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Transfer of resistance to PLRV titer buildup from *Solanum etuberosum* to a tuber-bearing Solanum gene pool

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Summary. Hybrids between *Solanum etuberosum* and *S. pinnatisectum* harboring resistance to titer buildup of potato leafroll virus (PLRV) were reciprocally crossed with tuber-bearing wild species *S. acaule* and *S. verrucosum*. A total of 47 hybrids with *acaule* were obtained with the aid of embryo rescue and sterile culturing of embryos from imbibing seeds. All but two hybrids with *acaule* had low pollen stainabilities or were pollen sterile. Hybrid seeds from crosses with *verrucosum* were easily obtained, and the triploid progenies were sterile. Hybrid progeny were screened for resistance to PLRV infection by viruliferous green peach aphid and for resistance to titer buildup. Although hybrids did not exhibit resistance to infection, PLRV was not detectable using ELISA. Virus was detected, however, by graft transmission to *Datura stramonium*. Crosses of fertile *acaule-etuberosum-pinnatisectum* hybrids with *S. phureja*, a cultivated diploid, using the latter as pollen parent, produced berries but seed did not complete development and was aborted. Rescue of immature embryos 25 days after pollination by excision from berries and sterile culture produced vigorous, pot-cultured plants. Segregation of susceptible (virus detected) and resistant (virus undetected) progenies suggests simple inheritance.

Key words: Embryo culture – Interspecific hybridization – PLRV resistance – Potato breeding

Introduction

Potato leafroll virus (PLRV) is an important pathogen affecting potato crops, particularly in developing countries where it causes severe yield reductions (Hooker 1977, 1980). It is particularly damaging in tropical and subtropical areas where green peach aphid, *Myzus persicae* Sulz. (GPA), populations are present throughout the year. The virus is spread over long distances by windborne alate aphids. Aphid transmission also occurs among tubers during unrefrigerated seed storage (Parker et al. 1983). Infected tubers, carried over between crops, appear as diseased volunteers and serve as virus sources (De Bokx 1974; Hooker 1977; Thomas 1983). *Datura stramonium*, used by some workers as a test and propagator host, can be a weed host of the virus (Hooker 1980).

Virus infection is managed with general success by seed certification systems in developed countries (Bishop 1967). However, these systems are expensive and the cost of shipping certified seed to growing areas is prohibitive in many developing countries. Where clean seed is expensive or unavailable, the best solution is resistant varieties. However, no source of immunity to PLRV in potato is known. Resistance to PLRV presently available in cultivated forms is expressed as resistance to infection and is of limited value (Brown 1980a, b).

Solanum etuberosum, a wild non-tuber-bearing diploid Chilean species, has resistance to frost, potato virus Y and PLRV (Brown 1980b; Hermsen 1980; Jones 1979). Initial hybridization studies were restricted to interspecific crosses within the series Etuberosa, while direct hybridization with most tuber-bearing *Solanum* was unsuccessful (Ramanna and Hermsen 1979, 1981, 1982). However, Hermsen and Taylor (1979) successfully crosses *S. etuberosum* with a wild, tuber-bearing, diploid Mexican species, *S. pinnatisectum*. Although the F_1 hybrids were sterile, the doubled allotetraploids were fertile (Hermsen et al. 1981).

The doubled interspecific hybrid allotetraploid *etuberosum-pinnatisectum* ($4x$-EP) was tested for resistance to PLRV (CIP 1982). Of 54 tested, 14 had resis-

tance to PLRV in the form of a repression of titer buildup (Brown 1984).

This paper reports the introgression of resistance to PLRV titer buildup derived from *E. etuberosum* to tuber-bearing hybrids by means of crosses with wild species and primitive diploid cultivated species, and describes progress in the overall goal of transferring this trait to cultivated tetraploid potato.

Materials and methods

Hybridization

Four wild and two cultivated potato species were utilized in crosses (Table 1). The synthetic allotetraploid hybrid clones, *S. etuberosum* × *S. pinnatisectum* (4*x*-EP), provided by Hermsen and Taylor (1979) were the source of PLRV resistance for crossing experiments with wild and cultivated clones. A breeding derivative involving *S. verrucosum* (coded TV^5), a wild, self-compatible diploid species developed by Hermsen and coworkers (1981), was used to avoid nuclear cytoplasmic sterility. The wild tetraploid species, *S. acaule*, was also utilized. Hybrids resulting from reciprocal crosses between 4*x*-EP, TV^5 and *S. acaule* were further crossed to diploid and tetraploid cultivars, reciprocally.

