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2n gametes in the potato: essential ingredients for breeding and germplasm transfer

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Abstract 2n gametes are the result of meiotic mutations occurring during micro – and mega-sporogenesis. They have been identified in several plant species of different taxa. The potato is probably the crop plant where they have been most intensively studied and also more appropriately used for the genetic improvement of cultivated genotypes. This paper reviews how 2n gametes allow potato breeders to broaden the genetic basis of the cultivated *Solanum tuberosum*, introducing both new genes for the improvement of traits of interest and allelic diversity to maximize heterozygosity. We provide molecular and breeding evidence that, in the potato, 2n gametes represent a unique tool to transfer target genes from wild forms to the cultivated tetraploid gene pool. In fact, species directly crossable to *S. tuberosum* haploids can be exploited through sexual polyploidization crossing schemes. For those which have developed crossability barriers, specific crossing schemes based on ploidy bridges can be designed. In this paper we also give possible hypotheses to explain conflicting results on the genetic control and meiotic mutations responsible for 2n-gamete formation in the potato.

Keywords *Solanum* spp. · Meiotic mutations · Genetic control · Resistance to stress · Ploidy bridges

Introduction

Meiosis is a fundamental and unique event which, under normal conditions, leads to chromosome-number reduction in the gametes. It is characterized by specific cytological features and integrated events controlled by a large number of genes, usually dominant, which are stage-, site- and time-specific. The normal pattern of meiosis can be drastically modified by several mutations (Kaul and Murthy 1985). Those leading to the production of gametes with an unreduced chromosome number (2n gametes) have been found in several plant species, including *Brassica* spp., *Citrus* spp., *Fragaria* spp., *Malus* spp., *Manihot* spp., *Medicago* spp., *Solanum* spp., and *Trifolium* spp. (Veilleux 1985).

The cultivated potato (*Solanum tuberosum*, $2n=4x=48$) and its many cultivated and wild relatives is the crop species where 2n gametes have been not only more intensively studied and characterized but also more appropriately used for the genetic improvement of cultivated genotypes. For a review of the use of 2n gametes in basic and applied research, see Peloquin et al. (1999).

The production of 2n gametes has been fundamental to the origin and population biology of polyploid potatoes (den Nijs and Peloquin 1977; Iwanaga and Peloquin 1982; Bretagnolle and Thompson 1995). In the evolutionary scheme proposed by den Nijs and Peloquin (1977), crosses involving both n and 2n gametes linked all ploidy levels of *Solanum* species, providing the opportunity for gene flow all the way from diploid to hexaploid species. 2n gametes also represent unique tools for genetic studies. This is important given that the tetrasomic inheritance of the tetraploid potato makes such studies very difficult. A number of mating designs based on the use of 2n gamete-producing diploid and tetraploid parents were proposed to study the inheritance of complex traits (Tai 1994) and for covariance analyses (Haynes 1992). 2n gametes have been used to determine gene-centromere map distances, such as those of isozymes and the yellow tuber flesh color loci (Douches and Quiros 1987), and those of genes conferring resistance to viruses and nematodes (Wagenvoort and Zimnoch-Guzowska 1992). They have also been employed to infer the physical location of QTLs controlling total tuber yield (Buso et al. 1999a).

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A very important feature of 2n gametes is that they make the potato the best organism in which to manipulate all sets of chromosomes for breeding purposes. They allow breeders to broaden the genetic diversity, introducing both new genes for the improvement of traits of interest and allelic diversity to maximize heterozygosity in tetraploid varieties. In this paper we provide molecular and breeding evidence that in the potato 2n gametes represent essential tools for the improvement of the cultivated potato. We also discuss the genetic consequences associated with 2n gamete production, and give possible hypotheses to explain conflicting results on the meiotic mutations responsible for 2n gamete formation.

Meiotic mutations leading to 2n gamete production

Cytological mechanisms and genetic control

Mutations causing 2n gamete formation may affect chromosome pairing, centromere division, spindle formation or cytokinesis during micro- and mega-sporogenesis. The first meiotic mutants in *Solanum* were discovered by Hanneman and Peloquin (1969). The unusually high seed set obtained from 4*x*×2*x* crosses, as well as the tetraploid chromosome number of the resulting progeny strongly suggested that a meiotic mutation systematically leading to 2n pollen formation was present in the diploid parents. The different characteristics of 4*x* F_1 progeny from different diploid parents also indicated that various meiotic mutations were involved in 2n pollen formation.

