1	3; 5	2; 3
[(*crc*2 × (*gon* × *tbr*)-2)]-1	2	0	2	4	1
[(*crc*2 × (*gon* × *tbr*)-2)]-3	1	0	1	3	1
[(*crc*6 × (*gon* × *tbr*)-2)]-1	1	0	1	5	1
[(*crc*6 × (*gon* × *tbr*)-1)]-1	1	0	1	4	1
Triploids					
(*crc*3 × *tbr*1)-1	1; 2	0; 0	0; 0	1; 1	4; 4
(*crc*5 × *tbr*1)-1	1	0	0	2	1
(*crc*6 × *tbr*1)-1	1	0	0	2	5
(*crc*6 × *tbr*1)-2	1	0	0	1	3
(*crc*6 × *tbr*1)-3	1	0	1	3	1
(*crc*6 × *tbr*1)-5	1	0	0	1	4
(*crc*6 × *tbr*1)-7	1	0	0	1	3
(*crc*6 × *tbr*4)-1	1	0	0	2	1
(*crc*15 × *tbr*3)-1	1; 1	0; 0	0; 0	1; 2	1; 1

[a] Two samples of tubers harvested at different dates
[b] The different classes are 0 (0 mg/kg), 1 (1–50 mg/kg), 2 (51–100 mg/kg), 3 (101–150 mg/kg), 4 (151–200 mg/kg), and 5 (>200 mg/kg)

were fully resistant. One triploid hybrid was highly susceptible and 1 diploid hybrid slightly susceptible, and on both these hybrids sporulating lesions developed.

Steroidal glycoalkaloid. Only tomatidenol glycoside and tomatidine glycoside were found in all 8 of the *crc* genotypes, and only solanidine glycoside was found in *tbr* (Table 6). Tomatidine glycoside concentrations were relatively high with absolute values of 260–530 mg/kg fresh tuber weight. In the hybrids not only were the glycoalkaloids of the parents detected, but also a new component, demissidine glycoside, and in fairly high amounts (Table 6), up to 230 mg/kg fresh tuber weight. In 1 diploid hybrid solasodine glycoside was also detected, although at a low level. Table 6 shows that the alkaloid level of the hybrids was intermediate between that of the parents, with a tendency to be lower than the mid-parent value. Among the hybrids a great range of variation was observed.

Discussion

The experiments reported here show that the species *crc* can be crossed with diploids of *tbr*, (*gon* × *tbr*), *phu*, and *ber*. The climatic conditions at the time of crossing appear to be an important factor (Table 1). Unfavorable climatic conditions may have been one of the factors why earlier attempts to obtain interspecific hybrids with *crc* and/or *qum* were either unsuccessful (van Soest et al. 1983) or met with only limited success (International Potato Center 1985; Hawkes and Hjerting 1989).

In the experiments reported here both diploid and triploid hybrids were obtained (Table 1). Since *crc* is thought to have 1 EBN, and *tbr*-2x, (*gon* × *tbr*), *phu*, and *ber*, 2 EBN, the EBN hypothesis would predict the absence of diploid hybrids and the presence of only triploid hybrids, to which the *crc* parent would contribute unreduced gametes. The presence of two *crc* genomes in each triploid was supported by the observation of two *crc*-like nucleolar chromosomes (Fig. 2B). Ehlenfeldt and Hanneman (1988) also obtained both diploids and triploids from a 1 EBN × 2 EBN cross. They have suggested that the diploids may have arisen through irregularities of the central cell (proliferation or endomitosis of nuclei). The percentage of unreduced *crc* gametes will probably have a large effect on the success rate of crosses between *crc* and *tbr*-2x, *phu*, and *ber*. In the accession of *crc* used here no unreduced pollen could be detected. If such 2n-pollen had occurred, the reciprocal crosses would also probably have resulted in interspecific triploid hybrids. It is likely that except for the development of functional endosperm depending upon EBN/ploidy level, no other insurmountable crossing barriers exist between *crc* and the species of series *Tuberosa* used here.

With the species *pnt*, chosen because of having 1 EBN and mentioned by Hawkes and Hjerting (1989) as probably having affinities to *crc*, no success was obtained. As only 5 genotypes of *pnt* were available and no more than 60 crosses were made, no definite conclusions can be drawn. The cross *pld* × *crc* was carried out on a large scale, and the crossing barrier(s) are likely to occur after fertilization, as pollen-tube growth in the ovary was observed (Table 1). *Pld* could be crossed easily to *pnt* (Louwes and Hoekstra 1989) and therefore probably also has 1 EBN. Consequently, for the combination *pld* × *crc*, the development of the endosperm is probably, not a bottle-neck, but other serious post-fertilization barriers occur.

