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Fertilization fitness and offspring ploidy in 3x × 2x matings in potato

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Abstract
The main objective of the current research was to study the reproductive behaviour of artificial triploid potato hybrids between wild *Solanum commersonii* and the cultivated potato *Solanum tuberosum*. When used in 3x × 2x crosses, triploids gave aneuploid progenies with somatic chromosome number ranging from 29 to 36. Fertilization fitness data suggested that the survival rate of gametes produced by the triploid parents may be related to their chromosome number. In addition, consistent with molecular data, our results indicated that fitness of gametes and chromosome number of progenies are influenced by the genome dosage of interspecific triploids. Since a main route to polyploidy formation is via 2n gametes and triploids, our study may contribute to a better understanding of polyploid plant reproduction, evolution and breeding.

Keywords: *Endosperm balance number, fertilization fitness, aneuploid, AFLP markers, potato*

Introduction

The production of artificial hybrids between related species represents a powerful approach for producing new genetic variability useful for both breeding purposes and genetic studies. It allows introgression of noteworthy genes from wild species into the cultivated gene pool; production of genotypes combining specific chromosomes from two parents; studies on the genomic alterations (e.g. changes in gene expression or genome content) that accompany hybrid formation; and testing of evolutionary hypotheses on plant speciation (Rieseberg & Willis 2007; Jin et al. 2008; Peruzzi 2008; Jansky 2009; Soltis & Soltis 2009; Galván et al. 2010). In this regard, information on the frequency of progenies with a specific chromosome number is important. Also of interest is the evaluation of the competitive ability of gametes and its relationship to the variation in chromosome number in succeeding generations. Interspecific hybridization is particularly attractive for improving characteristics of the tetraploid ($2n = 4x = 48$) cultivated potato *Solanum tuberosum*, which has about 200 wild relatives possessing several important resistance and quality traits that could be introgressed into the cultivated gene pool (Spooner & Salas 2006). Many of these wild species are sexually isolated from *S. tuberosum*. According to the endosperm balance number (EBN) hypothesis (Johnston et al. 1980), each potato species has an inherent EBN varying from 1 to 4. Successful hybridization occurs when a 2:1 maternal to paternal EBN ratio is present in the hybrid endosperm. In the absence of spontaneous (e.g. through 2n gametes) or artificial (e.g. through colchicine) variation in ploidy, a 2:1 EBN ratio occurs only when parents have the same EBN. If the EBNs are mismatched, the hybrid endosperm breaks down and embryo degeneration occurs. In potatoes, the EBN represents a strong isolating mechanism between species and, complementing the role of 2n gametes, allows speciation of polyploids (Carputo et al. 2003; Ortiz et al. 2009). An EBN-like system has been hypothesized also for several other species (Levin 2002).

Among wild potato species, *S. commersonii* ($2n = 2x = 24$) is of great interest as it possesses several desirable traits such as resistance to environmental stress (low temperatures) and pathogens (*Ralstonia solanacearum*, *Pectobacterium carotovorum*). Since *S. commersonii* is a 1EBN species and *S.*

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tuberosum is either 4EBN (at the tetraploid level) or 2EBN (at the diploid level), endosperm breakdown occurs following direct crosses between these species. To overcome EBN barriers, Carputo et al. (1995) doubled the somatic chromosome number (and as a consequence the EBN) of *S. commersonii,* generating 4x(2EBN) clones that were sexually compatible with 2x(2EBN) *S. tuberosum*. Following crosses between 4x(2EBN) *S. commersonii* and 2x2EBN) *S. tuberosum – Solanum phureja* hybrids, 3x(2EBN) hybrids were produced. These triploids were successfully used in breeding programmes and also in basic research to produce aneuploids through 3x × 2x crosses.

This article is a continuation of a previously published paper by Carputo (1999). Our objectives were to assess the competitive ability of female gametes produced by *S. commersonii–S. tuberosum* triploids and the pattern of chromosome number variation in derivatives from 3x × 2x crosses. Evaluation of fertilization fitness, a concept developed by Lu and Kato (2001), and genetic analysis with amplified fragment length polymorphism (AFLP) molecular markers were used as tools in this study.

Materials and methods

Plant material

The study was carried out on 72 offspring generated from 3x × 2x matings between four triploid *S. commersonii–S. tuberosum* hybrids used as females (code: A1, B3, B10 and C1) and diploid *S. phureja* used as pollinator (clone IVP35) because of its high male fertility. Triploid hybrids, *S. tuberosum* cv. Blondy, *S. phureja* and *S. commersonii* (PI 243503) were also included as controls in the molecular analysis. Interspecific 3x × 2x hybrids were coded as follows: MCF (C1 × IVP35); MCL (B10 × IVP35); MCM (B3 × IVP35); and MCN (A1 × IVP35).