Cytological analyses by Mok and Peloquin (1975a) allowed the identification of three distinct meiotic mutations during microsporogenesis, parallel spindles (ps), including also fused and tripolar spindles, premature cytokinesis-1 (pc1), and premature cytokinesis-2 (pc2), all inherited as simple Mendelian recessives (Mok and Peloquin 1975b). When these mutations are present at the end of meiosis, dyads with two 2n microspores are formed. Watanabe and Peloquin (1991) reported that ps is the most common mutation leading to 2n pollen production in the potato. Cytological investigations of different genetic material confirmed this finding, and correspondence between the anomalies observed at metaphase-II and 2n pollen was reported (Brown 1988; Watanabe and Peloquin 1993; Barone et al. 1999).

However, in some cases genotypes which exhibited parallel spindles at metaphase II did not produce 2n pollen (Ramanna 1979; Masuelli et al. 1992; Barone et al. 1997). We found a discrepancy between spindle anomalies and 2n pollen formation in diploid, triploid and pentaploid hybrids with different genetic backgrounds (Table 1). The diploid *Solanum phureja-Solanum tubersoum-Solanum chacoense* hybrids also segregated 1:1 for 2n pollen, even though they all exhibited ps. Some authors have hypothesized that only fused, but not parallel, spindles result in 2n pollen formation or that the production of 2n pollen behaves as a quantitative trait (Jacobsen 1980; Veilleux et al. 1982; Ramanna 1983). We agree with Mok and Peloquin (1975a), and believe that parallel, fused and tripolar spindles are the expression of the same gene, and that spindle orientation is a necessary condition for 2n pollen formation. In fact, only a tripolar orientation of spindles would guarantee the formation of a triad. We hypothesized that ps is not the only condition for 2n pollen formation, and that a second mechanism acting at the end of cytokinesis may be involved (Carputo et al. 1995). Analysis of α-tubulin and F-actin distribution during the cytokinesis of meiotic mutants provided evidence that microtubular cytoskeleton abnormalities are involved in the formation of 2n pollen (Genualdo et al. 1998). This finding confirms that, beside anomalous spindle orientation at metaphase-II, deviations in the cytokinetic process may be necessary for the formation of pollen with an unreduced chromosome number. A simple genetic control of these deviations at the end of microsporogenesis may also be hypothesized based on the 1:1 segregation we observed in *S. phureja-S. tubersoum-S. chacoense* hybrids. Another possibility to explain the discrepancy between parallel spindles and 2n pollen is given by Ortiz and Peloquin (1992). In studying the kind and amount of gene action which affects 2n pollen expressivity at the diploid level, they found that at least two epistatic genes besides ps are involved in the production of 2n pollen. When a complete lack of penetrance was observed, they hypothesized a complementary gene action of two dominant epistatic alleles with additive effects.

Meiotic mutations affecting megasporogenesis have also been found and described. Werner and Peloquin (1987) reported that omission of the second meiotic division (os) was the most common mutation of 2n egg for-

Table 1 Microsporogenesis analysis of *Solanum* hybrids with different genetic background: discrepancy between parallel spindles (ps) and 2n pollen

Pedigree of hybrids	Ploidy level	No. analyzed genotypes	No. genotypes with ps	No. genotypes with 2n pollen	Reference
phu-tbr[a]	2n=2*x*=24	50	50	50	Barone et al. 1999
phu-tbr	2n=2*x*=24	28	28	28	Unpublished results
phu-tbr-chc	2n=2*x*=24	30	30	16	Barone et al. 1997
cmm-phu-tbr	2n=3*x*=36	7	7	0	Carputo et al. 1995
cmm-phu- tbr	2n=5*x*±2=58–62	4	4	3	Unpublished results

[a] phu=*S. phureja*; tbr=*S. tuberosum*; chc=*S. chacoense*; cmm=*S. commersonii*

mation. When os is present, two 2n megaspores are produced, one degenerating and the other giving the functional spore. Another meiotic variant resulting in 2n eggs is the failure of cytokinesis after the second division, which results in the fusion of nuclear products of this division. Genetic analyses confirmed that these two meiotic mutations are also controlled by single recessive genes (Werner and Peloquin 1990). Other mutations leading to 2n egg formation may affect megasporogenesis. They cause an irregular anaphase-II and delayed meiotic divisions (Werner and Peloquin 1990), and an irregular spindle axis formation at metaphase-I, which can disturb the correct chromosome movement towards the two poles or cell-plate formation across the phragmoplast (Conicella et al. 1991).

