Cytological Analysis and Fertility of *Solanum commersonii* Dun. × *Solanum gourlayi* Haw. Triploid Hybrids

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Solanum commersonii Dun. (*comm*, $2n=2x=24$) is a wild diploid potato species endemic to Argentina, Brazil, Paraguay and Uruguay, which belongs to the series Commersoniana (Hawkes and Hjerting 1969). It is of potential value for potato breeding because it possesses resistance to adverse biotic and abiotic factors (see Hanneman and Bamberg 1986). This species cannot be directly crossed with other related diploid or higher ploidy tuber-bearing *Solanum* species, due to endosperm dysfunction in the hybrid seed (Johnston and Hanneman 1980, 1982). Therefore, it is necessary either to decrease the ploidy level of the related species or to increase the ploidy level of *comm* to obtain interspecific hybrids. The second alternative may be achieved through either asexual polyploidization (by colchicine treatment of somatic tissues) or sexual polyploidization (by functioning of $2n$ gametes).

In order to make this species accesible to potato breeding, crosses between several introduction of *comm* and the "bridge" species *Solanum gourlayi* Haw. (*grl*, $2n=2x=24$) were attempted to facilitate the ploidy level manipulations. The choice of *grl* was based on the affinities that this species has with the cultivated potato *Solanum tuberosum* L. ssp. *tuberosum* (*tbr*, $2n=4x=48$), given that both belong to the same taxonomic series Tuberosa (Hawkes 1990). Triploid hybrids were obtained from these crosses through the functioning of $2n$ eggs from *comm* (Masuelli 1990). In these triploids, meiosis was irregular; lagging and disperse chromosomes were observed in anaphase I and anaphase II. Despite all these irregularities, the average pollen stainability was 19.0%. Parallel spindles in anaphase II, leading to $2n$ pollen formation, were observed in these triploids, but the occurrence of this mechanism was not enough to account for the formation of all plump, stainable pollen grains.

For determining the possibility of introducing, by recombination, genes from *comm* into the cultivated potato using *grl* as a bridge species, an analysis of the meiotic behaviour, pairing affinity and fertility of the *comm* × *grl* triploid hybrids was carried out.

Material and methods

The genetic materials used in this study are presented in Table 1. The triploid F_1 hybrids were obtained from crosses between plants of the diploid species *comm*, which produced $2n$ eggs, and $2x$ *grl* (Masuelli 1990). Plant introductions of *S. infundibuliforme* Phil. (*ifd*, $2n=2x=24$), *S. acaule* Bitt. ssp. *acaule* (*acl*, $2n=4x=48$), *S. gourlayi* Haw. and *S. commersonii* Dun. were supplied by the Potato Germplasm Bank of the Agricultural Experimental Station (I.N.T.A.), in Balcarce, province of Buenos Aires, Argentina. These plants were grown in pots in a glasshouse, where the crosses were made.

After harvested, seeds were treated with 1500 ppm gibberellic acid (GA_3) to promote uniform germination.

Table 1. *Solanum* species and cultivars used in crosses

Species or cultivars	Ploidy	Introduction
F ₁ (<i>S. commersonii</i> × <i>S. gourlayi</i>)	3x	OKA 5258 X OKA 7660
<i>S. gourlayi</i>	2x	OKA 7680
<i>S. commersonii</i>	2x	OKA 5258
<i>S. infundibuliforme</i>	2x	OKA 7652 K
<i>S. acaule</i>	4x	OKA 5816
<i>S. tuberosum</i> ssp. <i>tuberosum</i> cvs.		
Huinkul	4x	cultivar
Sta. Rafaela	4x	cultivar

Table 2. Mean number per cell of various chromosome configurations, arm pairing frequencies ("C") and relative affinity values ("X") in three triploid *cmm* × *grl* hybrids

Genetic material	No. of cells	Chromosome configurations				“C”	“X”
		Univ.	Biv.		Triv.		
			Ring	Rod			
F ₁ <i>cmm</i> × <i>grl</i>	33	3.45 (1-7)*	4.42 (2-9)	4.63 (2-8)	4.85 (2-8)	0.965	0.699

* Range.