Cytological analysis and fertilization fitness

To analyze the somatic chromosome number of 72 3x × 2x hybrids, root tips were pre-treated with a 2 mM 8-hydroxyquinoline solution for 3 h at room temperature, fixed in ethanol-glacial acetic acid (3:1) and stored at -20°C. Digestion was carried out with an enzymatic solution (1% pectinase and 2% cellulase, Sigma, Aldrich, St. Louis, USA) for 40 min at 37°C. After digestion, the meristematic region was excised and macerated on a glass slide in fixing solution (methanol:acetic acid, 3:1). For chromosome DNA staining, 200 μl of Giemsa solution (0.5 ml 0.067 M Na_2HPO_4, 0.5 ml 0.067 M KH_2PO_4 and 0.1 ml of Giemsa dye, J.T. Baker, Deventer, Holland) were added to each slide. After 30 min, slides were washed with distilled water and air-dried. Chromosomes were counted on at least 10 mitotic metaphase cells per hybrid.

To evaluate the competitive ability of gametes from 3x female parents, fertilization fitness (Fi) and relative fitness (rFi) were estimated (Lu & Kato 2001). These parameters were calculated based on the theoretical production of gametes with different chromosome numbers, assuming a binomial distribution. Fertilization fitness is the ratio of the actual frequency (Ai) of gametes with a specific chromosome number to the theoretical frequency based on a binomial distribution of chromosomes in anaphase I of meiosis. Relative fitness represents the ratio of fertilization fitness of gametes with a specific chromosome number to fertilization fitness of gametes with the highest fitness. Both parameters are indicators of the fitness ability of gametes in fertilization and in the production of viable progeny (Lu & Kato 2001). The chromosome number of gametes produced by triploids was deduced by subtracting from the somatic chromosome number of the progeny the euploid gamete chromosome number of diploid parents (12). Actual frequencies (Ai), theoretical frequency (Ti), Fi and rFi were calculated using the formulas shown below.

(1) Actual frequency (Ai) of gametes with chromosome number $n = \mathrm{i}$:

$$\mathrm{Ai} = \mathrm{ni/N} \times 100;$$

ni = number of hybrids with a specific chromosome number;

N = total number of hybrids analyzed.

(2) Theoretical frequency (Ti) of gametes with chromosome number $n = 12 + i$ was calculated from the binomial expression: $(0.5 + 0.5)^{12}$

(3) Fertilization fitness (aFi) of gametes with chromosome number $n = \mathrm{i}$:

$$\mathrm{Fi} = \mathrm{Ai/Ti}.$$

(4) Relative fitness:

$$\mathrm{rFi} = \mathrm{Fi/hF}.$$

where hF = fertilization fitness of gametes with the highest fitness.

AFLP analysis

For AFLP analysis, 21 3x × 2x hybrids showing normal growth and no phenotypic aberrations were used. *S. commersonii*, *S. phureja* and *S. tuberosum*

were also included. Total DNA was extracted from frozen leaves using the DNeasy Plant Mini Kit (QIAGEN Gmbh, Hilden, Germany). AFLP analysis was carried out according to the method described by Vos et al. (1995) except that AFLP fragments were detected using a fluorometric system. A commercially available kit (Gibco-BRL AFLP analysis System I, Life Technologies, Gaithersburg, MD, USA) was used, in which *Eco*RI (*E*) and *Mse*I (*M*) are the restriction enzymes. All samples were screened with six AFLP primer pairs: *E*-ACT/*M*-CAC; *E*-ACT/*M*-CAG; *E*-AGG/*M*-CAG; *E*-AGC/*M*-CAA; *E*-AGC/*M*-CTA; *E*-AGG/*M*-CAA; *E*-AGG/*M*-CTT. Only *Eco*RI primers were labeled with 6-carbozy-fluorescein (6-FAM). AFLP fragments were separated on 6% denaturing polyacrylamide gels in 1× TBE buffer [100 mM Tris, 100 mM boric acid, 2 mM EDTA (pH 8.0), 6 M urea] and fluorescence was detected with a Typhoon 9210 scanner (Amersham). For each marker, only clear and unambiguous bands were scored as 1 for presence and 0 for absence. Markers present in only one species were classified as *S. commersonii* (CMM), *S. phureja* (PHU) and *S. tuberosum* (TBR) specific markers. To estimate the genetic similarity between hybrids, a dendrogram was constructed using the unweighted pair-group method (UPGMA) and Jaccard's similarity coefficient. Statistical analyses were carried out using NTSYS-pc (Numerical taxonomy system, version 2.1, Rohlf 2000).

