Ploidy level manipulations in potato through sexual hybridisation

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Summary

There is no better use of sexual reproduction in regard to breeding and genetic research than the ploidy level manipulations possible in the potato and its relatives. Unique reproductive characteristics of tuber-bearing Solanum species make possible: the production of gametes with unreduced chromosome number; the presence of an endosperm dosage system that regulates success of interploidy/interspecific crosses; the possibility to easily extract maternal haploids following crosses with S. phureja. This paper reviews results obtained in scaling genomic multiples up and down in potato, and relates these manipulations to breeding strategies for the genetic improvement of the cultivated potato. Several ploidy series have been developed, ranging from the monoploid to the hexaploid level. Cultivated tetraploids were scaled down to the diploid and monoploid level by haploidy. Scaling upward was achieved by sexual polyploidisation via 2n gametes that resulted in triploid, tetraploid, pentaploid, and hexaploid genotypes with a broad genetic base. Altogether, the success of ploidy level manipulations constitutes further proof that sexual polyploidisation played an important role in the polyploid evolution of Solanum species, and supports the idea that gene flow can be relatively easily accomplished through interploid and bridge crosses.

Key words: 2n gametes, endosperm, Solanum species, sexual polyploidisation, germplasm introgression

Introduction

Scaling up and down whole chromosome sets represents a powerful strategy to produce new genetic material for breeding purposes and genetic studies (Ramanna & Jacobsen, 2003). Among cultivated crops, the potato (Solanum tuberosum, 2n=4x=48) is one of the species where ploidy levels can more easily be manipulated and where this approach can have the widest multidisciplinary application. This is deemed very important given that the potato is unrivalled, among economic plants, in its abundance of related germplasm existing in nature at various ploidy levels (from diploid to hexaploid). Solanum species grow in a wide range of environments, from the southern part of the United States to southern Chile, and possess many desirable traits for potato breeding. The possibility of manipulating chromosome sets in the potato is essentially based on three biological features. First of all, there are several genotypes (mainly of Series Tuberosa) that produce gametes with an unreduced chromosome number (2n gametes) as the result of meiotic anomalies affecting either micro- or macrosporogenesis. The genetic control of these meiotic mutations is still an open question. According to some authors (reviewed in Peloquin et al., 1999), they are controlled by recessive genes with incomplete penetrance and variable expressivity. The presence of two genetic systems has also been hypothesised (Carputo et al., 1995). Analysis of alpha-tubulin and F-actin distribution during cytokinesis of meiotic mutants provided evidence that, besides spindle orientation, microtubular cytoskeleton abnormalities are involved in the formation of 2n pollen (Genualdo et al., 1998). Other scientists hypothesised that the production of 2n gametes behaves as a quantitative trait (Ramanna, 1983).

What is important in this context is that 2n gametes are the basis for sexual polyploidisation events. Bilateral and unilateral sexual polyploidisation (BSP and USP, respectively) can occur spontaneously in nature or can be induced artificially through controlled pollinations. BSP involves crosses where only one parent produces 2n gametes (either 2n pollen or 2n eggs). The second important biological characteristic of the potato is the endosperm balance number (EBN) (Johnston et al., 1980) that influences the success of interploidy/interspecific crosses. The EBN is a number varying from 1 to 4, and represents the “effective ploidy” of Solanum species. Cultivated S. tuberosum is 4EBN, whereas most wild species (either diploid or tetraploid) are 2EBN. According to the model developed, interploidy/interspecific crosses give a normal endosperm development only when there is a 2 : 1 maternal to paternal EBN ratio.
in the hybrid endosperm (Fig. 1). Thus, successful crosses occur only when the male and female gametes have the same EBN. Knowing the EBN of Solanum species, breeders can design crosses which selectively screen for 2n gametes. For example, a diploid species with EBN=2 and tetraploid S. tuberosum (4EBN) can be crossed only if the parent with lower ploidy produces 2n gametes. A tetraploid offspring with hexaploid endosperm is generated, and a strong block impedes triploid formation. The EBN model may represent an oversimplification of a complex biological phenomenon, and there are probably other unknown factors, besides EBN, which may be involved in the development of the endosperm. Exceptions to the 2 : 1 female to male EBN requirement have been found (Chavez et al., 1988; Camadro & Masuelli, 1995; Janssen et al., 1997; Carputo et al., 1998), and some of them may be due to these unknown factors. However, we strongly believe that, from the practical point of view of plant breeders, knowledge of EBN can be used in most cases as a predictor of 1) crossability between parents, and 2) ploidy level and EBN value of offspring.