A common feature of all the meiotic mutations described is that they have an incomplete penetrance and a variable expressivity. Environmental conditions have also been reported to have considerable effect upon the frequency of 2n pollen production (Veilleux and Lauer 1981; Veilleux et al. 1982; McHale 1983; Bani-Aameur et al. 1992). Recently, we have studied 2n pollen production in diploid *S. phureja-S. tuberosum* hybrids. Genotypes with a high 2n pollen production showed a variation of 2n pollen frequency at different collection times. Only clones with low (<5%) 2n pollen production maintained a constantly low level over the whole flowering period (Filotico et al. 1995). As in other papers, we were unable to determine one specific environmental factor influencing the frequency of 2n pollen.

Genetic consequences associated with 2n gamete production

Each meiotic mutation leading to 2n gametes is genetically equivalent to either first-division restitution (FDR) or second-division restitution (SDR) mechanisms (Peloquin 1983). Thus, for example, ps is equivalent to FDR, whereas os is equivalent to SDR. Recently, based on multilocus RFLP analysis of a 4*x* progeny coming from a 2n egg-producer clone, Bastiaanssen et al. (1998) hypothesized an additional mechanism (post-meiotic restitution, PMR) resulting in completely homozygous 2n gametes. This finding did not come from any cytological evidence, but only considered the genetic consequences of meiotic mutations. Similarly, genetic tests (half-tetrad analysis) were used to determine the mode of 2n gamete formation (Mendiburu and Peloquin 1979; Douches and Quiros 1988; Wagenvoort and Zimnoch-Guzowska 1992; Werner et al. 1992).

The genetic consequences associated with different cytological mechanisms are very important (Peloquin 1983; Hermsen 1984). With FDR, all loci from the centromere to the first crossover that are heterozygous in the parent will be heterozygous in the gamete, and 50% of loci between the first and the second crossover which are heterozygous in the parent will be heterozygous in the gamete. If crossing-over does not occur, then the intact genotype of the diploid parent will be incorporated into each 2n gamete. The SDR gametes do not maintain the original genic combinations of the diploid parents. In fact, all heterozygous loci from the centromere to the first crossover will be homozygous in the gametes, and all loci between the first and the second crossover, which are heterozygous in the parent, will be heterozygous in the gametes. FDR gametes are expected to strongly resemble each other and the parental clone they derive from. By contrast, SDR is expected to produce a heterogeneous population of highly homozygous gametes. Based on the frequency of single-exchange tetrads ($0<\beta<1$), Tai (1994) estimated that in a diploid clone which is heterozygous at a given locus, the frequency of 2n pollen will be $A1A1=\beta/4$; $A1A2=1-(\beta/2)$; $A2A2=\beta/4$. With SDR, the frequency of 2n pollen will be $A1A1=(1-\beta/2)$; $A1A2=\beta$; $A2A2=(1-\beta/2)$.

Based on cytological expectations, it was calculated that 2n gametes maintain and transmit about 80% of parental heterozygosity and a large amount of epistasis, whereas SDR 2n gametes transmit about 40% of heterozygosity and a smaller amount of epistasis. Recently, through the use of RFLP markers we verified in tetraploid progeny coming from $2x\times2x$ crosses that 31.8% and 71.4% of the heterozygosity was transmitted through SDR and FDR 2n gametes, respectively (Barone et al. 1995).

One-hundred percent heterozygosity and epistasis can be theoretically transmitted by 2n pollen produced by FDR without crossing-over when ps is associated with asynaptic mutants (Peloquin 1983), and therefore this should be the most desirable genetic condition. However, this does not hold for genes which have a predominant physical location between centromeres and proximal chiasmata, because SDR and FDR gametes are expected to be genetically equivalent for loci present in these regions. This is the case of genes controlling yield ability (Buso et al. 1999a). Thus, when $4x\times2x$ crosses are performed, the use of diploid parents which produce 2n pollen by FDR without crossing-over (and have almost 100% of the parental genotype) result in tuber yields similar to those obtained through FDR 2n pollen with crossing-over.

