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Application of interspecific sesquiploidy to introgression of PLRV resistance from non-tuber-bearing *Solanum etuberosum* to cultivated potato germplasm *

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Summary. Hybridization of synthetic allotetraploids of *S. pinnatisectum* with *S. etuberosum* (4*x*-EP) with *S. acaule* ($2n=4x=48$) resulted in two individuals that were highly fertile, in contrast to all other progenies. The unique individuals are hexaploids, $2n=72$, while the other progenies are tetraploids, $2n=48$. They are thought to be the products of a union between $2n$ eggs of *S. acaule* and normal $1n$ microspores of 4*x*-EP. The fertile hexaploids (designated 6*x*-AEP) produced abundant selfed seed and viable hybrids with cultivated diploid potato, *S. phureja,* when developing embryos were rescued from berries and cultured before transplanting to pot culture. The extreme variability in chromosome constitution of the hybrids with *S. phureja* and selfed progenies indicates that addition and substitution lines of etb chromosomes bearing genes of interest to breeders could easily be produced from this material. The production of sesquiploids, as the 6*x*-AEP hybrids are called, is discussed as a useful bridging step in the introduction of alien genes from genomes that share little homology with the cultivated genome.

Key words: Breeding recombination – Disease resistance – Interspecific hybridization – Wide crosses – Alien addition lines

Introduction

The transfer of traits from a wild to a cultivated plant species by means of interspecific sexual hybridization is often hindered by lack of homology between genomes. The probability of successful introduction of small amounts of exotic genome via recombination is directly proportional to the affinity between genomes. Moreover, combinations of species with different genomes leads to hybrids possessing imbalanced meiosis and inviable gametes. This sterility reduces the probability of gene exchange even further. Sterility is sometimes overcome by doubling the chromosome number which provides homologous pairing. The existence of balanced pairing between identical homologues after chromosome doubling, however, has the crucial disadvantage of reducing the homeologous pairing necessary for intergenomic gene transfer. The challenge is to promote recombination between heterologous genomes in meiotic milieus conducive to viable gametogenesis. One scheme seldom employed is sesquiploidy: the construction of hybrids possessing the sporophytic or $2n$ complement of one species, preferably from the cultivated gene pool, and the haploid or $1n$ set of the wild species from which useful traits are to be extracted. The presence of the $2n$ complement is achieved through the union of a $2n$ gamete (i.e., a gamete with the same chromosome number as the parent) from one parent with the normal reduced $1n$ gamete of another. The combination of the two in such proportions can foster the partitioning of the $2n$ complement in a balanced fashion, overshadowing the deleterious effects of the unequal partitioning of the $1n$ complement. The term sesquidiploid was first applied by Webber (1930) to such a hybrid between *Nicotiana tabacum* and *N. sylvestris*. It appeared again in a review by Goodspeed and Bradley (1942) in reference to interspecific *Nicotiana* spp. hybrids. In all cases, the chromosome number of the hybrid indicated the functioning of $2n$ eggs, and unexpectedly high fertility was found despite the lack of pairing partners for the exotic genome. Recently a sesquidiploid intergeneric

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hybrid was described that constituted a major breakthrough in the transfer of traits from *Solanum lycopersicoides* into *Lycopersicon esculentum*, or cultivated tomato (Rick et al. 1986). Further work has established the occurrence of trisomics and euploid recombinant phenotypes presumably incorporating *Solanum lycopersicoides* genes into *esculentum* chromosomes (DeVerna et al. 1987).

Among the non-tuber-bearing *Solanum* species there are many traits that would be valuable in cultivated potato. Non-tuber-bearing species of the series Etuberosa are separated from cultivated potato by strong crossing and genome homology barriers. *Solanum etuberosum*, a diploid Chilean member of this series, has attracted interest from potato breeders because it has resistance to frost, potato virus Y and potato leafroll virus (Jones 1979; Hermsen 1980; Brown 1980). The transfer of resistance to potato leafroll virus (PLRV) from *S. etuberosum* (etb) to *S. tuberosum*, cultivated potato, is a very important goal. 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 1981). However, Hermsen and Taylor (1979) crossed etb successfully with a Mexican, tuber-bearing, wild diploid species, *S. pinnatisectum* (pnt). The production of this new interspecific hybrid was the first step in making the valuable traits of etb accessible to potato breeders. Although the F1 hybrids were sterile, the doubled allotetraploids were fertile (Hermsen et al. 1981). The present paper reports on the production of a sesquitetraploid hybrid with the allotetraploid wild species, *S. acaule* (acl). Data will indicate the value of this kind of hybrid to transfer traits from etb.

Materials and methods

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 over tomato. The crossability among the species utilized was determined by fruit set, number of plump seeds per berry, and seed germination.

Embryos were excised from ovules developing in berries between 3 and 4 weeks after pollination. Berries were surface sterilized by immersion in a filtered solution of 5% sodium hypochlorite for 10 min. Berries were dissected in a laminar flow hood and the intact embryos 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 the culture room maintained at 16°–22 °C with a photoperiod of 14 h provided by cool-white fluorescent light.

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).