The last essential biological feature of the potato is that maternal haploids (sporophytes with gametic chromosome number) can be easily extracted following crosses with pollinator clones of S. phureja. Haploids represent living gametes of a given genotype, and their production makes it possible to scale genomes down by half in a single step. It is believed that haploids originate through parthenogenesis from unfertilised egg cells of the female parent, and that both sperm nuclei fuse with the polar nuclei of the central cell of the female gametophyte to form the primary endosperm nucleus (von Wangenheim et al., 1960). Following analysis with RFLP markers, Clulow et al. (1991) hypothesized that S. tuberosum haploids may also originate from a process of chromosome elimination from zygotes deriving from S. tuberosum × S. phureja crosses. Wilkinson et al. (1995)

Fig. 1. Ploidy of embryo and endosperm, and female to male EBN ratio in the hybrid endosperm after intra(inter) ploidy and intra(inter) EBN crosses between Solanum species differing in ploidy and EBN. The EBN and ploidy of both central cells and eggs of the female gametophyte, and EBN and ploidy of sperm nuclei are shown.
demonstrated that somatic translocations allow stable incorporation of *S. phureja* DNA, thus explaining the presence of pollinator DNA in haploids obtained.

Conventional breeding strategies for the genetic improvement of the tetraploid cultivated potato are quite straightforward, and over the years they have changed little. They are essentially based on phenotypic recurrent selection, involving crosses between tetraploid varieties and/or advanced clones, and then field evaluation and selection. However, due to the simplicity with which in the potato whole chromosome sets can be manipulated, breeding at other ploidy levels is possible. Particular importance has been attached to the diploid level, in that it is a genetically simple and efficient approach for the use of diploid species. Also of note is the progress made in the last few decades in the field of molecular biology. It may give a further significant contribution to genome manipulations. Indeed, both in situ hybridisation (GISH and FISH) and molecular markers are useful supports to verify the products of sexual crosses, and to assist selection. In many examples reported in the literature in situ hybridisation has been used to identify intergeneric (*D’Hont et al.*, 1995; Garriga-Calderé *et al.*, 1999) or interspecific somatic and sexual hybrids in different genera (Lim *et al.*, 2001), including the genus *Solanum* (*Dong et al.*, 1999). In situ hybridisation can be particularly pertinent for the study of the transmission of extra chromosomes in aneuploids obtained from crosses involving odd ploidy levels. Therefore, depending on the genetic distance of intercrossed *Solanum* species, in situ hybridisation techniques could be more or less easily applied for this purpose. When no useful probes are available to distinguish whole genomes and/or chromosomes, various molecular markers may be used. In ploidy manipulations aiming to transfer genes from the wild incongruent species *S. commersonii* to the cultivated *S. tuberosum* genetic background, recombination events between homeologous chromosomes of these two species were clearly evident through the use of RFLP and AFLP markers (Barone *et al.*, 2001). In addition, *S. commersonii*-specific AFLP markers proved to be particularly efficient to select hybrids with a low wild genome content.

This paper will discuss results obtained in scaling genomic multiples up and down in potato, and relates these manipulations to interploid/interspecific germplasm transfer in the fourth most important food crop in the world. Only ploidy manipulations obtained through sexual hybridisation are discussed. Those achieved through in vitro tissue culture (protoplast fusion, anther culture, regeneration from explants) will not be reviewed here.

**Development of Ploidy Series**

Several ploidy series have been developed to sexually transfer genetic diversity from wild germplasm to the cultivated gene pool. They range from the monoploid to the hexaploid level.