Use of 2n gametes in potato breeding

Several features of the potato provide the basis for successful use of 2n gametes in breeding. First, the cultivated potato is foremost among crop plants in its abundance of wild and cultivated relatives, most of which are diploids ($2n=2x=24$). They represent a unique receptable of allelic diversity and useful genes. Second, haploids (sporophytes with a gametophytic chromosome number) can be routinely extracted from cultivated tetraploid potato, and represent an important source of 24-chromosome genotypes in the *S. tuberosum* form. Third, *Solanum* species have poorly differentiated genomes (Peloquin et al. 1989a), thus allowing the production of fertile hybrids after interspecific crosses. Fourth, in the potato, as in other polysomic polyploids, the genetic variance for several polygenic traits (e.g. tuber yield), is almost entirely non-additive, depending on intra- and inter-locus interactions (Mendoza and Haynes 1974). Thus, breeding for polygenic traits should be oriented towards maximizing heterozygosity and maintaining valuable epistatic combinations.

Based on these features, Peloquin et al. (1989b) proposed an alternative approach to the traditional 4*x*×4*x* crossing scheme for potato breeding. It involves the use of haploids (2n=2*x*=24) of common cultivars, which are crossed to diploid wild or cultivated *Solanum* species to capture their allelic diversity. Hybrids that produce 2n gametes are then used in sexual polyploidization schemes to generate tetraploid progeny, and thus return to the ploidy level of cultivated potatoes. Triploids are rare due to the so called “triploid block” (Marks 1966). The significance of 2n gametes in these crossing schemes lies not only in the possibility of returning to the 4*x* level, but also in their ability to transmit non-additive genetic effects (heterozygosity and epistasis) from the 2*x* parent to the 4*x* offspring. This last aspect is important given that n gametes from diploids are only capable of transmitting additive effects, whereas n gametes from tetraploids can only transmit a certain number of first-order intralocus interactions. Epistasis is not transmitted due to the disruptive effects of meiosis.

Sexual polyploidization

Two types of polyploidization can be used, unilateral sexual polyploidization (through 4*x*×2*x* or 2*x*×4*x* crosses), which requires functioning of either 2n pollen or 2n eggs, and bilateral sexual polyploidization (through 2*x*×2*x* crosses), which requires the functioning of both 2n pollen and 2n eggs. The choice of the breeding scheme to be used depends on the mode of 2n gamete formation present in the parent and, in the case of unilateral sexual polyploidization, on the fertility of the 4*x* parent.

Due to the high level of intra- and inter-locus interactions transmitted by FDR gametes and on the wide occurrence of 2n pollen, much emphasis has been given to the 4*x*×2*x*-FDR breeding approach to produce superior potato genotypes. Table 2 presents tuber yields of tetraploid families obtained in southern Italy from 4*x*×2*x* and 4*x*×4*x* crosses involving two selected *S. phureja*-*S. tuberosum* diploid hybrids producing 2n pollen and five tetraploid cultivars with different genetic backgrounds. On average, 4*x*×2*x* families gave a tuber yield higher than the 4*x*×4*x* families (7.5 and 6.3, respectively), with the contrast between family groups being significant (F=7.03, P<0.05). These results are remarkable also considering that the 4*x*-parents used had a broad genetic base.

Our results are in agreement with previous papers in which high yields of 4*x*×2*x* families as well as a high degree of heterosis for tuber yield were reported (Mok and Peloquin 1975c; Mendiburu and Peloquin 1977; Tai 1994; Ortiz 1997; Buso et al. 1999b). Superiority of FDR 2n gametes in giving more uniform and productive tetraploids than SDR gametes has been reported as well (Mok and Peloquin 1975c; De Jong and Tai 1991; Hutten et al. 1994; La Rotonda et al. 1999). Darmo and Peloquin (1991) and Ortiz et al. (1991) also reported a high yield stability of 4*x*×2*x* genotypes. The high degree of allelic diversity transmitted by FDR 2n pollen may explain the stability of 4*x*×2*x* genotypes, the two traits being positively correlated (Amoros and Mendoza 1979). Concilio and Peloquin (1991) and Buso et al. (1999b) showed that another advantage of the 4*x*×2*x* breeding scheme is that it can increase the efficiency of cultivar development. The authors found that the percentage of clones with high yielding ability and good quality traits selected in relatively small 4*x*×2*x* populations was much higher than that reported using 4*x*×4*x* crosses.

