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Characterization of the Natural Genetic Diversity of Argentinean Potato Species and Manipulations for its Effective Use in Breeding

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ABSTRACT

The common potato, *Solanum tuberosum* L. ssp. *tuberosum*, is a tetraploid allogamous species, clonally propagated for commercial purposes. Advances in breeding of this species are usually slow due to its narrow genetic base and tetrasomic inheritance, in contrast to other polyploid species of agronomic importance (i.e. wheat, tobacco) that have disomic inheritance with simpler genetic ratios. However, it has approximately 200 wild and cultivated closely related species that constitute an invaluable source of genes for desirable attributes in breeding. Most of these species are diploids that cannot be directly crossed to the the common potato due to the presence of pre- and post-zygotic hybridization barriers. These barriers, however, can be incomplete and, if properly identified, strategies can be devised to introgress desirable genes into the cultivated pool. At the Laboratory of Genetics, in Balcarce, studies are carried out (some of them in collaboration with other potato groups) since 1981 to characterize wild Argentinian potato germplasm using genetic, cytogenetic, biochemical and molecular approaches, in order to: (1) identify breeding barriers and establish their genetic bases and (2) develop strategies to circumvent them. Information, of both basic and applied value, is regularly generated. In the process, genetic materials are obtained (pre-breeding) that can be of use in breeding.

Keywords: hybridization barriers, heterosis, ploidy level manipulations, tuber-bearing Solanums, wild species

CONTENTS

INTRODUCTION

The common potato, *Solanum tuberosum* L. ssp. *tuberosum* ($2n=4x=48$; 4 EBN[1]), also known as the “Irish potato”, has approximately 200 wild and cultivated relatives. Although there is lack of agreement on their exact number (Hawkes 1990; Spooner and Castillo 1997), it is much higher than the number of relatives of any other important food or feed crop. These species are grouped into section *Petota* Dumortier, that includes two subsections: *Estolonifera* and *Potatoe* (Hawkes 1994); the former contains diploid species that bear only stolons, whereas the latter includes all tuber-bearing species, generically known as “potatoes”, that produce both stolons and tubers. Potato species range in ploidy from diploid to hexaploid, with a basic chromosome number of $x=12$. Although most of them are diploid, there are also triploid, tetraploid, pentaploid and hexaploid species, and a few of them are mixtures of cytotypes.

Wild and cultivated potatoes are grouped into 16 taxonomic series. Series *Tuberosa* includes the seven cultivated species: diploid *S. stenotomum*, *S. phureja* and *S. ajanhuiri*; triploid *S. chaucha* and *S. juzepczukii*; tetraploid *S. tuberosum* ssp. *andigenum* Hawkes and ssp. *tuberosum*; and pentaploid *S. curtilobum*, as well as the wild species most closely related to them (Hawkes 1994). More recently, Huamán and Spooner (2002) proposed to denominate all cultivated forms as *S. tuberosum*. In this paper, I will follow Hawkes’ (1994) denomination because I consider that it is more appropriate from the breeding point of view.

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[1] Hypothetical genetic factors that when present in a 2:1 proportion in the endosperm - provided by the female and male parent, respectively- determine the normal development of this tissue (Johnston *et al.* 1980)

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Invited Mini-Review

Species in section *Petota* are widely distributed along the Americas. They grow spontaneously from Southwestern United States to Southern Chile and, towards the East, in Argentina, Paraguay, Uruguay and Brazil, in a variety of contrasting micro- and macroenvironments (Hawkes 1990). The higher number of species per degree of latitude is found in the highlands of Central Mexico and the high Andes from Peru to Northwest Argentina (Hijmans and Spooner 2001).

Based on morphological and molecular studies, Spooner and Castillo (1997) and Van den Berg *et al.* (2002) stressed the necessity of re-evaluation of the taxonomic series and superseries in the section. More recent works from our Laboratory (Bedogni and Camadro 2008; Erazzú *et al.* 2009; Masuelli *et al.* 2009) indicate that hybridization in potato is a frequent and recurrent phenomenon that taxonomists usually have not taken into account when assigning taxonomic status to wild germplasm collections. Our results, in general, are in accordance with Ugent's (1966) general assertion that potato species can be regarded as a single biological species from which taxonomists have to delineate taxonomic units somehow arbitrarily to facilitate the breeders' work. But although spontaneous interspecific hybrids are widespread in overlapping distribution areas, we have ample evidence that the biological species concept cannot be directly applied to potato species (Camadro *et al.* 2004; Masuelli *et al.* 2009) as I will relate in short.