In vitro pollen germinability was conducted in some interspecific hybrids using a hanging-drop method (Mortenson et al. 1964). Pollen from freshly dehisced anthers was tapped into a droplet of germination solution consisting of 20% w/v sucrose and 50 ppm boric acid dissolved in distilled water. This was incubated 18 h at 15 °C and the number of pollen grains with a developing pollen tube was noted relative to ungerminated grains.

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

Meiotic studies were done by a regular aceto-orcein squash method. Buds were fixed in 3:1, ethyl alcohol: glacial acetic acid for 24 h, washed in 70% ethanol and then placed in fresh 70% ethanol for long-term storage at 4 °C. Fixed single anthers were cut into three or four pieces in a drop of 1% aceto-orcein and then pressed with a mounted needle until the pollen mother cells were released. Debris was scraped to the side and a coverslip was placed over the droplet without pressure. After gentle heating the coverslip was squashed to spread the chromosomes. Different stages of meiosis were observed under ×150, ×300, ×600 and ×1500.

Results

Examination of root tip chromosomes revealed that 29 of the 31 progeny resulting from crossing acl with 4*x*-EP (designated 4*x*-AEP) were tetraploids, $2n=48$ (Table 1). Of these 29, 5 progenies had some $2n$ pollen and averaged 7.2% stainable pollen. Twenty-four remaining progenies had unstained, aborted pollen. In contrast, 2 hexaploid individuals, $2n=72$ (designated 6*x*-AEP), produced highly stainable pollen averaging 80.0% (Table 2). No seed resulted from selfs of 4*x*-AEP hybrids nor recip-

Table 1. Somatic chromosome number of hybrids between acl and 4*x*-EP, 6*x*-AEP, and 2*x*-phu, and selfs of 6*x*-AEP.38 and 6*x*-AEP.59

Cross \ Somatic chromosome No.	37	38	44	45	47	48	50	51	54	55	56	58	59	60	61	62	64	65	66	67	68	69	70	71	72	73	74	75	77	N_{total}
4*x*-acl × 4*x*-EP						10																			2					12
6*x*-AEP.38 selfed					1														1											2
6*x*-AEP.59 selfed			1						1				1	1		1	1	1			2	3		1		1	1	1		16
6*x*-AEP.38 × 2*x*-phu	1	2		1			2	2	1	1	1	1	1	1	2	4	3	1	1	1		4	1			1		1	1	34

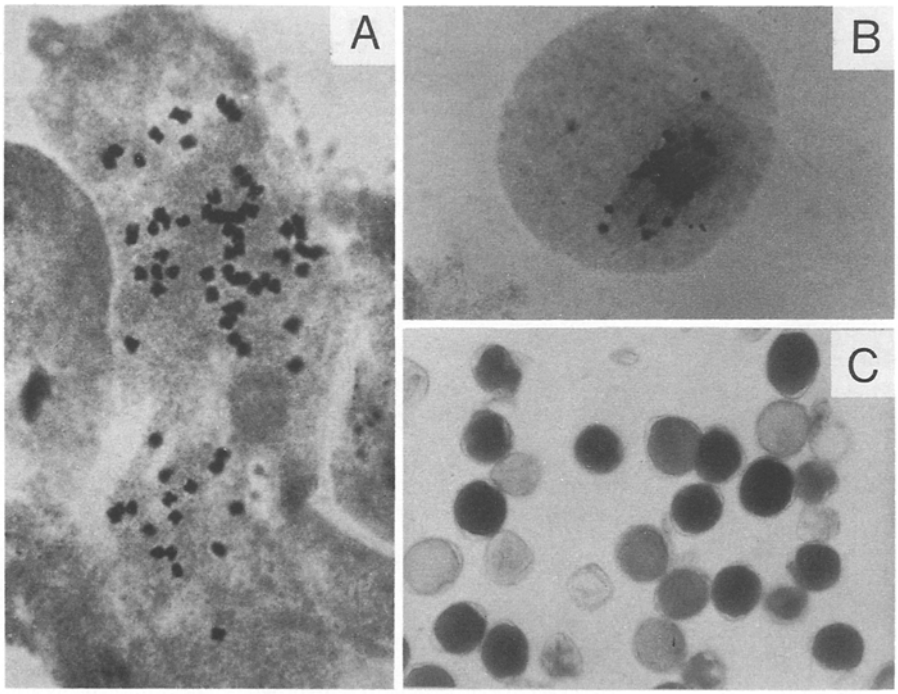

Fig. 1. **A** Root tip chromosomes (1000 ×); **B** metaphase I of microsporogenesis (400 ×); and **C** stained pollen (400 ×) of 6*x*-AEP hybrid No. 38

Table 2. Pollen stainability and germination of species and hybrids including sesquitetraploid 6*x*-AEP hybrids

Species	No. clones	Ploidy level	Mean pollen stainability	Mean pollen germination
etb	5	2*x*	85.6	63.1
pnt	5	2*x*	73.2	51.3
4*x*-EP	5	4*x*	79.6	55.0
acl	3	4*x*	87.0	74.6
4*x*-AEP	24	4*x*	0.0	0.0
4*x*-AEP	5	4*x*	7.2	3.9
6*x*-AEP	2	6*x*	80.0	56.0
AEPP	19	variable	12.5	1.3