Bilateral sexual polyploidization can be employed when superior 2n egg- and 2n pollen-producing hybrids are available. This method potentially allows the combination of multiple traits from two diploid interspecific hybrids into tetraploid genotypes through 2*x*×2*x* crosses. It also allows transmission of high levels of heterozygosity into 4*x* progeny, especially when the 2*x* hybrids used have unrelated genetic backgrounds. Werner and Peloquin (1991) reported a 30% increase in tuber yield of 4*x* families from 2*x*×2*x* crosses in comparison to control cultivars and advanced selections. In 2*x* (SDR)×2*x* (FDR) crosses, SDR and FDR complement each other in terms of the transmission of heterozygosity. We used RFLP markers to evaluate transmission of heterozygosity in a 4*x* progeny coming from 2*x* (*S. tuberosum*-*S. chacoense*)×2*x* (*S. phureja*-*S. tuberosum*) interspecific clones (Table 3). As expected, several multiallelic loci were identified. The level of the heterozygosity index (LH), calculated on the basis of the number of probe/enzyme combinations showing heterozygosity, indicated

Table 2 Total tuber yield (kg per plot of 2.7 m^2) of families from 4*x*×2*x* and 4*x*×4*x* crosses evaluated in Southern Italy. Values are means of three replications. LSD$_{0.05}$ for comparing two families means=2.2

4*x* parents	UP88-P5 (2*x*)	UP88-P7 (2*x*)	Chiquita (4*x*)	Mean
Carmine	8.6	7.9	5.8	7.4
Pamina	6.6	8.8	5.6	7.0
Atlas	7.6	6.6	6.3	6.8
Jasmine	9.0	8.6	7.7	8.4
AVRDC-1287.19	5.4	6.1	5.9	5.8
Mean	7.4	7.6	6.3	

Table 3 Estimation through RFLP markers of level of heterozygosity (LH) in a 4x (*S. tuberosum*×*S. chacoense*)×(*S. phureja*×*S. tuberosum*) progeny obtained from 2*x*-SDR×2*x*-FDR crosses

Class of LH value[a]	Genotypes (no.)	LH mean (%)	LH range (%)	Genotypes with multiallelic loci (no.)
0–20%	1	–	–	0
20–40%	4	30.9	28.6–37.5	1
40–60%	7	52.6	42.8–57.1	1
60–80%	13	73.6	68.3–79.1	8
80–100%	5	87.6	83.2–90.8	3

[a] LH=(number of probe/enzyme combinations showing heterozygosity per total number of probe/enzyme combinations) x100. The total number of probe/enzyme combinations used was 23

that most genotypes (18 out 30) had higher than 60% heterozygosity, and that in several (5) this value was close to 90%.

True potato seed

Sexual polyploidization can also be employed for producing potato from true seed (TPS). Disease elimination, as well as low storage and transport costs, are some of the advantages associated with the use of true seed. Tuber yield and uniformity, however, are generally lower than those obtained through seed tubers (Pallais 1991). Besides the advantages of the allelic interactions transmitted, FDR 2n gametes are highly uniform, and thus unilateral sexual polyploidization is expected to produce vigorous and fairly homogeneous progeny in crosses with tetraploids (Ortiz 1997). We evaluated a number of TPS families coming from 4*x*×4*x* and 4*x*×2*x* crosses, and from open-pollinations in southern Italy (Carputo et al. 1994). The highest tuber yields were recorded in 4*x*×2*x* TPS families grown from seedling tubers, averaging 37 t/ha and 42 t/ha when class A (tuber diameter 25–35 mm) and class B (tuber diameter 35–45 mm) seedling tubers were planted, respectively. Shonnard and Peloquin (1991) and Concilio and Peloquin (1991) also reported high tuber yields of 4*x*×2*x* families compared to those of 4*x* clones and 4*x*×4*x* families, respectively.