In Argentina, and according to different taxonomists, the number of wild species vary from 35 (Hawkes and Hjerting 1969) to 23 (A. Clausen, pers. comm.). An important number of accessions of these species (population samples available mostly as botanical seeds) and clones of tetraploid landraces are stored at the Potato and Forages Germplasm Bank of the National Institute of Agropecuarian Technology (INTA), of the Agropecuarian Experimental Station (EEA), in Balcarce, Buenos Aires province (http//balcarce.inta.gov.ar/banco_germop/); duplicates of these accessions are also available at germplasm banks of other countries (i.e. USA, Germany).

The wide ecological adaptation of this germplasm and the fact that it has been exposed to natural selection for very long periods of time is of relevance for the genetic improvement of cultivated genotypes because they are a reservoir of desirable genes for agronomic, culinary and industrial purposes (Ross 1986).

To put the huge amount of genetic variability present in the wild germplasm into a useful form for breeding, it is necessary to understand, in the first place, the basic principles of potato cytogenetics, genetics and reproduction.

CYTOGENETICS

Diploid potato species are highly fertile because they regularly form bivalents in meiosis; they have, therefore, disomic inheritance. In these species, the paired chromosomes segregate normally in Anaphase I – one chromosome to each pole – giving rise to gametophytes (pollen and embryos sacs) and gametes (generative cells and egg cells, respectively) with reduced (n=x) balanced chromosome numbers. Polyploid species, however, can form either bivalents or multivalents in meiosis; in the latter, either each chromosome or each chromatid can be the unit of segregation, resulting in different genetic ratios (Burnham 1962). Polyploids with bivalent pairing have disomic inheritance, as diploids, and produce balanced gametes (n=2x, 3x, and so on, according to whether they are tetraploid, hexaploid or have higher ploidies), whereas polyploids with multivalent pairing have polysomic inheritance, with complex genetic ratios, and produce gametes with balanced and unbalanced chromosome numbers (n=x, x+1,………2x-1, 2x, 2x +1…, and so on) and, consequently, sterility problems.

Regular pairing is mainly determined by chromosome (genome) homology. Following a review of the literature, Matsubayashi (1991) concluded that: (1) species in the 16 potato taxonomic series, irrespective of their ploidy levels, are phylogenetically related to one another by possessing a common genome, A, regarded as the basic genome; (2) this genome is modified to different degrees due to cryptic structural differences in the chromosomes; (3) polyploid species differ from one another by virtue of their additional genomes, which are structurally differentiated to a greater or lesser extent and are given different genome formulae; (4) five different genomes, A, B, C, D, and E, are recognized.

Thus, since genome differentiation is not of a great magnitude in potatoes, interspecific hybrids – generated both spontaneously and by controlled crosses – are, in their majority, vigorous and fertile (Ramanna and Hermsen 1979; Masuelli and Camadro 1992). Thus, in the absence of other barriers, it is feasible to incorporate small chromosome segments from the wild species to the cultivated forms to introgress heritable resistances and to broaden their genetic base.

2N GAMETES

Normal sporogensis results in the formation of gametophytes and gametes, as previously mentioned, with numerically reduced (n or gametophytic) chromosome numbers. However, when random pre- or post-meiotic chromosome duplication events occur or mutations appear that affect the normal course of meiosis, gametes with unreduced (2n or sporophytic) chromosome numbers can be formed. These types of gametes and gametophytes have been extensively studied in a number of genera, including potatoes, and have been generally attributed to the action of one single recessive gene or a few genes (Mok and Peloquin 1975; Werner and Peloquin 1990; Camadro *et al.* 1992/93).