Results and discussion

The 3x × 2x progeny produced in this study showed high variability in terms of chromosome number that ranged from 29 to 36 (Figure 1). The average value was $2n = 32.6 \pm 2.4$; 39% of hybrids showed a somatic chromosome number of $2n = 33$, 32% displayed $2n = 31$–32, and the remaining 29% displayed $2n = 29$–30 and $2n = 34$–36. The frequency distribution of genotypes with various chromosome numbers was significantly different from the expected random distribution (χ^2 757.2, $P < 0.01$). The inferred gametic chromosome numbers of fertilized female eggs are listed in Table I. Actual frequencies ranged from 4% (for $n = 17$) to 39% (for $n = 21$). According to the literature, 3x × 2x matings usually give trisomics ($2n = 2x + 1$), as reported in *Asparagus* (Ozaki et al. 2004), *Hordeum* (Thomas & Pickering 1988), *Pennisetum* (Dujardin & Hanna 1988), *Solanum* (Wagenvoort & Lange 1975; Kessel & Rowe 1975) corn (Premachandran & Sarkar 1992), wild rye (Wei et al. 1995) and *Brassica* (Zhang et al. 2006). However, our matings did not produce trisomics but progeny with a high number of extra chromosomes. Indeed, fertilization successfully occurred only with gametes possessing a chromosome number from $n = 17$ to $n = 24$. To compare the competitive ability of egg cells produced by our 3x female parents, the fertilization fitness (Fi) and relative fertilization fitness (rFi) were calculated (Table I). The highest Fi was found in gametes with

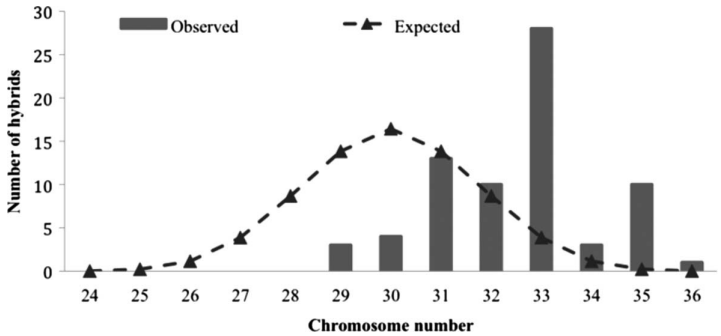

Figure 1. Number of observed *vs.* expected hybrids following 3x × 2x crosses based on chromosome number.

Table I. Actual and theoretical frequency, fertilization fitness and relative fitness of the maternal gametes with specific chromosome numbers involved in 3x × 2x crosses.

	Chromosome number (n)												
	12	13	14	15	16	17	18	19	20	21	22	23	24
Actual frequency (%)	0	0	0	0	0	4.2	5.7	18.1	13.9	38.9	4.2	13.9	1.9
Theoretical frequency (%)	0.01	0.28	1.44	4.88	10.93	17.43	20.69	17.43	10.93	4.88	1.44	0.28	0.01
Fertilization fitness (Fi)	0.00	0.00	0.00	0.00	0.00	0.24	0.27	1.04	1.27	7.98	2.88	50.00	100.0
Relative fitness (rFi)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.08	0.03	0.50	1.00

Note: To calculate the actual frequency, it is considered that the diploid parent produced gametes with $n = x = 12$.