All pollinations were made in an insect-proof screenhouse. Crosses were performed on cut inflorescences maintained in containers filled with a saturated aqueous thiram solution, or on potato scions grafted on tomato stocks. The crossability among the species utilized was determined by fruit set, no. of plump seeds per berry and seed germination (Brown et al. 1984).

In vitro embryo culture

Embryos were excised from immature imbibing seeds when lack of seed development or germination became a problem. In addition, embryos were excised from ovules developing in berries between 3–4 weeks after pollination. Berries and harvested dried seeds were surface sterilized by immersion in a filtered solution of 5% sodium hypochlorite for 10 min. Intact embryos were explanted to 9 cm petri dishes containing the following nutrient agar media: Murashige and Skoog (MS), MS fortified with 15% coconut milk (MS+CM), Nitsch and Nitsch (NN) and NN enriched with 15% coconut milk (NN+CM). Petri dishes were sealed with parafilm and transferred to a culture room maintained at 16°–22 °C with a photoperiod of 14 h, provided by cool-white fluorescent light.

Cytological analysis

Pollen fertility. Pollen fertility was estimated by stainability in all parental genotypes and their hybrid derivatives. Fresh pollen collected 1 day after anthesis was stained with aceto-carmine-glycerol (Marks 1954; Mortensen et al. 1964).

Chromosome counts. Fixation of root tips was done with glacial acetic acid (saturated with ferric acetate):ethyl alcohol (1:3). Fixed root tips were transferred to 70% ethanol and placed in a refrigerator at 5 °C. Root tips were hydrolyzed in 1N HCl at 60 °C for 10 min, stained in aceto-orcein for a minimum of 30 min, squashed in a drop of 45% acetic acid with coverslip, and warmed gently over a spirit lamp to destain cytoplasm.

Inoculation of PLRV with green peach aphids

Parents and progenies were inoculated with PLRV-bearing GPA. Parents were represented either by rooted, vegetatively propagated cuttings or by seedlings derived from selfed seed where applicable. The term "seedling", as used here, refers to primary plantlets derived from true seed. Hybrid progenies were inoculated as seedlings, as was open-pollinated DTO-33 (OP DTO-33), which served as a susceptible control.

The "T" isolate of PLRV, obtained in Peru, was maintained over several vegetative generations in the potato cultivar 'Ticahuasi' (CIP 1978). Virus inoculum was prepared by transmission by GPA to 4-week-old greenhouse grown *Datura stramonium* seedlings.

The *D. stramonium* seedlings developed interveinal chlorosis and were allowed to grow 4 weeks, during which time virus titer, as determined by enzyme-linked immunosorbent assay, ELISA (Clark and Adams 1977; Salazar 1983), and GPA populations reached high levels and provided viruliferous aphids for transfer to the plants that were to be challenged with virus.

Test plants were inoculated 6 weeks after sowing or rooting. Apterous aphids (25/plant) were caged on individual plants in nylon screen cylinders that covered the entire plant. Aphids were killed after 10 days by spraying the plants with 0.1% w/v aqueous solution of methidamophos, transplanted into 10 cm pots and grown in an insect-free screenhouse with temperatures fluctuating between 18°–25 °C. Symptomatology was noted.

Test plants were indexed for PLRV by graft inoculation to a diagnostic host, *Datura stramonium*, 1 month after the killing of aphids. The original potato test plants, scions, and *Datura* stocks were indexed by ELISA 1 and 2 months after grafting. The *Datura* stocks were assayed again after 3 months. Only ELISA was used to index the selfed progeny of hexaploid *S. acaule* × 4*x*-EP hybrids (6*x*-AEP) and hybrids of these with *S. phureja* (AEPP). The later results were expressed as susceptible (i.e., virus detected) versus resistant (i.e., virus undetected).

Results

Crossability of 4x-EP with S. acaule, S. verrucosum (TV5) and cultivated diploid and tetraploids

Over 600 reciprocal pollinations made between the allotetraploid 4*x*-EP hybrid and cultivated tetraploid *S. tuberosum* and diploid *S. phureja* did not produce seeds. Parthenocarpic berries were produced, however, when 4*x*-EP was the pistillate parent. In contrast, abundant berry set and seed production were obtained on self-pollinated 4*x*-EP plants used as a control.