A fundamental trait enabling better use of diploid parents for TPS production is that they should exhibit a high and stable 2n pollen frequency, independent of environmental conditions. High stability in 2n pollen production over 3 years was found in one *S. phureja*-*S. tuberosum* clone. Stable expression of 2n pollen production in this clone was explained on the basis of the presence of two cytological mechanisms (parallel spindles and premature cytokinesis) leading to 2n pollen formation (Barone et al. 1999). We hypothesized that penetrance and expressivity of the gene controlling anomalous spindle orientation may be more greatly affected by the environment than that of the gene controlling premature cytokinesis, and thus the presence of two mechanisms guarantees a high stability of 2n pollen production.

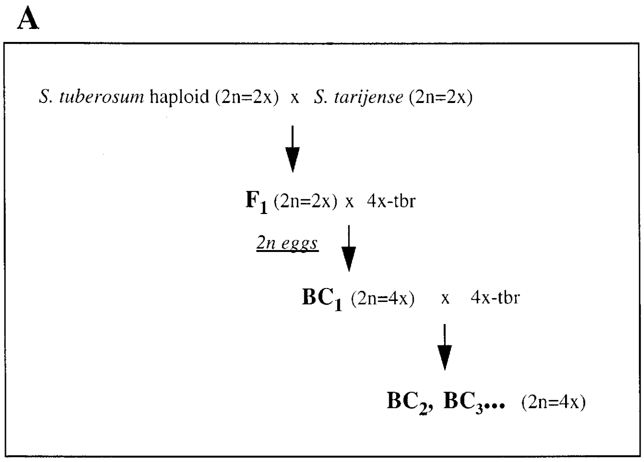
Use of 2n gametes in potato germplasm introgression

Besides providing the allelic diversity necessary to reach high heterosis, *Solanum* species also represent a rich, unique and diverse source of genetic variation (Hanneman 1989). Genes for resistance to biotic and abiotic stresses, and characteristics such as high solid content, good chipping quality, and tuber color can be easily found in these species. 2n gametes represent a unique tool to transfer target genes from wild, unadapted forms to the cultivated tetraploid gene pool. Species directly crossable to *S. tuberosum* haploids can be exploited through sexual polyploidization crossing schemes. For those which have developed crossability barriers, specific crossing schemes have to be designed.

Exploitation of wild species crossable with *S. tuberosum* haploids

The availability of so many species with a 24-chromosome complement, the ease with which most of these species can be crossed which *S. tuberosum* haploids, and the widespread occurrence of meiotic mutations leading to 2n gametes, strongly favor sexual polyploidization crossing-schemes for germplasm introgression from species which are crossable with *S. tuberosum* haploids.

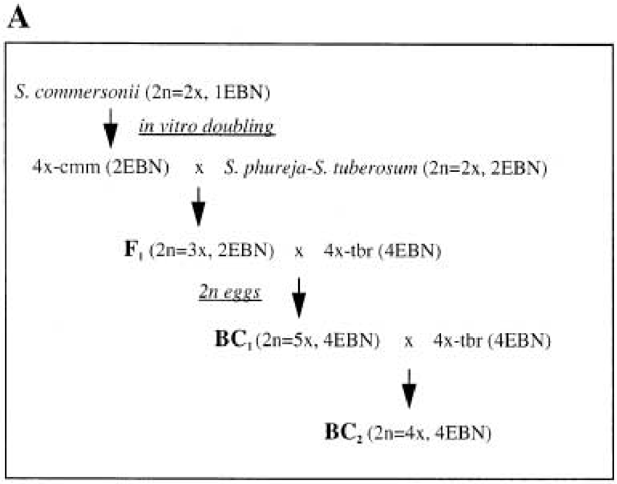
Interspecific diploid hybrids producing 2n gametes are routinely selected at the Mlochòw Research Center in Poland, where one of the biggest potato breeding program in the world is carried out. The hybrids are then used by breeders to transfer important traits (resistance to viruses, *Phytophthora infestans* and *Erwinia* spp., as well as chipping quality and high starch content) from wild and primitive species into *S. tuberosum* (Zimnoch-Guzowska and Dziewonska 1989; Zimnoch-Guzowska et al. 1999). It is remarkable that the genetic background of these hybrids includes several wild species, so that multiple traits can be simultaneously transferred to the tetraploid level. Also remarkable is the fact that most tetraploid parental lines developed at the Mlochòw Research Center originate from 4*x*–2*x* crosses (Zimnoch-Guzowska et al. 1998). 2n pollen-producing haploid-wild species hybrids with resistance traits have also been developed at the International Potato Center in Lima, Peru (Ortiz et al. 1994). These diploid hybrids contain the germplasm of several species, including *Solanum stoloniferum*, *S. chacoense*, *Solanum sparsipilum*, *Solanum microdontum* and *Solanum vernei*. Production of diploid hybrids and use of the 4*x*–2*x* breeding approach to transfer resistance traits has been reported by other authors. Watanabe et al. (1992) introgressed the resistance to *Ralstonia solanacearum* from *S. chacoense* and *S. sparsipilium*. Ortiz et al. (1997) transmitted the resistance to the potato cyst nematode *Globodera pallida* from *S. vernei*, *S. sparsipilum* and *Solanum andigena* haploids into tetraploid clones using 2n pollen. Other examples are reported by Ortiz (1998).



