

Ploidy Levels Affect Phenotype and Cytogenetic Traits in *Zea mays* ssp. *mays* ($2n=20$ or 40) and *Zea mays* ssp. *parviglumis* Hybrids

María del Carmen Molina^{1,*} and María Dina García²

¹ Instituto Fitotécnico de Santa Catalina, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CIGEN (UNLP-CONICET-CIC), Garibaldi 3400, C.C.4, 1836 Llavallol, Buenos Aires, Argentina

² Facultad Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Ruta 4 Km 2, C.C. 95, 1832 Lomas de Zamora, Buenos Aires, Argentina

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Summary The aim of this study was to analyse phenotype and cytogenetic behaviour of *Zea mays* ssp. *mays* and *Zea mays* ssp. *parviglumis* (Zpa) hybrids with different ploidy levels. Maize $2n=20$ (Zm20) and Zpa hybrid plants (MPa20) were obtained naturally, they showed regular meiosis and fertile progeny (+80%). Otherwise, maize $2n=40$ (Zm40) and Zpa hybrids (MPa30) were obtained by embryo rescue, they showed irregular meiosis and sterile progeny (0–8%). Meiotic configurations more frequently observed in the species and the hybrids were: Zm20 (10II); Zm40 (3.24II+8.34IV); Zpa (0.46I+9.76II); MPa20 (0.40I+9.54II+0.05III+0.65IV); MPa30 (5.97I+5.93II+4.05III). After colchicine treatment the number of IV increased in Zm20, Zpa and MPa20 and 10III were observed MPa30, because of homoeologous chromosomes pairing. In conclusion, Zm20 (AmAm BmBm) and Zpa (ApaApa BpaBpa) are allotetraploids with 2 homoeologous genomes. Whilst in the hybrids, the homoeologous genomes A pair in all cases, genomes B only do if their homologous competing during pairing, does not exist except in colchicine-treated plants which also show homoeologous genomes B pairing.

Key words *Zea*, Embryo rescue, Maize hybrids, Ploidy level, Genomic formulae, Somatic embryogenesis, Incompatible crosses, Colchicine.

The genus *Zea* comprises 2 perennial species, *Zea perennis* ($2n=40$) and *Zea diploperennis* ($2n=20$), and one annual species, *Zea mays* ($2n=20$), which embraces 3 subspecies: *mexicana*, *parviglumis* and *mays* (Doebley and Iltis 1980, Doebley 1990).

Therefore, interspecific crosses within the genus *Zea* result in hybrids with somatic chromosome numbers of $2n=20$, $2n=30$ or $2n=40$. Further, hybrids with somatic chromosome numbers $2n=30$ or 40 can also be obtained from crosses between maize inbreds ($2n=40$) and some other *Zea* species. Morphologic and cytogenetic traits that characterise each of these levels, are similar irrespective of the parents (Molina and García 1999a). Molina and García (1999b) confirmed that ploidy level or the chromosome number contributed by each parent considerably affect the phenotype and meiotic behaviour of maize ($2n=20$ or 40) and *Z. perennis* ($2n=40$) hybrids. In such hybrids of the genus *Zea*, the most frequently observed meiotic configurations were: i) $2n=20$: 10II; ii) $2n=30$: 5III+5II+5I; iii) $2n=40$: 5IV+10II. The $2n=20$ – 40 hybrids (balanced gametes) showed fertile pollen and viable seeds, whilst $2n=30$ hybrids (unbalanced gametes) were highly sterile.

There is cytological evidence supporting the polyploid condition of the genus *Zea* (Molina and Naranjo 1987, Naranjo *et al.* 1990, Molina and García 1994, 1997, 1999a, b). Bennett (1983),

* Corresponding author, e-mail: mcmgen@yahoo.com

Moore *et al.* (1995) and Gale and Devor (1998) also confirmed that maize is a complete tetraploid, whose chromosomes can be grouped in 2 genomes. Maize ($2n=20$), *Z. perennis* and their hybrids showed cryptic homoeology when cells were treated, in pre-zygotene, with a dilute solution of colchicine for 12 h (Poggio *et al.* 1990, Naranjo *et al.* 1994, Molina and García 1999b). Quadrivalents appeared up to 5 in colchicine treated maize plants and their number increased in *Z. perennis* and in their hybrids. It was also suggested that the genus has homoeologous genomes that probably do not pair when a *Ph* like gene is present (Poggio *et al.* 1990, Molina and García 1999b).