Although there are a number of cytological mechanisms that can lead to the formation of 2n gametes in potatoes (Mok and Peloquin 1975; Iwanaga and Peloquin 1979; Ramanna 1979 and 1983; Okwagwu and Peloquin 1981; Jongedijk 1985; Camadro 1986; among others), the products of the meiotic modifications can be genetically equivalent to those formed either by first division restitution (FDR) or by second division restitution (SDR). 2n gametes would transmit different levels of intra- and inter-locus interactions to the next generation, depending on the genotype of the plant, the distance between the locus/loci of interest and the centromere (related to the number of effective crossovers that can take place in the region) and the mode of 2n gamete formation. For a given locus with two alleles, and considering the formation of both non-exchange and single-exchange tetrads, FDR gametes will transmit 75% of the heterozygous genotype intact to the next generation vs. 50% of SDR gametes (Mendiburu *et al.* 1974). If tetrads with more than one effective exchange are formed in potatoes, FDR gametes will transmit even a higher proportion of the heterozygous genotype of the plant, being this the reason why they are preferred over SDR gametes in breeding highly heterozygous (and, consequently, heterotic) cultivars. These meiotic mutants may exhibit incomplete penetrance and/or variable expressivity, both of which can be significantly modified by genetic, environmental, and developmental factors (Peloquin *et al.* 1999).

Functional 2n gametes have been detected in a large number of potato species (den Nijs and Peloquin 1977; Camadro and Peloquin 1981; Watanabe and Peloquin 1989; among others). These gametes allow for gene flow and introgression between diploid and polyploid species, circumventing endosperm barriers as will be explained in this paper.

GENETICS

There are four main genetic differences between polysomic inheritance (i.e. in tetrasomic tetraploids) and disomic inheritance (i.e. in disomic tetraploids or higher ploidy species and in diploids) as can be seen in **Table 1**.

S. tuberosum ssp. *tuberosum* and ssp. *andigenum* are both typical tetrasomic polyploids, with tetravalent pairing

Table 1 Basic differences between diploids and polysomic polyploids (modified from Carputo *et al.* 2005).

	Inheritance	
	Disomic	**Tetrasomic**
	S. chacoense	**Common potato**
	2n=2x=24	**2n=4*x*=48**
Genomes	DD*	TTTT
Cytogenetics	only bivalents	quadrivalents
Genetics	disomic	tetrasomic
Reproductive behavior	autogamous	allogamous
Fertility	high	reduced
Breeding	homozygosity at each locus; inter-locus interactions	heterozygosity at each locus; intra- and inter-locus interactions

*D = disomic; T = tetrasomic

Table 2 Gametic and zygotic genotypes for a locus with two alleles (A, a) in diploid and tetraploid species.

Ploidy	**Gametes**	**Zygotes**
2x	A, a	AA, Aa, aa
4x	AA, Aa, aa	AAAA, AAAa, AAaa, Aaaa, aaaa

at meiosis and tetrasomic inheritance. In contrast, several tetraploid and hexaploid species behave as disomic polyploids, with regular bivalent pairing at meiosis (see Dvorák 1983). Most wild Argentinian species, however, are diploid and the only polyploid species are tetraploid *S. acaule*, hexaploid *S. oplocense*, and the tetraploid cytotype of *S. gourlayi*. Of these, only *S. acaule* has disomic inheritance (Camadro *et al.* 1992).

The possible gametic and zygotic genotypes in diploids and tetraploids with only two different alleles at a given locus (i.e. A and a), are presented in **Table 2**.

In diploids, gametes are hemizygous because they carry only one allele of each locus (A or a) and zygotes can be either homozygous (AA or aa) or heterozygous (Aa). In tetraploids, in contrast, gametic genotypes are identical to the zygotic genotypes of diploids because they carry two alleles per locus and, therefore, heterozygous gametes can exhibit heterosis (Simon and Peloquin 1976); moreover, heterozygosity in zygotic genotypes is not genetically equivalent to heterozygosity in diploid genotypes because tetraploid zygotes carry four alleles per locus instead of two. Thus, another terminology has to be used to take into account the number of dominant alleles at a given locus in a polyploid. For example, in a tetraploid there are five possible zygotic genotypes: quadruplex (four dominant alleles), triplex (three), duplex (two), simplex (one) and nulliplex (none). Thus, intra-locus interactions between different alleles can occur only in zygotes of diploids but both, in gametes and zygotes of tetraploids, providing the opportunity for rich interaction patterns, particularly for loci with more than two alleles, which are very frequent in the common potato. For example, with four different allele at a given locus (1) in diploids, there are no possible interactions in gametes and only one in zygotes, (2) in tetraploids, there are 11 possible interactions: six of first order (between two alleles), four of second order (betwen three alleles) and one of third order (between four alleles) (Mendiburu *et al.* 1974).