B

Genotype	Ploidy	Number of genotypes			
		Tested	Resistant	Intermediate	Susceptible
F_1	2n = 24	3	2	1	0
BC_1	2n = 48	5	2	2	1
BC_2	2n = 48	50	11	16	23

Fig. 1 **A** Breeding scheme used to introgress useful genes from the 2*x* wild species *S. tarijense* to the 4*x* cultivated *S. tuberosum* (*tbr*). **B** Resistance to *Erwinia carotovora* subsp. *carotovora* of selected F_1 diploid, BC_1 and BC_2 tetraploid hybrids obtained through unilateral sexual polyploidization (data of F_1 and BC_1 hybrids are from Carputo et al. 1997)



B

Genotype	Ploidy	Killing temperature (°C)	
		NAC	**ACC**
F_1 (B10)	2n = 36	-4.1	-7.5
BC_1 (P3)	2n = 60	-2.5	-5.5
cmm	2n = 24	-4.9	-9.5
phu-tbr	2n = 24	-2.6	-4.4

Fig. 2 **A** Breeding scheme used to introgress resistance to low temperature and acclimation capacity from 2*x*(1EBN) *S. commersonii* to the cultivated 4*x*(4EBN) S. *tuberosum* (*tbr*). A tetraploid clone of *S. commersonii* (4*x*-cmm, 2EBN) was crossed to diploid *S. phureja-S. tuberosum* hybrids (2n=2*x*, 2EBN) to obtain F_1 triploids, further backcrossed to tbr. **B** Freezing tolerance (°C) of non-acclimated (NAC) and acclimated (ACC) F_1 and BC_1 selected *S. commersonii-S. tuberosum* hybrids and their diploid parents (cmm and phu-tbr) (from Carputo et al 2000)

Using unilateral sexual polyploidization, we recently achieved the transfer of resistance to tuber soft rot by *Erwinia* spp. from wild species into the cultivated gene pool. Breeding for tuber soft-rot resistance is important in that the degree of resistance is very low in cultivated varieties. Sources of resistance were found in one clone of *Solanum tarijense* (Carputo et al. 1996), which was crossed to a *S. tuberosum* haploid (Fig. 1). Transfer of resistance from the 2*x* level of F_1 hybrids to the 4*x* level of BC_1 hybrids was successful through 2*x*×4*x* crosses involving 2n eggs. It should be pointed out that at each generation (F_1, BC_1, BC_2) only genotypes with good tuber characteristics were screened for resistance. This selection was applied to speed up the breeding program. The introgression of resistance to *Erwinia* spp. has now advanced to resistant BC_2 4*x* hybrids for further backcrosses.