The hybrids involving 4*x*-EP and two wild species are referred to as triple hybrids and have the species composition of [acl × (etb × pnt)], [(etb × pnt × acl], [ver × (etb × pnt)], and [(etb × pnt) × ver]. These are coded AEP, EPA, VEP, EPV, respectively, using the first letter of the species involved.

The crossing *S. acaule* ($2n=48$) as the pistillate parent with 4*x*-EP (AEP), 6 berries resulted from 217 pollinations, while 34 berries developed from 224 pollinations with 4*x*-EP (EPA) as the pistillate parent. The former yielded 26.7 and the latter 2.7 plump seeds per berry (Table 2).

Table 1. Potato species used to transfer PLRV resistance from wild to cultivated species

Species	Abbreviation	Series	Ploidy[b]	Origin	Source[a]
S. etuberosum	etb	Etuberosa	2*x* Sc	Chile	PI
S. pinnatisectum	pnt	Pinnatisecta	2*x* Si	Mexico	PI
S. verrucosum	ver	Demissa	2*x* Sc	Mexico	IvP
S. acaule	acl	Acaulia	4*x* Sc	Peru	CIP
S. phureja	phu	Tuberosa	2*x* Si	Colombia	CIP
S. tuberosum	tbr	Tuberosa	4*x* Sc	Peru	CIP
etb × pnt	EP	hybrid	4*x* Sc	Netherlands	IvP

[a] PI = United States Department of Agriculture, Potato Introduction Station, Sturgeon Bay/WI; IvP = Agricultural University, Institute of Plant Breeding, Wageningen, The Netherlands; CIP = International Potato Center, Lima, Peru
[b] Sc = self-compatible; Si = self-incompatible

Table 2. Crossability of 4*x*-EP with *S. acaule* and *S. verrucosum*

Cross	No. of pollinations	% berry set	No. of seeds			Plump seeds/berry	Plump seeds/ pollination
			Total	Shrivelled	Plump		
acl × 4*x*-EP	217	2.7	153	17	136	26.7	0.6
4*x*-EP × acl	224	15.1	673	581	92	2.7	0.4
acl ×	40	80.0	3 194	280	2 914	91.0	72.8
TV^5 × 4*x*-EP	510	56.0	5 727	2 517	3 210	11.2	6.3
4*x*-EP × TV^5	228	38.1	931	910	21	0.2	0.1
(TV^5) ×	83	89.1	3 898	931	2 967	40.1	35.7

More than half of the 510 pollinations performed in crosses between TV^5 as the pistillate parent and 4*x*-EP (VEP) resulted in berry formation with an average of 6.3 plump seeds per berry (Table 2). In contrast, only 21 plump seeds were produced from 87 berries resulting from 228 reciprocal pollinations (EPV). Post-fertilization breakdown appeared to be occurring in the latter cross as indicated by many shrivelled seeds and heavy parthenocarpic berry set.

In vitro embryo culture to obtain AEP, EPA, and EPV triple hybrids

Some AEP seeds were initially obtained from crosses. Although normal embryos were observed in the testa, the seed did not germinate despite pretreatment with gibberellic acid. Embryos were excised from seeds sown in sterile culture, and these developed into normal plants. In addition, the testa of VEP seeds disintegrated upon drying, leaving the embryos naked. These did not germinate when placed in petris with moistened filter papers. Normal germination occurred, however, when the seeds were sown immediately after extraction, before drying.

Sterile excision resulted in successful rescue of embryos from berries. Sixty-five coiled embryos were excised from 3 to 4-week-old berries from the AEP cross from with 31 seedlings developed (Table 3). Out of 53 crescent-shaped embryos excised from the reciprocal cross EPA, 18 seedlings resulted. From the cross VEP, more than 400 coiled embryos were excised and 275 seedlings raised. However, the reciprocal cross, EPV, resulted in only 21 torpedo-shaped embryos out of 87 berries harvested, from which 15 seedlings were grown (Table 3). In culturing these embryos no significant differences were noted in growth on Murashige and Skoog or Nitsch and Nitsch medias, with or without coconut milk added.