Table 3. Chromosome behavior in metaphase II and at the tetrad stage in three *cmm* × *grl* triploid hybrids

Hybrids	No. of cells	Metaphase II			Tetrad stage			
		Cells with lagg. (%)	No. of lagg. per cell		No. of cells	Tetrads with micro-cells (%)	No. of micro-cells per tetrad	
			$\bar{X}$	range			$\bar{X}$	range
115/19	214	75.7	3.0	0-8	233	46.8	1.7	0-4
115/2	100	84.0	2.6	0-8	110	60.0	1.3	0-3
342/2	102	87.2	2.3	0-6	105	33.3	1.5	0-1

Table 4. Results of crosses between the 3 × *cmm* × *grl* hybrids and species of *Solanum* with different ploidy levels

Cross	Pollinations	Fruit set		Total No. of seed	Seed germination (%)	Chromosome numbers
		No.	%			
F1 (<i>cmm</i> × <i>grl</i>) × <i>ifd</i> ¹	47	30	63.8	22	18.8	29-31 (4)*
F1 (<i>cmm</i> × <i>grl</i>) × <i>acl</i> ¹	7	0	0	0	—	—
F1 (<i>cmm</i> × <i>grl</i>) × <i>grl</i> ¹	42	10	23.8	23	13.0	26-29 (3)
F1 (<i>cmm</i> × <i>grl</i>) × <i>cmm</i> ²	186	51	27.4	11	37.7	24-25 (3)
F1 (<i>cmm</i> × <i>grl</i>) × <i>tbr</i> ³	303	45	14.8	0	—	—

* Number of plants

¹ *ifd* = *acl* = *grl* = 2EBN² *cmm* = 1EBN³ *tbr* = 4EBN

Ploidy level determinations were made on root tips of young seedlings pre-treated with an 8-hydroxyquinoline solution (0.29 g/l) for four hours and then fixed in a 3:1 (ethanol:acetic acid) solution for at least 24 hr. Prior to examination, root tips were hydrolized for 10 min in 1 N HCl at 60°C. Root tips were squashed in a 1% acetocarmine solution.

Pollen stainability was estimated by staining the pollen with Alexander's solution (Alexander 1969) and observing in a light microscope. A minimum of 200 grains were counted for each plant.

For meiotic analysis, buds were fixed for 48 hr in 3:1 (ethanol:acetic acid), and stained with alcohol-hydrochloric acid carmine (Snow 1963) for one week.

Mean arm pairing frequency ("C")—calculated as the sum of the observed rod bivalents per cell plus twice the number of ring bivalents and trivalents per cell, all divided by twice the basic chromosome number of the species- and relative affinity ("X") in the interspecific *comm* × *gri* triploid hybrids were calculated by the methods of Alonso and Kimber (1981). The value of "X" is the value of the relative affinity of the most closely related chromosome which gives the smallest sum of squares of differences (SSD) between the observed and expected meiotic figures.

Female fertility was estimated by the number of seeds produced from crosses of the *comm* × *gri* triploids with parents with different ploidy and Endosperm Balance Numbers (EBN) (Table 4).

The EBN are hypothetic genetic factors whose ratio in the endosperm between those contributed by the male and female progenitors affect the development of this tissue (Johnston *et al.* 1980).

Results

Meiosis was studied in three triploid *comm* × *gri* hybrids. All plants showed abnormal meiosis. In pachytene, although bivalents were observed in some meiocytes, small structural differences could be detected (Fig. 1).

First meiotic division

Meiotic configurations were analyzed in metaphase I. In general, the chromosomes in these hybrids showed a tendency to clump at this stage. The mean frequencies per cell of univalents, rod and ring bivalents, and trivalents are given in Table 2, along with the "C" and "X" values (Alonso and Kimber 1981).