GENETIC VARIABILITY

Homoploid hybridization (Masuelli *et al.* 2009) and sexual polyploidization (Peloquin *et al.* 1999) are the two major forces in potato evolution, since they generate great genetic variability, fitness, and heterozygosity in the populations (Carputo *et al.* 2003). Also, the pattern of variability in potato species is strongly influenced by their two alternative means of reproduction – sexual (by seeds) and asexual (by stolons and tubers) – and by the fact that most diploid species are obligate outcrossers, as will be explain in short. Therefore, the breeding system, in general, provides for success and opportunism, both in maintaining superior genotypes in stable environments and for the rapid evolution of new forms under varying environmental conditions (Camadro *et al.* 2004). Hybridization and subsequent gene flow within and between ploidy levels often result in exceedingly complicated patterns of variation (Bedogni and Camadro 2009; Erazzú *et al.* 2009; Masuelli *et al.* 2009), creating an extensive assemblage of inter-related plants.

There is a lack of agreement on the taxonomic treatments of the group as a consequence of these particularities and, also, to the various taxonomic concepts and practices of leading potato taxonomists (Spooner and Van den Berg 1992; see Camadro *et al.* 2004 as an example). Making matters even more complex, in my opinion, the biological species concept is inadequate to accommodate the phenotypic variants in the group and discussions among leading potato geneticists and taxonomists to advance in that regard are meager (see Masuelli *et al.* 2009).

BREEDING BARRIERS

As Camadro *et al.* (2004) have pointed out, there are a number of factors that restrict or prevent gene exchange, allowing the maintenance of a very high number of potato species but also providing the opportunity for gene flow and introgression. If breeders are aware of the existence of these barriers and their genetic control, they can devise strategies for the effective use of this germplasm in the improvement of the cultivated pool.

According to Hawkes and Hjerting (1969), potatoes are mainly separated by external hybridization barriers such as physical separation of populations in time or space, adaptation of populations to specific ecological niches, or combinations of barriers that produce genetic discontinuity among populations. These external barriers are usually reinforced by internal barriers that can be either pre-zygotic (those resulting from incompatible or incongruent biochemical signals between pollen and pistil during the progamic phase that leads to seed formation) or post-zygotic (those leading to embryo and/or endosperm abortion, due to contrasting genetic systems in the uniting gametes or to genetic imbalance) (see Camadro *et al.* 2004). These barriers can be either unilateral (UI, in only one way of a given cross) or bilateral (BI, in both ways of the cross). Hybrids are formed when there are no barriers between the parental species, or when the barriers are incomplete or can be circumvented. However, even when hybrids are formed, other post-zygotic barriers can be expressed if the progenitors are not closely related, such as hybrid weakness, male (most common) and/or female sterility, and hybrid breakdown in the F_2 and other segregating generations (Camadro *et al.* 2004).

POLLEN-PISTIL INTERACTIONS

In flowering plants, sexual reproduction involves a series of very complex events that take place upon pollination: pollen hydration and germination, pollen tube growth along the style and in the ovary, penetration of the ovule and the embryo sac, and double fertilization to give rise to zygote and endosperm. These events, that have some degree of independent control, constitute the progamic phase that leads to sexual seed formation (de Graaf *et al.* 2001).

In potatoes, abnormal pollen-pistil relations can result from: (1) self-incompatibility, due to identity of alleles at the S (for self-incompatibility) locus (see Frankel and Galun 1977) and (2) incongruity, as described by Hogenboom (1973) in tomatoes and referred to as cross-incompatibility by Camadro and Peloquin (1981) in potatoes; the first occurs as a result of identity whereas the second is a corollary of evolutionary divergence.

Most diploid potato species are insect-pollinated obligate outcrossers because they possess a multiallelic S-locus with gametophytic expression. Polyploid polysomic species, as the common potato, can be self-compatible when pollen grains carry different S-alleles due to a phenomenon known as "competition interaction" (see Frankel and Galun 1977) that can either weaken or suppress the incompatibility reaction and result in seed formation. In autogamous disomic polyploids (i.e. tetraploids), fixed heterozygosity (McKey 1970) – that is, for duplicated loci, simultaneous homozygosity for different alleles and heterozygosity (i.e. AA.aa) – can occur. In fact, this phenomenon has been proposed to explain self-compatibility and, therefore, the autogamous behavior of tetraploid *S. acaule*, a species that grows at very high altitudes, where pollinating insects are not present, on the basis of observations regarding the aspartate aminotransferase locus (Camadro *et al.* 1992).