Ploidy and pollen fertility of triple hybrids

Root-tip chromosome counts of AEP and EPA families showed 45 clones with 48 chromosomes, confirming the tetraploid nature of these hybrids. However, the triple hybrids, AEP-38 and AEP-59, were found to be hexaploids with $2n = 72$ (6*x*-AEP). Their mode of origin will be the topic of a future publication.

Pollen stainability in the parental species *S. acaule* and in the interspecific parental hybrid 4*x*-EP was over 70%. With eight exceptions, the tri-specific hybrids AEP and EAP were male sterile. The 6*x*-AEP hybrids, specifically AEP-38 and AEP-59, had pollen stainability of 80%. Five AEP hybrids showed a mean pollen stainability of 3.0% and one EPA clone of 7.2%. The size of the pollen was discretely bimodal suggesting that 2*n* restitution was occurring. The remaining AEP and EPA hybrids were pollen sterile.

Table 3. In vitro embryo rescue from developing berries of triple species hybrids

Cross	Embryo type	No. excised embryos	No. embryos in culture medium[a]	No. seedlings	Code of seedlings
acl × 4*x*-EP	small-coiled	65	15 NN	9	4*x*-AEP
			17 NN + CM	8	4*x*-AEP
			17 MS	7	4*x*-AEP
			16 MS + CM	6	4*x*-AEP
4*x*-EP × acl	crescent-shaped	53	12 NN	6	4*x*-EPA
			14 NN + CM	4	4*x*-EPA
			15 MS	3	4*x*-EPA
			12 MS + CM	5	4*x*-EPA
TV_5 × 4*x*EP	large-coiled	403	182 NN	141	3*x*-VEP
			168 MS	134	3*x*-VEP
4*x*-EP × TV_5	torpedo-shaped	20	20 NN	15	3*x*-EPV

[a] NN = Nitsch and Nitsch; NN + CM = Nitsch and Nitsch + coconut milk; MS = Murashige and Skoog; MS + CM = Murashige and Skoog + coconut milk

Table 4. Results of ELISA tests of parental clones, hybrid progeny and *Datura stramonium*

Parents and hybrids	No. clones	ELISA 1 mo.[a]	ELISA 2 mos.[a]	ELISA of grafted *Datura*			ELISA of grafted scions	
				1 mo.[a]	2 mos.[a]	3 mos.[a]	1 mo.[a]	3 mos.[a]
S. etuberosum	100	(−)						
S. pinnatisectum	100	(+)						
4*x*-EP	5	(−)						
4*x*-EP selfed	125	(−)						
2*x*-ver	8	(+)*		(+)*			(+)*	
2*x*-ver selfed	200	(+)*						
4*x*-acl	3	(+)						
4*x*-acl selfed	75	49 (+), 26 (−)						
4*x*-6*x*-AEP	31	(−)	(−)	(−)	15 (+), 16 (−)	(+)*	(−)	(−)
4*x*-EPA	16	(−)	(−)	(−)	10 (+), 6 (−)	(+)*	(−)	(−)
3*x*-VEP	200	(−)	(−)	(−)	120 (+), 80 (−)	(+)*	(−)	(−)
3*x*EPV	15	(−)	(−)	(−)	7 (+), 8 (−)	(+)*	(−)	(−)
Control								
DTO.33	50	(+)*		(+)*			(+)*	
6*x*-VEP	10					(−)	(−)	

[a] (−) = Negative in ELISA test; (+) = Positive in ELISA test; * = showed visual symptoms for PLRV

Table 5. PLRV resistance of AEPP, VEPP and self-hexaploid offspring

Family	No. clones	PLRV resistance	
		Resistant	Susceptible
AEPP	52	17	35
VEPP	1	–	1
AEP.38 selfed	18	10	8
AEP.59 selfed	53	25	28

Crossability and self-fertility of triple EPA, AEP, VEP and EPV hybrids

Self-pollination among 16 hybrid EPA clones and 29 AEP clones did not result in berry formation, indicating male and female sterility. More than 1,000 cross-pollinations between the triploid hybrids VEP and EPV, and 2*x* and 4*x* cultivated clones produced only two plump seeds and small seedless berries. Self-pollinations of VEP and EPV did not result in berry formation. Crossing of EPA and AEP hybrids with 2*x* and 4*x* cultivated clones in both directions mostly yielded parthenocarpic berries.