Generally, the bivalents and trivalents had a tendency to orient themselves on the equatorial plate but the univalents were scattered haphazardly on a continous bipolar spindle. The trivalents observed were mostly "Y" or "V" shaped but few were "Q" shaped; chains of three were also observed. The metaphase configurations observed are shown in Fig. 2. These triploids were analyzed using the method of Alonso and Kimber (1981). The "C" value was high but the affinity value "X" was intermediate.

Lagging chromosomes and chromatin bridges were observed in anaphase I (Fig. 3). The number of chromosomes in the poles was counted. In Fig. 6 a comparison is given of the observed values with the theoretical ones expected from a binomial distribution $(0.5+0.5)^{12}$.

Figs. 1–4. Microsporogenesis in *comm* × *gri* triploid hybrids. 1. Pachytene bivalents showing unpaired regions (arrow) (750×). 2. Chromosome associations at metaphase I, 7 III + 5 II + 5 I (670×). 3. Chromatin bridge in anaphase I, meiocytes with 21 and 15 chromosomes in each pole (670×). 4. Tetrads with micro-cells (670×).

Although the distribution patterns of these two values are similar to each other, the χ^2 -test did reveal that significant differences exist between both. The observed values were above the expectation at the points of 12 to 15 and 21 to 24 chromosomes and below at the points of 16 to 20 chromosomes.

Second meiotic division

The behavior of chromosomes in metaphase II and the number of tetrads with micronuclei for each of the three clones analyzed are summarized in Table 3.

Seventy-five to 87.2 per cent of the cells in metaphase II had lagging chromosomes, with the mean numbers of 2.3 to 3.0 laggards per cell, their maximum number being 8. At the tetrad stage, the number of tetrads with micro-cells (Fig. 4) varied between 33.3 and 60.0%; the maximum number of microcells observed per meiocyte was 4.

Male and female fertility

Pollen stainability was determined by counting as fertile all pollen grains stained and normally shaped. The triploids 115/9, 115/2 and 342/2 had 31.7, 29.5 and 2.5% of pollen stainability respectively. Pollen grain size of the triploid hybrids was variable: well stained and turgid pollen grains had diameters that, on the average, were 18% larger than those of typical n grains of the diploid parents (Fig. 5).

Fig. 5. Pollen grains with different size of the $cmm \times grl$ triploid hybrid (480 $\times$).

Fig. 6. Chromosome distribution in anaphase I in $cmm \times grl$ triploid hybrids.

Only three crosses between the $cmm \times grl$ triploid hybrids and species of *Solanum* with different ploidy level were successful (Table 4). The chromosome numbers of the different hybrids obtained were variable. Crosses of the $cmm \times grl$ triploid hybrids with *ifd* and *grl* produced plants with $2n=26$, 29 and 31 chromosomes, and with *cmm* produced plants with $2n=24$ and 25 chromosomes.

Discussion

a. Genomic Relationship Between *S. commersonii* and *S. gourlayi*

The triploid $cmm \times grl$ hybrids showed, in metaphase I, configurations similar to those of the autotriploids of *cmm* studied by Von Olah (1938).

According to the Alonso and Kimber's (1981) model, by definition, these triploids would be 2:1 hybrids because they were produced by the functioning of $2n$ eggs of *cmm* and n generative nuclei of *grl*; therefore, high values of "X" (relative affinity) would be expected. This relative affinity, by definition, ranges from 0.5 when all the genomes are equally related to each other (3:0 types) to 1.0, when two genomes pair to the exclusion of the other (2:1 types).

The intermediate value of "X" at a high value of "C" for the *cmm* × *grl* hybrids indicates a situation where, as would be expected, the two genomes of *cmm* have a close homology, and the *grl* genome has a high probability of pairing with the *cmm* genomes. In the same way, Alonso and Kimber (1981) suggested that values of $X < 0.7$ can, in most circumstances, be considered equivalent to random pairing.