$n = 24$ (Fi 100), followed by those with $n = 23$ (Fi 50). In gametes with $n = 19$–22, Fi was higher than 1, indicating that they were competitive to some extent. By contrast, in those with Fi < 1 ($n = 17$ and 18) the competitive ability was low. To calculate the rFi, we considered the highest Fi (100) for gametes $n = 24$. rFi showed a distribution similar to that of fertilization fitness, ranging between 0 and 1. These data confirmed that the competitiveness of gametes might be related to chromosome number since any increase in chromosome number corresponded to changes in the fertilization fitness. Similar results have been found by Lu and Kato (2001) in *Brassica*. Indeed, in a 3x × 2x interspecific cross combination, they found that gametes with euploid chromosomes showed higher fertilization fitness than aneuploid gametes. Based on meiotic analysis, the authors hypothesized that the difference between actual and theoretical frequency of gametes may result from selective pressure at the embryo developmental stage. Although differential survival rates can also affect the frequency of certain types of gametes, a similar hypothesis may explain our results. This would be supported by EBN knowledge and meiosis of the triploids parent, as already suggested by Carputo (1999). It can be inferred that our triploids produced gametes with various chromosome numbers, as expected from the theoretical frequencies reported in Table I. However, since 3x × 2x matings in this study were performed between parents with the same EBN, only eggs with 17–24 chromosomes were successfully fertilized by male gametes and thus showed the highest fertilization fitness. These eggs are more likely to possess the same EBN value (i.e. 1) as gametes produced by the 2x(2EBN) male parent. Our molecular data are consistent with this possibility. Indeed, UPGMA analysis (Figure 2) showed that all aneuploid hybrids and their triploid parents were more similar to wild *S. commersonii* than to *S. tuberosum* and *S. phureja*. Moreover, frequencies of *S. commersonii* AFLP markers in aneuploid hybrids were higher than in *S. tuberosum* and *S. phureja* (data not shown) suggesting that they may derive from eggs possessing a high number of *S. commersonii* chromosomes. Previous studies indicated that in potato EBN is controlled by either three (Ehlenfeldt & Hanneman 1988) or two loci (Camadro & Masuelli 1995) with additive effects. Therefore, in gametes produced by triploids, the likelihood of inheriting all genes increases with a greater number of chromosomes. Since a *S. commersonii* haploid genome has an EBN of 0.5 and each haploid genome has an additive effect on EBN, eggs with several *S. commersonii* chromosomes may be more likely to have all genes controlling EBN and hence an EBN of 1, compatible with 1x gametes produced by the diploid male parent. This might explain the highest fitness of gametes with $n = 24$ chromosome, which are those with the highest probability of having all genes involved in this regulatory system. Recently, to explain the parent-of-origin effects during angiosperm seed development, Dilkes and Comai (2004)

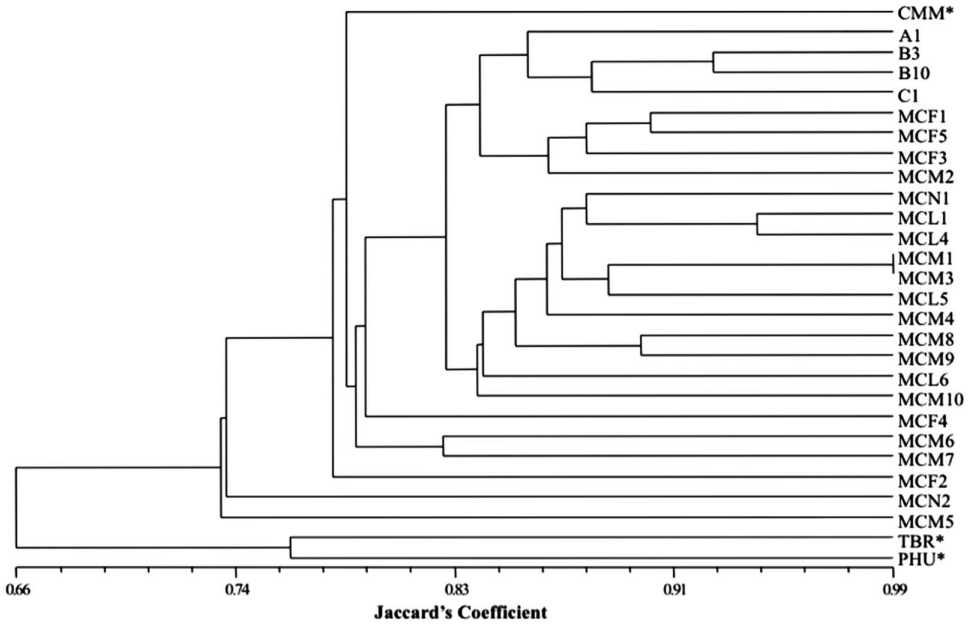

Figure 2. UPGMA cluster analysis using AFLP fingerprinting data of 3x × 2x aneuploid hybrids (MC) and their triploid (A1, B3, B10 and C1) and diploid (PHU*, TBR* and CMM*) parents. Similarity on the *x*-axis is based on Jaccard's coefficient. *PHU = *S. phureja* clone IVP35; TBR = *S. tuberosum*; CMM = *S. commersonii*.

confirmed the presence of a dosage-sensitive gene mechanism in which the dose of a gene product contributes to endosperm development.

In conclusion, our results provide evidence that in triploids, gametes with the highest fertilization fitness were those with the highest chromosome number and that this could be related to the EBN incompatibility system. Our findings not only support the EBN model but also provide genetic material that, with the availability of molecular tools, can be useful for studying the genetic control of EBN. We are currently developing *S. commersonii* chromosome-specific markers in order to assess the genome dosage in 3x × 2x hybrids. Towards this goal, we are looking for single nucleotide polymorphisms (SNPs) among single-copy conserved ortholog sequences (COSII markers) and are using the high resolution melting (HRM) approach to rapidly scan our hybrids.

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