**The monoploid level (2n=x=12)**

To produce monoploids from the tetraploid potato through pollinations with *S. phureja*, two successive cycles of chromosome number reduction are needed. Breukelen *et al.* (1975) first reported on monoploid extraction from one haploid genotype of *S. tuberosum* cv. Gineke. Monoploids from *S. tuberosum* and *S. tuberosum*-*S. phureja* genotypes were also extracted by Uijtwaal *et al.* (1987). The very large number of monoploids they obtained (more than 500) allowed them to determine the effect of maternal genotype, pollinator, physiological stage of plant material, and environment on the frequency and vigour of monoploids. Although most work has been done with *S. tuberosum*, monoploids have also been produced in other species, such as *S. verrucosum* (Breukelen *et al.*, 1977). Monoploid production can represent an interesting strategy to obtain homozygous lines useful for selecting mutant phenotypes, and for genetic studies. A well known example of the use of monoploids is given by the work done at the University of Wageningen on the selection of new starch mutants in potato, the amylose–free mutants (*amf*) (Jacobsen *et al.*, 1991). Recently, Hoogkamp *et al.* (2000) from the same University reported on the identification of two vigorous *amf* monoploids that produced microtubers.

One main bottleneck in the use of potato monoploids in breeding programmes is that they are sterile. Thus, strategies alternative to sexual hybridization must be followed for their utilisation. Wenzel *et al.* (1979) proposed a scheme including two cycles of haploid extraction (from 4x to 2x, and from 2x to 1x level) followed by chromosome doubling of monoploids. In this way homozygous diploid genotypes are produced. The resynthesis of highly heterozygous tetraploid genotypes is then accomplished by protoplast fusion.

**The diploid level (2n=2x=24)**

The diploid level is the naturally occurring level of most (about 80%) wild *Solanum* species noteworthy for potato breeding. The cultivated *S. tuberosum* has been scaled to the diploid level by 4x × 2x crosses with *S. phureja*. It should be pointed out that the *S. phureja* pollinators used are the same as those involved in monoploid production. Starting from the pioneering work of Hougas & Peloquin (1960), several studies have been undertaken on the production, characterisation and use of haploids from tetraploid genotypes. They allowed identification of
various factors affecting haploid frequency, the selection of haploids combining resistance traits with good agronomic performances (Concilio, 1992; Hutten et al., 1995; Carputo et al. 1996), and the determination of inbreeding effects (De, Maine, 1995).

Haploids have been mainly produced from S. tuberosum, even though examples exist of haploid extraction from S. acaule (Camadro et al., 1992), S. andigena (Samitsu & Hosaka, 2002), and somatic hybrids (Rokka et al., 1995). Haploids represent unique material not only for potato breeding, but also for genetic and evolutionary studies, and for germplasm introgression. For a review on the use of haploids see Peloquin et al. (1990). From the breeding standpoint, S. tuberosum haploids are widely used in sexual/somatic interspecific hybridisation programmes. First of all, they represent an essential tool to efficiently capture the genetic diversity of 2x(2EBN) Solanum species through haploid × wild species crosses. Hybrids involving species such as S. multidissectum, S. tarijense, S. sancta-rosae, S. chacoense have been produced (Table 1). The hybrids developed have 50% wild genome, and can be used in USP or BSP crossing schemes to re-establish the chromosome number of the cultivated potato, and gradually reduce the wild genome content. Breeding at diploid level has the great advantage of disomic rather than tetrasomic inheritance patterns, and requires a smaller population size (Peloquin et al., 1999). It should be pointed out that haploid-wild species hybrids represent a tool to indirectly evaluate tuber characteristics of wild species that lack tuberisation under long days (Yerk & Peloquin, 1989), as well as phenotypic correlations among traits of interest (Serquen & Peloquin, 1996). S. tuberosum haploids also served as essential ingredients to generate somatic hybrids between S. tuberosum and wild species with various ploidy and EBN, such as S. acaule, S. commersonii, S. brevidens, S. bulbocastanum. Table 1 reports some examples on the use of S. tuberosum haploids to transfer noteworthy traits through somatic hybridisation.

Besides haploidisation, the diploid level can also be reached through crosses involving triploid parents (generally 3x × 2x crosses). It has been widely reported that 3x × 2x progenies normally have a chromosome number close to that of the diploid parent, and euploid as well as aneuploid genotypes were produced through this approach (Vogt & Rowe, 1968; Lee et al., 1972; Wagenvoort & Lange, 1975). In the case of 3x × 2x crosses between triploids with EBN=2 and diploids with same EBN, progenies with a high number of extra chromosomes were obtained (Carputo, 1999). To explain these results, Carputo (1999) hypothesised that in these intra-EBN crosses the 2 : 1 EBN requirement performs negative selection for gametes with a low chromosome number and EBN, and positive selection for gametes with a high chromosome and EBN number.