Fluorescence microscopy has revealed up to four sites of incompatible pollen-pistil relations in intra- and interspecific crosses between and within ploidy levels; i.e. in 4x *S. gourlayi*-6x *S. oplocense* crosses and backcrosses (Camadro and Peloquin 1981), 4x *S. tuberosum* ssp. *tuberosum*-2x *S. okadae* crosses (Camadro *et al.* 2008), 2x populations with germplasm of *S. chacoense* and 2x cultivated *S. phureja* (Camadro *et al.* (1998), 2x *S. kurtzianum* and *S. ruiz-lealii*, as well as between the latter two species and 2x *S. chacoense* (Raimondi and Camadro 2003; Raimondi *et al.* 2003); 2x *S. maglia*-2x *S. kurtzianum* crosses (Ispizúa *et al.* 1999), and even between morphological groups of 2x *S. spegazzinii* (Erazzú *et al.* 1999, 2009). Certain genotypic combinations in the previous examples exhibited more than one incompatibility site, and a few compatible pollen tubes in otherwise incompatible combinations also grew until the base of the style and among the ovules; in the latter, reduced number of seeds were obtained, revealing that pollen-pistil incompatibility barriers can be incomplete. Uniform pollen-tube growth arrest in the upper third of the style has also been observed in certain non-reciprocal interspecific tuber-bearing × tuber-bearing and tuber-bearing × non-tuber bearing crosses involving other diploid species (Fritz and Hanneman 1989).

The first to propose the action of specific genes independent of the *S*-locus in cross-incompatible combinations in potatoes were Grun and Radlow (1961) and Grun and Aubertin (1966) and, later, Hermsen and Sawicka (1979), Camadro and Peloquin (1981), Sala (1993), Masuelli and Camadro (1997), Camadro *et al.* (1998), Erazzú *et al.* (1999), among others. Camadro and Peloquin (1981), based on the Incongruency Model developed by Hogenboom (1973, 1979) in *Lycopersicon*, proposed a genetic model with dominant Cross Incompatibility (CI) genes in styles that prevent fertilization by pollen carrying specific dominant complementary genes. This model, which assumes segregation for both types of loci, accounts for the results of the observed UI and BI incompatibility in inter- and intraspecific crosses and also accommodates the results obtained in similar crosses in *Asparagus* between species that do not possess a self-incompatibility system (Marcellán and Camadro 1996). Studies have been initiated in our laboratory to identify the molecular basis of the pre-zygotic breeding barriers.

ENDOSPERM

Johnston *et al.* (1980) proposed that the success of a cross in potato, in the absence of pre-zygotic barriers, does not depend on the real ploidy (chromosome number) of the parents but on a 2:1 balance of genetic factors (EBN = Endosperm Balance Numbers) in the endosperm; these factors are provided by the female parent in the two nuclei of the central cell and by the male parent in the nucleus of the generative cell that fertilizes the central cell. The EBN or "effective ploidy" and the real ploidy do not necessarily coincide. Potato species have been assigned 1, 2 or 4 EBN on the basis of their crossing behavior (number of normal seeds/fruit and chromosome number of the progeny) in otherwise compatible pollen-pistil genotypic combinations of intra- and inter-ploid crosses (Johnston and Hanneman 1980; Hanneman and Bamberg 1986; Ochoa 1992; Hanneman 1994). Thus, and provided that pollen–pistil interactions are normal, 4x species with 4 EBN –as the common potato– can directly cross only with species with the same EBN, independently of their real ploidy (i.e. 4x or 6x) –as reported for 4x *S. gourlayi* and 6x *S. oplocense* (Camadro and Peloquin 1981)– and with species with 2 EBN only if the latter produce functional 2n gametes –as reported for 4x *S. acaule* (Camadro and Espinillo 1990)– or if its ploidy level is reduced by half by haploidization, as in crosses with *S. gourlayi*, *S. chacoense* and *S. spegazzini* (Santini *et al.* 2000).

The EBN in potato is apparently under control of few genes with additive effects (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995). However, the molecular bases have not been established yet but they are the object of a current collaborative research study (Masuelli *et al.* 2006) between our laboratory and the potato group led by Dr. R.W. Masuelli in Mendoza, Argentina.

The EBN concept is relevant for taxonomic and phylogenetic studies (Hawkes and Jackson 1992) and is also of great value for potato breeders because the success or failure of a cross and the chromosome number of the resulting progeny can be predicted in advance; in this way, strategies can be developed or applied to circumvent this barrier (Johnston and Hanneman 1982).