Mattheij et al. (1992) obtained tetraploid and near-tetraploid somatic hybrids between *crc* and *tbr*-2x. The comparison between the efficiency of sexual hybridization and that of somatic hybridization cannot be made, since in the somatic hybridization experiment only one *crc* genotype and one *tbr*-2x genotype was used. But both sexual and somatic hybridization are likely to have good perspectives as backcrosses with the cultivated parent were successful at all ploidy levels and no great differences for disease resistances and steroidal glycoalkaloid contents were found (Mattheij et al. 1992). An advantage of using somatic hybridization might be that no 2n-gametes are required in *crc*. Still, there are differences in the possible use of the diploid, triploid, or tetraploid hybrids in a breeding program, of which some aspects will be discussed here. For the diploids the strategy might be to start with intercrossing to obtain genotypes with a combination of favorable alleles and to select against unwanted alleles, since this is most effectively carried out at the diploid level. Afterwards, several backcrosses with the cultivated species, (initially) also at the diploid level, will be necessary. Fertility is needed in all cases, and more crossing experiments must be carried out to test whether the fertility of the diploid hybrids is sufficient.

The triploid hybrids carry a double *crc* genome, and therefore the relative frequency of favorable *crc* alleles in the *crc* × *tbr* progeny is likely to be the highest. However, it is unknown if there are triploid hybrids with the functional male fertility necessary for intercrossing, and resulting progenies from back and/or intercrosses will be aneuploid with probably a further reduction in fertility.

The tetraploid somatic hybrids were very fertile as a female parent in crosses with both diploid and tetraploid pollinators, but they were male sterile. It needs to be investigated whether somatic hybridization of other genotypes results in male-fertile somatic hybrids. An advantage of using the tetraploid somatic hybrids is its direct application in a tetraploid potato breeding program, but progress in selection against unwanted alleles is slow at the tetraploid level.

Hawkes and Hjerting (1989) consider series *Circaeifolia* to be rather primitive and isolated. The present results support this rather isolated position, since in many cases *crc* and its interspecific hybrids behave differently from other species and interspecific hybrids. First of all, diploid hybrids from a 2x-1 EBN × 2x-2 EBN cross such as *crc* × *tbr*-2x are rare. Secondly, the occurrence of univalents in the M I in the diploid hybrids (Table 2) is unusual for interspecific diploid hybrids, since 12 bivalents are normally found in the M I (Singh et al. 1989). Only Ramanna and Hermsen (1979) have reported a high degree of failure of chromosome pairing in diploid F_1 genotypes of the cross between *S. etuberosum* (non-tuberous) and *S. pinnatisectum*. They observed on average 16–18 univalents in the M I. In the diploid hybrids of *crc* × *tbr*-2x fewer univalents were observed, and it seems likely that in addition to genome interaction resulting in univalents, some degree of affinity also exists between the chromosomes of *crc* and *tbr*. Namely, multivalents were observed in both the triploid (Table 2) and tetraploid somatic hybrids (Mattheij et al. 1992), and there were also indications for pairing of the nucleolar chromosomes of *crc* and *tbr*-2x. Thirdly, female fertility (Table 4) of the triploid hybrids was quite high, which is rather unusual for triploids (Prakken and Swaminathan 1952; van Suchtelen 1976). Furthermore, the functional female gametes of the triploid hybrids were aneuploid (Table 5), whereas it has often been found that mainly female gametes with approximately the somatic number of chromosomes are functional (Lamm 1945; Wagenvoort and Lange 1975; van Suchtelen 1976). Also, the pollen fertility of the triploid hybrids was much higher than that which has been reported for other triploid interspecific hybrids (Müntzing 1933; Prakken and Swaminathan 1952; Marks 1965; Abdalla 1970).

The high transmission rate of resistance to Pa2 and Pa3 to the F_1 is encouraging and similar to the results of Chavez et al. (1988 b) with *cap*, another species from series *Circaeifolia*, but again is exceptional in comparison with other series. Chavez et al. (1988 b) screened 21 interspecific F_1 families originating from 19 series for Pa2 and Pa3 resistance, and *cap* showed the highest transmission of resistance, being 100% for the cross *cap* × *lgl* and 88% for *cap* × *cmm*. They were able to cross *cap* × *lgl* with *tbr*-2x, but only 29% of the progeny was resistant to Pa2 and 14% to Pa3. In order to transfer the nematode resistance to *tbr*, the direct cross between *crc* and *tbr*-2x may offer better perspectives. Resistance to *P. infestans* was tested for only few genotypes, but the results justify a more detailed study. The absence of solanidine glycoside in *crc* (Table 6) and the presence of tomatidenol and tomatidine glycoside is exceptional for a potato species (Schreiber 1979; van Gelder et al. 1988). The great range of variation present in the hybrids for the amount of steroidal glycoalkaloids (Table 6) suggests that selection for low glycoalkaloid content should be possible in this material, which is in conformity with the research of Ross

et al. (1978) and van Gelder and Scheffer (1991) who used hybrids of other wild species with the cultivated potato.

The experiments presented here have shown that the interesting genepool of *S. circaeifolium* subsp. *circaeifolium* can be made accessible for potato breeding and research programs through sexual and somatic hybrids. Further experiments are needed to examine the fertility of the hybrids and to clarify the observed differences in female and male fertility of the hybrids, and to study the transfer of important characters to *S. tuberosum*.

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