The triploid level (2n=3x=36)

Triploids played a key role in the evolutionary pathway leading to polyploid evolution in several Angiosperms (Ramsey & Schemske, 1998). In the case of tuber-bearing Solanum species, they probably represent a link between 2x and 4x species in the process of polyploid evolution. In 2x(2EBN) × 2x(1EBN) crosses, the required 2 : 1 ratio in the endosperm favors 2n gametes of the parent with lower EBN, resulting in the formation of a viable

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Table 1. Examples of wild Solanum species sexually or somatically hybridised with S. tuberosum haploids to transfer resistance traits from wild Solanum species to the S. tuberosum gene pool

<table>
<thead>
<tr>
<th>Wild species</th>
<th>Ploidy of offspring</th>
<th>Target resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual hybridisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. chacoense</td>
<td>2x</td>
<td>Tuber soft rot</td>
<td>Zimnyak-Guzowska &amp; Tejciowska, 1993</td>
</tr>
<tr>
<td>S. malacoreticulatum</td>
<td>2x</td>
<td>Tuber soft rot</td>
<td>Carputo et al., 19976</td>
</tr>
<tr>
<td>S. phureja, S. stenotomum</td>
<td>2x</td>
<td>Tuber soft rot</td>
<td>Wolters &amp; Collins, 1994</td>
</tr>
<tr>
<td>S. sancta-rosae</td>
<td>2x</td>
<td>Frost</td>
<td>Taccini et al., 1996</td>
</tr>
<tr>
<td>S. sancta-rosae</td>
<td>2x</td>
<td>Nematodes</td>
<td>Iwanaga et al., 1989</td>
</tr>
<tr>
<td>S. sancta-rosae</td>
<td>2x</td>
<td>Bacterial wilt</td>
<td>Watanabe et al., 1992</td>
</tr>
<tr>
<td>S. sancta-rosae, S. berthaultii, S. chacoense</td>
<td>2x</td>
<td>Bacterial wilt</td>
<td>Jansky &amp; Rouse, 2000</td>
</tr>
<tr>
<td>S. sancta-rosae, S. bertaultii, S. phureja</td>
<td>2x</td>
<td>Tuber soft rot</td>
<td>Andrivon et al., 2003</td>
</tr>
<tr>
<td>Somatic hybridisation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S. sanchezii</td>
<td>6x</td>
<td>Potato virus X</td>
<td>Yamada et al., 1997</td>
</tr>
<tr>
<td>S. brevidens</td>
<td>4x, 6x</td>
<td>Potato virus Y</td>
<td>Redzic et al., 1994</td>
</tr>
<tr>
<td>S. commersonii</td>
<td>4x, 6x</td>
<td>Frost</td>
<td>Cardi et al., 1993</td>
</tr>
<tr>
<td>S. tuberosum</td>
<td>4x, 6x, 8x, 10x</td>
<td>Late blight</td>
<td>Zimnyak-Guzowska et al., 2003</td>
</tr>
<tr>
<td>S. stenotomum</td>
<td>4x, 6x</td>
<td>Bacterial wilt</td>
<td>Fock et al., 2001</td>
</tr>
</tbody>
</table>
endosperm associated with a triploid embryo.

Triploids have been artificially produced mainly as bridge ploidies to overcome incompatibility barriers. Intra-EBN crosses were designed to generate triploids from 4x(2EBN) x 2x(2EBN) crosses between noteworthy tetraploid species S. acaule, S. fendleri, S. hjertingii, S. papita, S. polytrichon, S. stoloniferum and 2x(2EBN) genotypes (Brown, 1988; Brown & Adiwilaga, 1990; Adiwilaga & Brown, 1991). Most triploids produced pollen grains with an unreduced chromosome number that allowed their use in subsequent breeding efforts. Another possible means of producing triploids is through somatic chromosome doubling of 2x(1EBN) species to synthesise 4x(2EBN) genotypes, compatible with 2x(2EBN) parents. This strategy was followed to exploit 2x(1EBN) - 2x and 3x × 4x crosses and allowed the production of progenies with various chromosome complements.