In general, meiosis in these triploid hybrids was irregular. Although bivalents were observed in pachytene, small structural differences could be detected in some instances. These small structural differences have been also observed in progenies of crosses between various species of *Solanum* (Hermesen and Ramana 1976, Ramana and Hermesen 1979, Marks 1968). In general, and on the basis of the fertility and the normal cytological behaviour of the hybrids, it is accepted that the genomes of the diploid potatoes species are poorly differentiated. Dvorak (1983) claimed that it is not the structural differentiation of genomes but the rapid evolution of "noncoding" nucleotide sequences separating genes (nonstructural chromosome differentiation) that is primarily responsible for reduced pairing between chromosomes of different species.

b. Meiotic behaviour and fertility

Chromosomes in anaphase I showed a tendency to pass together as a group to one pole, rather than to be distributed at random. The observed frequencies of chromosome distribution were superior to the expected at the points of 12 to 15 chromosomes and 21 to 24 chromosomes. Therefore, it is possible that unbalanced pollen grains with $n = x + 1 = 13$, $x + 2 = 14$, $\dots$, $2x - 3 = 21$, $2x - 2 = 22 \dots$ may be stainable and of normal shape. In this sense, the number of cells with laggards in metaphase II was superior to that of tetrads with microcells. Therefore, laggard chromosomes in metaphase II might have been included in normal nuclei at the end of meiosis. The plants obtained from crosses between the *cmm* × *grl* triploids with parents with different ploidies, must have arisen from the fertilization of eggs with $n = x + 2 = 14$ to $n = x + 7 = 19$ chromosomes from *cmm* × *grl* triploids by $n = x = 12$ pollen grains from *ifd* and *grl*, and eggs with $n = x = 12$ and $n = x + 1 = 13$ chromosomes from the triploids by $n = x = 12$ pollen grain of *cmm*, respectively.

c. Concluding remarks

A high homology between the *cmm* and *grl* genomes was established through a meiotic analysis. These results are in agreement with those reported by Matsubayashi (1983), who found high genomic homology between diploid species of the *Tuberosa* and *Commersoniana* taxonomic series. Therefore, it is possible that the loss of certain chromosomes of one species in the gametes could be partially compensated by the presence of chromosomes from the other species. In this way, some gametes with unbalanced chromosome numbers could be functional.

The results of this study give an indication that there is a close genetic affinity between *cmm* and *grl*. Therefore a high degree of gene exchange between the genomes of these species is expected to occur in the triploid hybrids. It is likely that important genes of *cmm* could be transferred, in this way, to the cultivated forms, through crosses with *tbr*. However, the crosses attempted between triploids and *tbr* were unsuccessful.

The EBN hypothesis proposed by Johnston *et al.* (1980) could explain the impossibility to obtain descent from the *cmm* × *grl* triploids × *tbr* crosses. According to this hypothesis, only 2n eggs of the triploids fertilized with n pollen from *tbr* would lead to normal endosperm development in the hybrid seed. It is possible that these triploids did not produce 2n eggs or that the frequency of 2n eggs was very low, and remain undetected. Therefore, in order to evaluate this possibility, a large number of pollinations with these and other *cmm* × *grl* triploid plants would have to be carried out in further experiments.

Summary

Meiotic analysis, pairing affinity and fertility studies were carried out in *Solanum commersonii* Dun. (*cmm*, $2n=2x=24$) by *Solanum gourlayi* Haw. (*grl*, $2n=2x=24$) triploid hybrids, obtained through the functioning of $2n$ eggs of *cmm*. Mean arm pairing frequency ("C") and relative affinity ("X") were calculated according to Alonso and Kimber (1981). The "C" value was high but the "X" value was intermediate. Pollen stainability for three triploid hybrids was 31.7, 29.5 and 2.5 respectively. Crosses between the *cmm* × *grl* triploids, used as females, and various species of *Solanum* with different ploidy were performed. Hybrids with 24 to 31 chromosomes were obtained from these crosses. The results of this study give an indication that the *cmm* and *grl* genomes are highly homologous. Therefore a high degree of gene exchange between the genomes of these species is expected to occur in meiosis. The feasibility of utilizing *grl* as a "bridge" species, for transferring important genes of *cmm* to the cultivated potato, *Solanum tuberosum* L. are discussed.

Key words: Triploid hybrids, *Solanum*, meiotic analysis.

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