NUCLEAR-CYTOPLASMIC MALE STERILITY

In the absence of pollen-pistil and embryo/endosperm barriers, other post-zygotic barriers can act both in and controlled crosses. Hybrids that flower can be male and/or female sterile. The sterility, which can take diverse phenotypic expressions such as anther indehiscence, sporad formation, anther-style fusion, deformed flowers, and shrivelled microspores, among others (Grun 1979; Abdalla and Hermsen 1972; Larrosa *et al.* 2005; Marfil *et al.* 2009), can be controlled by nuclear or cytoplasmic genes. But, more frequently, sterility in potatoes and other species of agronomic importance is the result of specific interactions between genetic factors present in the nucleus of one species and in the cytoplasm of the other species that are involved in a cross (Grun 1990).

Nuclear-cytoplasmic interactions leading to male sterility in potatoes is well-documented in F_1 hybrids betwen various wild and cultivated species. As Camadro *et al.* (2004) have informed, male sterility has been reported as the result of interactions between *S. tuberosum* cytoplasm and nuclear genetic factors of the wild species *S. sanctae-rosae*, *S. infundibuliforme*, *S. raphanifolium*, *S. curtilobum*, *S. acaule*, *S. commersonii*, *S. chacoense*, *S. spegazzini* and *S. stoloniferum* (Lamm 1941, 1953; Brown 1984; Hermunstad and Peloquin 1985; Tucci *et al.* 1996; Santini *et al.* 2000; Carputo *et al.* 2003) and in other cultivated potatoes (Ross *et al.* 1964; Grun 1973; Hanneman and Peloquin 1981), *S. verrucosum* cytoplasm and nuclear genes of *S. phureja*, *S. chacoense*, and *S. tuberosum* (Buck 1960; Abdalla and Hermsen 1972), and *S. demissum* cytoplasm and nuclear genes of *S. tuberosum* (Dionne 1961), *S. tuberosum* cytoplasm and the 4x cytotype of *S. gourlayi* as well as in natural populations of this species, *S. infudibuliforme* and their interspecific hybrids (Larrosa *et al.* 2005).

Information about the genetic bases of nuclear-cytoplasmic male sterility is generally limited. However, it has been proposed that this phenomenon is under control of a single or a few dominant genes from wild and cultivated diploid species in interaction with *S. tuberosum* cytoplasm (Ross *et al.* 1964; Hanneman and Peloquin 1981; Iwanaga *et al.* 1991; Kaul 1988; Ortiz 1998, among others). However, the molecular basis of the nuclear-cytoplasmic male sterility remains unknown, although it is known that is controlled by mtDNA, as was also reported for other species (Breiman and Galun 1990; Cardi *et al.* 1999).

Abnormal flowers, as the observed by Raimondi *et al.* (2005) in *S. ruiz-lealii* and by other potato geneticists and breeders in wild and cultivated potato species (Roig 1956, Frusciante pers comm., our own experience) could not be associated to male sterility. They have been attributed to changes in the methylation status of DNA (Marfil *et al.* 2009), which result in epigenetic changes (that is, changes in gene expression in contrast to the genetic changes, which occur at the nucleotide sequence level). Although much research is needed in that respect, the epigenetic variation could be eventually of value in potato breeding.

BREEDING

The common potato and its closely related wild species have numerous characteristics of agronomic interest such as resistance/tolerance to water and temperature stresses, nematodes, harmful insects, fungi, bacteria and viruses, as well as desirable culinary and industrial qualities like high dry matter content, low reducing sugars and vitamin C (Huamán *et al.* 2000; Santini *et al.* 2000; Davies *et al.* 2002; Oltmans and Novy 2002; Jansky and Peloquin 2005). In fact, Ross (1986) reported that a large number of European and North American cultivars have resistance/tolerance to diverse pathogens and harmful insects introgressed from various diploid and polyploid wild species.