Several examples exist where triploids have been produced from 2x(1EBN) - 2x(2EBN) crosses where the parent with lower EBN produced 2n gametes. In order to transfer traits of interest from 2x(1EBN) species into S. tuberosum, triploid hybrids were obtained between 2x(1EBN) S. brevidens (Johnston & Hanneman, 1982) and S. commersonii (Carputo et al., 1997a). Triploids produced from 4x × 2x crosses were female fertile in both 3x × 2x and 3x × 4x crosses, and allowed the production of progenies with various chromosome complements.

The tetraploid level (2n=4x=48)

This is the naturally-occurring level of cultivated potato and some of its wild relatives like S. fendleri, S. acaule, S. oxycarpum, S. stoloniferum. The origin of tetraploid S. tuberosum has been explained by sexual polyploidisation involving diploid wild species. Hawkes (1990) proposed an evolutionary pathway where the cultivated S. andigena derived from cultivated 2x(2EBN) S. stenotomum and the 2x(2EBN) weedy species S. sparsipilum through bilateral polyploidisation. S. tuberosum derived from S. andigena as a result of artificial selection for tuberisation under long day conditions. Somatic doubling of diploids is theoretically another pathway leading to tetraploids. However, the genetic consequences of 2n gametes indicate that sexual polyploidisation results in greater variability, fitness, and heterozygosity than somatic doubling. Carputo et al. (2003) suggested that the mechanism of 2n gamete formation and the frequency of 2n gamete forming genes in tetraploids and their ancestral diploid species is evidence of the involvement of sexual polyploidisation in the evolution of tetraploid potatoes.

Several methods have been set up to synthesise new tetraploid potatoes. Both USP and BSP are effective due to the heterosis for tuber yield of the progeny and their broad application. In addition, the availability of so many traits in the wild germplasm, the widespread occurrence of 2n gametes, and the ease with which diploid 2n gamete-producers hybridise with 4x potato strongly favour these approaches as a valuable scheme for germplasm transfer. The most difficult task for this purpose is that breeders must select desirable genotypes that also produce 2n gametes. The potential of these breeding schemes in potato has been demonstrated mainly from USP involving 2x S. phureja (Tai & De Jong, 1991; Clulow et al., 1995; Buso et al., 1999, 2002), and other species such as S. berthaultii (Darmo & Peloquin, 1991), S. tarijense (Carputo et al., 2000), S. sparsipilum (Serquen & Peloquin, 1996), S. vernei (Ortiz et al., 1997). The most successful use of USP has been by the potato breeding programme at Rozalin, Poland. Here 2x S. tuberosum – wild species hybrids with multiple disease resistance and desirable tuber quality are currently developed and used in 4x × 2x crossing schemes to develope parental lines and cultivars (Zimnoch-Guzowska, 2002). Also, in China potato cultivars obtained through USP are being released (Jin et al., 2004). By contrast, the potential of BSP has only been experimentally demonstrated, and to our knowledge no cultivar has been produced through this approach yet. Ortiz & Peloquin (1991) proposed to use BSP to produce inexpensive 4x hybrids through 2x × 2x crosses using bumblebees as pollinators.

Another interesting way to produce tetraploids is from interploidy intra-EBN crosses. Cases have been reported of tetraploids being produced after 5x(4EBN) × 4x(4EBN) crosses (Carputo, 2003). In fact, a pentaploid genotype can produce gametes with various chromosome complements, from 24 to 36. If 24-chromosome gametes have an EBN=2, these crosses produce tetraploid progenies. Similarly, 3x(2EBN) x 4x(4EBN) interploidy crosses can theoretically yield tetraploids. The two necessary conditions for this event are that the functional gametes of the 3x parent must have a 24-chromosome complement and an EBN=2. Watanabe et al. (1992) proposed a breeding strategy to produce tetraploids from 4x(2EBN) species. It involves sexual crosses between 4x(2EBN) species and 4x(4EBN) S. tuberosum followed by a second pollination and embryo rescue. The authors suggested that, to maximise the efficiency of
selection, haploids can be extracted from tetraploid offspring and selection can be made at diploid level.

**The pentaploid level (2n=5x=60)**

A direct method of producing a pentaploid is by 4x(4EBN) - 6x(4EBN) crosses. To introgress resistance to root-knot nematodes of hexaploid species *S. hougasii*, Janssen et al. (1997) produced resistant pentaploids that were successfully backcrossed as females to *S. tuberosum*. Pentaploids and aneuploid-pentaploids have also been produced following 3x(2EBN) - 4x(4EBN) crosses (Ehlenfeldt & Hanneman, 1984; Brown & Adiwilaga, 1990; Adiwilaga & Brown, 1991; Carputo, 2003). If this approach is used, triploid parents have to produce 2n gametes, functional balanced gametes in terms of chromosome number and EBN.