Table 3. Number of pollinations, berry set, and seeds per berry of diverse crosses involving 4*x*-AEP and 6*x*-AEP

Cross	No. pollinations	Berry set (%)	Seeds per berry
4*x*-AEP selfed	107	0	0
6*x*-AEP selfed	48	70.8	29.0
4*x*-AEP × 4*x*-tbr	134	15.6	0
4*x*-tbr × 4*x*-AEP	202	0	0
4*x*-AEP × 2*x*-phu	218	28.8	0
2*x*-phu × 4*x*-AEP	176	0	0
6*x*-AEP × 4*x*-tbr	97	20.6	0
4*x*-tbr × 6*x*-AEP	55	0	0
6*x*-AEP × 2*x*-phu	149	41.6	2.1[a]
2*x*-phu × 6*x*-AEP	116	0	0

[a] Excised from berries 20–25 days after pollination

rocal crosses with either diploid or tetraploid cultivated potato. The 6*x*-AEP hybrids, on the other hand, yielded an average of 29.0 selfed seeds per berry. Berry set with selfings and crosses of 6*x*-AEP to *S. phureja* (designated AEPP) was 70.8% and 41.6%, respectively (Table 3). AEPP embryos did not complete development and form a viable seed, but 2.1 embryos per berry could be extracted after excision into axenic culture.

Root tips of the two 6*x*-AEP clones contained 72 chromosomes, indicating the clones were hexaploids (Fig. 1 A). Metaphase I of pollen mother cells of 6*x*-AEP progenies showed a equatorial plate with well-aligned chromosomes, as well as chromosomes precociously migrating to the poles (Fig. 1 B). The succeeding stages proceeded normally without evidence of aberration. Sporogenesis led to the formation of viable pollen as evidenced by the high pollen stainability (Fig. 1 C), while the production of abundant seed upon selfing is confirmation of high frequency of functional male and female gametes.

The selfed progeny of 6*x*-AEP.38 and 6*x*-AEP.59 displayed a great range of somatic chromosome numbers, from $2n=44-2n=75$. This phenomenon was also apparent in AEPP hybrids (Table 1). The somatic chromosome number of 34 AEPP hybrids ranged from $2n=37-2n=77$ (Table 1). Twenty-nine survived and were grown in pots in the greenhouse. Pollen production of 19 of these hybrids was examined. Ten progenies had unstained aborted pollen, 3 progenies had less than 5% stainable pollen, and 6 had stainabilities ranging from 20%–55%, while overall pollen germination was 1.3% (Table 2).

Discussion

The high pollen stainability and germination of the 6*x*-AEP hybrids, 80.0% and 56.0%, respectively, indicated that it is clearly a channel through which etb genes can be passed. The fact that etb and pnt chromosomes lack pairing affinity would be expected to produce tetrads with imbalanced chromosome constitution that would abort development. Apparently the unique assemblage of the sporophytic complement of acl and the haploid complement of 4*x*-EP permitted a sufficient normalization of meiosis so that functional gametes appeared in high frequency. It may be postulated that balanced partitioning of acl chromosomes may have buffered the unequal distribution of etb and pnt to the gametes. Thus, sesquiploidy offers a unique opportunity to recover viable gametes even after abnormal chromosome pairing.

The occurrence of two 6*x* plants was not totally unexpected. The functioning of 2*n* eggs in acl has been reported in successful interspecific hybridization in previous work (von Wangenheim 1954). In any case, this example points out a new feature of 2*n* gametes as a tool to produce sesquiploids harboring genomes that do not have homology with the cultivated potato genome and have valuable genes to be extracted. Use of 8*x* acl plants, which can be produced by conventional somatic doubling of normal 4*x* acl, could achieve the same objective of getting sesquiploid plants by 8*x* acl-4*x* EP crosses. Therefore, a sesquiploid approach can be implemented even when 2*n* gametes are not available in the specific genetic material to be utilized. The construction of sesquiploids, such as the 6*x*-AEP hybrids reported here, sets the stage for deriving chromosome addition and substitution lines that can lead to the introgression of alien genes through recombination after several further sexual cycles. Selfing of the 6*x*-acl chromosomes would result in production of plants with complete sets of acl chromosomes in addition to extra etb chromosome(s) that carry PLRV resistance gene(s). This expectation has a good experimental basis as the 6*x* AEP had high seed set upon selfing (Table 3), a large variation in chromosome number (Table 1), and resistance to PLRV is probably simply inherited (Chavez et al. 1988). Only genetic factors from one or two etb chromosomes to be incorporated into the cultivated potato gene pool. The extremely broad range of chromosome numbers in the AEPP progenies is a useful start at sorting out unwanted alien chromosomes. The high pollen stainability of certain progenies augurs well for the success of future sexual cycles in introducing limited amounts of alien genes, the presence of which can be assured by repeated selection. The incorporation of resistance to titer buildup of PLRV from etb (Chavez et al. 1988) into cultivated potato will probably occur very rapidly as a result of sesquiploid methodology.

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