Self-pollination of 6x-AEP and embryo culture to obtain 6x-AEP × phureja hybrids

Selfing of the 6*x*-AEP clones (38 and 59) produced over 70% berry set and averaged 29 seeds per berry. Crosses of these hexaploids with *S. phureja* as the pollen parent yielded hybrids when embryo rescue was employed. Out of 149 pollinations, 65 berries were obtained from the

cross 6*x*-AEP × *S. phureja* (AEPP). Although most seeds in the berries were empty or shrivelled, an average of 2.1 intact embryos per berry was excised from 20 to 25-day-old berries and cultured. A total of 131 embryos was obtained of which 70 were crescent-shaped and 48 torpedo-shaped. Both types were cultured on Nitsch and Nitsch nutrient medium. A total of 89 grew to the plantlet stage, were transplanted to pots and grown in the glasshouse. Of the clones, 30 were unthrifty and died 2–3 weeks after transplanting; 59 survived and were screened for PLRV resistance.

PLRV resistance and aphid colonization

Aphids established colonies and reproduced on progeny of O.P. DTO-33, the susceptible control. Aphids multiplied rapidly on acl, ver, and pnt parental materials, but died on etb. The number of aphids decreased sharply after placement on AEP, EPA, VEP and EPV hybrid progenies. Testing indicated that all genetic materials containing etb, e.g. 4*x*-EP clones, 4*x*-EP selfed, AEP, EPA, VEP and EPV were infected by PLRV (Table 4). This was only verified upon graft challenge to *D. stramonium*. While ELISA detected virus in *D. stramonium* by 3 months after grafting, in no case did ELISA detect PLRV in tissues of etb-containing hybrid potato tissue. The *D. stramonium* showed clear interveinal chlorosis, symptoms of PLRV, besides testing positive for PLRV with ELISA.

Selfed progeny of the two 6*x*-AEP hybrids, AEP-38 and AEP-59, segregated 10:8 and 25:28 for resistant (virus undetected) versus susceptible (virus detected) to PLRV titer buildup. Hybrid progeny from the cross 6*x*-AEP × phu segregated 17:35 resistant:susceptible (Table 5).

No tuber set was found among etb, 4*x*-EP, EPV, or VEP at sea level on the Pacific Coast of Peru or in the Andean Highlands at 3,280 m elevation. However, AEP and EPA hybrids set tubers in both locations. The occurrence of tubers among hybrid clones was related to the plant habit. Non-tuberiferous clones showed a strong tendency toward perenniality with woody stems and little or no stolon development. In contrast, tuber-bearing hybrids were clearly herbaceous in habit and senesced upon setting tubers.

Discussion

Solanum verrucosum is the only diploid species from series Demissa growing in North America and is a link with the Tuberosa species of South America (Hawkes 1978). It may also be an important bridging species between 2*x* and 4*x* *Solanum* species on both continents (Hermsen 1980). Genotypes of *S. verrucosum* with cytoplasm of 2*x*-*S. tuberosum* (TV^5) were successfully crossed with *S. etuberosum* × *S. pinnatisectum* hybrids and produced triple species hybrids (VEP and EPV) resistant to PLRV. All were sterile because of genome nonhomology.

The South American tetraploid species has been utilized previously as a bridinging species in wide interspecific crosses (Hermsen and Ramanna 1973; Hermsen 1984). In line with this, the cross between the interspecific hybrid 4*x*-EP and *S. acaule*, reported here, has served as the bridge to transfer resistance to PLRV titer build-up from *S. etuberosum* to hybrid populations involving four different series: Acaulia, Etuberosa, Pinnatisecta and Tuberosa, i.e., the AEPP hybrid. The 4*x*-EP alone was not crossable to *S. phureja*, but its hybrid with *S. acaule*, 6*x*-AEP, was.

Embryos excised from seeds and young berries produced by crossing these species have provided viable hybrid plants. The lack of germination of hybrid seeds may be due either to strong dormancy, small embryo size or underdeveloped endosperm, especially in the case of EPV hybrids. In vitro excision of developing embryos saved time and overcame apparent developmental barriers, although seedlings from fully developed, but nongerminable harvested seed were also obtained with the intervention of embryo culture. The net result was that these crossability barriers were overcome, with the aid of in vitro manipulation, and vigorous, PLRV-resistant and sometimes fertile hybrids were produced. The segregation of resistance to titer buildup in selfed progeny of 6*x*-AEP and in AEPP hybrids suggests oligogenic control of resistance to titer buildup.