In most 4x × 3x crosses, pentaploids were produced through the use of 2n pollen. Use of triploids producing 2n pollen guarantees the production of euploid pentaploid progenies when the meiotic mutation responsible for 2n pollen formation is parallel spindle. In fact, after the chromosomes of the triploid distribute randomly into the Telophase I nuclei, the parallel spindle mechanism in the second division ensures symmetric incorporation of 36 chromosomes in each pair of 2n microspores. By contrast, when pentaploids are produced through 3x × 4x crosses, a high frequency of aneuploids may be found if the restitution mechanism leading to 2n egg production is omission of second meiotic division. In fact, in this case meiotic restitution in the 3x female parent involves poles with various chromosome numbers, from 12 to 24, as expected from the chromosome distribution at Anaphase I of the triploid. As a result of meiotic restitution, 2n eggs from 24 to 48 chromosomes are functional in the 3x × 4x crosses. The possibility that eggs with 24 chromosomes may operate in 3x × 4x crosses is very important from the breeding standpoint. Indeed, it guarantees the re-establishment of the tetraploid level of the cultivated potato after one single backcross, avoiding the production of a second odd ploidy bridge (Fig. 2). It should be pointed out that it is necessary that eggs with 24 chromosomes have an EBN of 2 to guarantee a balanced EBN ratio in the hybrid endosperm.

**The hexaploid level (2n=6x=72)**

This represents the natural ploidy level of a few wild species. Among them, *S. demissum* is by far the most commonly used species for potato breeding (Ross, 1986). This is probably due to the high resistance of this species to *Phytophthora infestans*, the causal agent of late blight. The best explanation for the origin of hexaploids is the union of 2n gametes from triploid genotypes. New hexaploids can be efficiently produced also by 4x(2EBN)-4x(4EBN) crosses if the parent with lower EBN produces 2n gametes. The F₁ hexaploid offspring produced through these matings can be used as a genetic bridge and crossed to *S. tuberosum*. Direct crossing between virus resistant 4x(2EBN) *S. acaule* and 4x(4EBN) *S. tuberosum* was possible through the functioning of 2n eggs (Camadro & Espinillo, 1990). Similarly, hexaploids were yielded from 4x(2EBN) *S. fendleri* × 4EBN *S. tuberosum* (Janssen et al., 1997). Hexaploids were also produced through bridge hybridisation by Bamberg et al. (1994). They crossed a synthetic 4x(2EBN) *S. commersonii* with 4x(2EBN) *S. acaule* to get fertile hybrids. These were crossed to *S. tuberosum* and, through the use of 2n gametes, produced hexaploid complex hybrids. The same authors successfully produced hexaploids by crossing F₁ hybrids of 4x *S. commersonii* × species of Series Longipedicellata with *S. tuberosum*. Finally, the hexaploid level may be potentially produced by 3x-6x crosses in which there is a selective screen for 2n gametes from the 3x parent.

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**Fig. 2.** Breeding strategy to transfer useful genes and allelic diversity from 2x(1EBN) and 4x(2EBN) species into cultivated *S. tuberosum* (tbr). 4x and 5x hybrids are produced if the 3x parent produces balanced gametes with 24 and 36 chromosomes, respectively.
Conclusion

Endosperm (through the EBN incompatibility system) and 2n gametes are the keys to ploidy level manipulations in potato. Many ploidy levels have been produced, representing useful material for genetic and evolutionary studies as well as breeding efforts. Similar manipulations can be used for genetic studies and breeding efforts of polyploid crops when 2n gametes and EBN-like systems have been identified (e.g. alfalfa, clover, oat). Of particular importance is the fact that odd ploidy levels often significantly contribute as a bridge to several ploidy levels, refuting the commonly held opinion that they represent dead-ends. The fact that all polyploid levels have been generated by sexual polyploidisation is also significant. This constitutes further proof that sexual polyploidisation played an important role in the evolution of ploidy in this species and explains the origin of the cultivated tetraploid potato.

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References


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