Argentina is a center of diversity of an important number of species some of which are sources of genes for desirable attributes. As previously described, the common potato cannot be directly crossed to diploid species (which, in their majority, have 2 EBN) because, being a tetraploid with 4 EBN, the hybrid endosperm is unbalanced and aborts. However, strategies have been devised to reduce its chromosome number by haploidization (Hougas and Peloquin 1958), cross it with other cultivated and wild species at the diploid level to produce a first round of heterosis and, finally, make use of 2n gametes from the diploid hybrids (preferably FDR 2n gametes, for the high percentage of heterozygosity than can transmit almost intact) to produce highly heterozygous (and heterotic) tetraploids for crop production. These manipulations that combine haploidization, selection, crossing and sexual polyploidization is known as the "analytical-synthetic" breeding scheme (Mendiburu *et al.* 1974), a scheme that has no drawbacks from a theoretical point of view but that cannot be easily applied in practical terms. Thus, in modern potato breeding, haploids ($2n=2x=24$) produced from commercial cultivars ($2n=4x=48$) are crossed to 2x species to broaden the genetic diversity through the introduction of new alleles for traits of interest and allelic diversity to maximize heterozygosity. Diploid hybrids that produce 2n gametes and that are pollen-pistil compatible with the common potato are then used in unilateral (4*x* x 2*x* and 2*x* x 4*x*), and bilateral (2*x* x 2*x*) sexual polyploidization crosses, to generate highly heterotic 4*x* progenies. The direction of the 4*x*-2*x* crosses will depend on the availability of diploid genotypes that produce 2n egg and/or 2n pollen, the mode of 2n gamete formation and the fertility of the parents involved.

The asexual reproduction system and the presence of 2n gametes in potato provide the unique possibility of accumulating non-additive genetic effects at the tetraploid level and the immediate fixation of heterosis (Mendiburu *et al.* 1974; Mendoza and Haynes 1976; Carputo *et al.* 2005). The extent of conservation of allelic interactions depends mainly on the mode of 2n gamete production (FDR vs. SDR), whereas the creation of new interactions depends mainly on the allelic diversity of the parents involved. With FDR 2n gametes, approximately 80% of the heterozygosity (and heterosis) present in the parents is transmitted and compounded in the upper level (Mendiburu *et al.* 1974). The value of these approaches in maximizing allelic interactions is well documented in potato, where highly heterotic tetraploid progenies have been produced between cultivated *S. tuberosum* and closely related tuber-bearing species (Tai and de Jong 1991; Werner and Peloquin 1991; Darmo and Peloquin 1991; Peloquin and Ortiz 1992; Ortiz 1998; Buso *et al.* 1999) to produce 4x offspring and Ortiz *et al.* (1991) reported that besides the allelic diversity transmitted, the 4*x* x 2*x* breeding scheme is more effective than the traditional 4*x* x 4*x* scheme because, in addition to the previously mentioned, fewer replications and locations are necessary for progeny evaluation.

In Argentina, wild potato species have a very wide geographical distribution and overlap in many areas with commercial potato crops and experimental plots of transgenic cultivars. In spite of this, there are no data generated in the country on gene flow between the cultivated and related wild species.

Gene flow can take place by dispersion of pollen, seeds and/or asexual propagules. It can be affected by pre- and/or post-zygotic internal hybridization barriers, which can reduce or even prevent hybridization. Moreover, gene flow by pollen can also be affected by environmental factors such as temperature, rains and predominant winds during the blooming season. Therefore, the results obtained in one environment are not necessary extrapolable to others.

This information is of fundamental importance for the establishment of isolation distances and strategies to prevent gene flow and its potential negative consequences (depending of the transgene) on the ecosystem (Camadro 2004). Actually, we are conducting a study that takes into account various variables to try to obtain a more precise risk estimation: two ploidy levels, various species and diploid genotypes that produce 2n gametes and are pollen-pistil compatible among them, in a design that has control of the environmental variables. Preliminary results obtained in one season, with two cultivars ('Huinkul MAG' and 'Spunta') and using SSR markers, revealed that hybrid seeds could be obtained at 40 m from the pollen source (the maximum distance established in the experiment) and that gene flow was not influenced by the direction of the predominant winds (Capurro and Camadro 2009).

CONCLUSIONS

In Argentina, there is an enormous source of genetic variability for potato improvement in the wild potato germplasm. To put it in a valuable form for breeding, it is necessary to continue with its genetic characterization and evaluation studies. Although various internal barriers can hindered or even prevent intra- and/or interspecific hybridization, some of these barriers can be incomplete; thus, the knowledge of their genetic bases is of fundamental importance for parental selection and the application of particular breeding strategies (i.e. ploidy level manipulations). The molecular bases of both pre- and post-zygotic breeding barriers are still unknown, but studies are progress, in association with other Argentinian laboratory, to dilucidate them. Shortly, we will have an assessment of gene flow risk in the field, under experimental conditions.

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