Only tetraploid clones (AEP and EPA) with *S. acaule* genomes set tubers. However, the tiploid hybrid families (VEP and EPV) did not tuberize despite possessing two genomes from the taxa *S. pinnatisectum* and *S. verrucosum*, which tuberize under 12 h daylength. This association of annuality and tuber formation is an important observation. There was a discrete simultaneous change to annuality and tuberifery during the introgression of PLRV resistance from *S. tuberosum*. This association may have evolutionary significance, since the series Etuberosa type is assumed to be ancestral to the tuberiferous series.

The Endosperm Balance Number (EBN) hypothesis for *Solanum* species has been used to assign numbers to species based on interspecific crossability patterns (Johnston and Hanneman 1980, 1982; Johnston et al. 1980). As long as the endosperm maternal to paternal EBN ratio is 2:1, taxa with the same number producing reducing, or 1 *n*, gametes should be readily crossable. Species *S. etuberosum* and *S. pinnatisectum* have been assigned an EBN of 1. Unimpeded hybridization between the two is, therefore, predictable. In another study, crossability between *S. brevidens* and *S. commersonii*, two EBN = 1 species, has been confirmed (Ehlenfeldt and Hanneman 1984). EBN incompatibility may be remedied

by somatic or sexual polyploidization. The former was used by Hermsen and coworkers (1981) to raise the EBN of the 2*x*-EP hybrid from 1 to 2 by producing the 4*x*-EP allotetraploid. Crossability of 4*x*-EP with *S. acaule* (EBN = 2) and *S. verrucosum* (EBN = 2) was expected.

The appearance of 6*x*-AEP, however, seems to violate EBN rules. Fertilization of putative 2*n* eggs of *acaule* with normal 1*n* pollen of 4*x*-EP is expected to result in nonfunctional endosperm. The success of crossing the 6*x*-AEP, which should have EBN of 3, with *S. phureja,* EBN = 2, is also unexpected. Hermsen and Ramanna (1973) produced triploid hybrids by crossing *S. acaule* (EBN = 2) and *S. bulbocastanum* (EBN = 1), a combination which also should result in nonfunctional endosperm by EBN theory. Hermsen (1984) has pointed out that an imputed lack of crossability between two species may only be ascertained by large scale crossing, using a range of genotypic variation under varing environmental conditions. It is also possible that crossability patterns predicted by the EBN hypothesis may be circumvented by embryo rescue.

Studies of meiotic chromosome pairing of VEP triploids and doubled hexaploids by Ramanna and Hermsen (1981) revealed that potential for gene transfer from the *S. etuberosum* genome to the cultivated A genome via recombination is considerable. The achievement of hybrids showing fertility and crossability to cultivated potato in this study augurs well for the successful transfer of PLRV resistance from *S. etuberosum* to future potato cultivars.

Protoplast fusion between *S. tuberosum* and another member of the series Etuberosa, *S. brevidens*, has been achieved (Austin et al. 1985; Barsby et al. 1984; Fish et al. 1987). The objective was to transfer PLRV resistance from *S. brevidens* to cultivated potato. Although the resistance needs further characterization, this approach appears to be a rapid means of introgressing genes of Etuberosa into the cultivated tetraploid gene pool. Based on follow-up studies of fertility, crossability and agronomic traits, these fusion hybrids and backcross derivatives appear to be very useful breeding materials (Austin et al. 1986; Ehlenfeldt and Helgeson 1987).

The relative ease of producing these multiseries hybrids provides ready access to needed traits even in nontuberiferous *Solanum* spp. Production of fertile hexaploid AEP hybrids via union of normal 1*n* pollen of 4*x*-EP with 2*n* *S. acaule* eggs points out a mechanism by which meiosis, in hybrids with haploid complements of nonhomologous genomes, can be sufficiently normalized to allow further cycles of sexual improvement.

Primitive traits, such as lack of tuber formation, can be replaced by tuber-bearing habit along with annuality in discrete steps. The rapidity of conversion encourages further efforts to draw other resistances to biotic and abiotic factors from non-tuberiferous *Solanum* species.

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