Exploitation of sexually isolated species

Sexual polyploidization represents the easiest way to efficiently introgress useful genes from wild *Solanum* species into cultivated varieties. However, it cannot always be employed. In fact, a number of *Solanum* species cannot be routinely crossed to 4*x S. tuberosum* or its haploids due to post-zygotic barriers caused by Endosperm Balance Number (EBN) differences between parents. The EBN represents the effective ploidy of *Solanum* species (Hanneman 1994), and is a fundamental concept for potato breeding. In fact, interspecific crosses are successful only when the parents employed have the same EBN, and thus give a 2:1 female: male EBN ratio in the hybrid endosperm (Johnston et al. 1980). To overcome EBN barriers, 2n gametes can be used to design breeding schemes aimed at equalizing the EBN of parents. In 4*x*(2EBN)–4*x*(4EBN) matings, for example, 2n gametes produced by the parent with lower EBN guarantees the required 2:1 female:male EBN ratio. The F_1 hexaploid offspring produced through these matings can be easily used as a genetic bridge and crossed to *S. tuberosum*. Direct crossing between virus-resistant 4*x*(2EBN) *Solanum acaule* and 4*x*(4EBN) *S. tuberosum* was possible through the functioning of 2n eggs (Camadro and Espinillo 1990). Similarly, the transfer of resistance to *Meloidogyne* spp. from 4*x*(2EBN) *Solanum fendleri* to 4EBN *S. tuberosum* was achieved through 2n eggs (Janssen et al. 1997). Hexaploid bridges were resistant to *Meloidogyne*, as were all the BC_1 hybrids deriving from 6*x*×4*x* crosses.

2n gametes are also essential ingredients in breeding schemes where triploid bridges are produced. In order to transfer resistance genes from 2*x*(1EBN) and 4*x*(2EBN) species into a *S. tuberosum* form, triploid hybrids were obtained between 2*x*(1EBN) *Solanum brevidens* and 2*x*(2EBN) *S. chacoense* (Ehlenfeldt and Hanneman 1984), 4*x*(2EBN) species of the series Longipedicellata and 2*x*(2EBN) *S. tuberosum* haploids (Adiwilaga and Brown 1991), 2*x*(1EBN) *Solanum circaefolium* and 2*x*(2EBN) *S. tuberosum* haploids (Louwes et al. 1992), and 2*x*(1EBN) *S. commersonii* and 4*x*(4EBN) *Solanum gourlayi* (Masuelli et al. 1992). A necessary condition for using these triploids efficiently is that they produce 2n gametes. In this case, 2n gametes have a double function, i.e. (1) equalizing the EBN between parents, and (2) producing functional, balanced gametes from a genotype with an odd ploidy chromosome number.

We have recently designed a breeding scheme based on the use of 2n gamete-producing triploids to introgress resistance to low temperature and acclimation capacity from *S. commersonii* into *S. tuberosum*, which lacks these traits (Fig. 2). *S. commersonii* has an EBN=1 and thus cannot be crossed to 2EBN *S. tuberosum* haploids. After doubling its ploidy, and thus its EBN, we produced 2EBN triploids hybrids through 4*x*(2EBN) *S. commersonii*×2*x*(2EBN) *S. phureja*-*S. tuberosum* crosses (Carputo et al. 1995). 2n eggs of the triploids made it possible to produce BC_1 pentaploids, easily crossed to *S. tuberosum* to continue the backcross program (Carputo et al. 1997). One triploid hybrid (B10) showed a degree of resistance to low temperature and acclimation capacity comparable to that of *S. commersonii* (Fig. 2). Finding this genotype with such levels of resistance is very important. It means that the barriers which prevent the exploitation of *S. commersonii* are overcome and that useful genes of this noteworthy species are now available in the form of 3*x*(2EBN) genotypes. BC_1 hybrids are being produced and evaluated with the aim of obtaining genotypes combining both traits.

Prospects for the future

Due to the growing interest in exploiting exotic germplasm, sexual polyploidization can be successfully employed both for increasing the allelic diversity and for transferring useful traits from wild related species into cultivated potatoes. The results obtained so far in the potato stimulate further research on formation mechanisms and the potential use of 2n gametes also in other species. Besides the classical approaches, new molecular techniques can be employed for a better understanding of the meiotic mutations leading to 2n gamete formation. Molecular markers have been used to map one gene (*ds*) controlling desynapsis in potato (Jacobs et al. 1995) and to locate the *pc* gene on chromosome IV of the potato RFLP map (Barone et al. 1999). This could open up new prospects in the use of 2n gametes for potato breeding. It should be possible to clone these genes in desirable genotypes and use linked markers for the assisted selection of superior 2n gamete-producing genotypes.

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