The purpose of this work was to analyse the phenotypic and cytogenetic behaviour of *Zea mays* ssp. *mays* × *Zea mays* ssp. *parviglumis* hybrids with different ploidy levels and to use premeiotic colchicine treatment as a test for the possible presence of homoeologous genomes in maize and *Zea mays* ssp. *parviglumis*.

Material and methods

Plant materials: Three inbred lines (A188, B73, SC66) and one cultivar ('Colorado Klein') of *Zea mays* ssp. *mays* $2n=20$ (Zm20); 3 inbred lines of *Zea mays* ssp. *mays* $2n=40$ (Zm40) (N107B, N104B and N103A, supplied by Maize Genetics Cooperation Stock Center, Urbana, Illinois), *Zea mays* ssp. *parviglumis* $2n=20$ (Zpa), the hybrids MPa20 (Zm20 × Zpa) $2n=20$ and MPa30 (Zm40 × Zpa) $2n=30$ were used for these studies.

MPa30 embryos were excised 21, 23 or 30 days after pollination and cultured on the basic medium of García *et al.* (1992) supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin (García and Molina, in press) to obtain MPa30 hybrid plants.

Colchicine treatment was done according to Poggio *et al.* (1990). The stems were cut and kept for 12 h in a 0.5×10^{-4} M colchicine solution. Before anther fixation, the stems were placed in tap water for 24 h. Control and treated plant materials were fixed in 3 : 1 (absolute ethanol : acetic acid) solution.

Anthers were squashed in 2% acetic hematoxylin (Nuñez 1968) and pairing configurations observed at diakinesis-metaphase I. Only cells with well spread plates were scored.

Results

Phenotype

MPa20 hybrid plants were obtained following normal seed germination. Such plants were 3 m high and had a few tillers (average of 2 tillers per plant). They showed continuous flowering in the greenhouse and produced 96% fertile pollen, distichous ears which disarticulate at maturity (Fig. 1C), and 90% viable seeds. Clorantia (shoot development from the male flower) was observed on these plants (Fig. 2) under unfavourable conditions of light and temperature (Gallardo 1904). These shoots could be easily rooted in sand.

Zm40 × Zpa crosses produced non viable seeds because of failure of endosperm development. Therefore, MPa30 hybrid plants were obtained by embryo rescue. Such plants were 2 m high and showed an average of 8 tillers per plant. They had photoperiod requirements to flower, with a short day quantitative response and a critical photoperiod of 13 h. These hybrids produced 40 to 80% fertile pollen grains, 15% viable naked caryopses. Ears were small, with 2 to 4 grain rows disposed on a rigid rachis (Fig. 1D).

Meiotic analysis

The most frequent meiotic configurations observed at diakinesis-metaphase I were:

Zm20: Cells invariably showed 10II (Table 1, Fig. 3A). In 40% of the cells secondary associations, separation of the chromosomes into 2 groups of 5 each, and an average of 14 chiasmata/cell

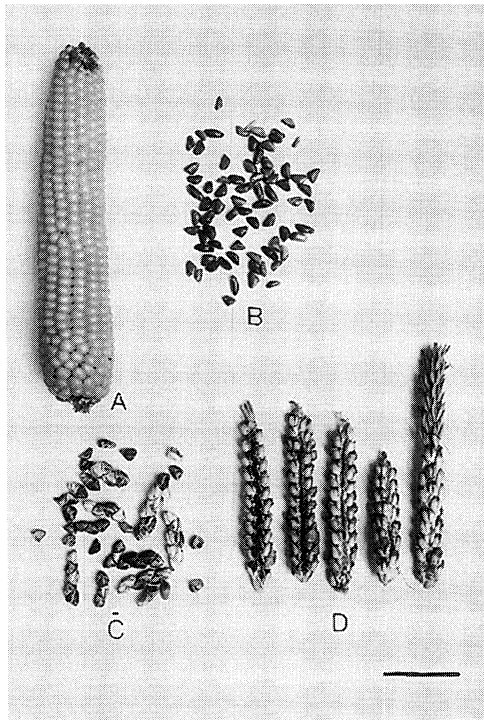


Fig. 1. Ears of *Z. mays* ssp. *mays*, *Z. mays* ssp. *parviglumis* and their hybrids. A) Zm20, B) Zpa, C) MPa20, D) MPa30. Scale=5 cm.

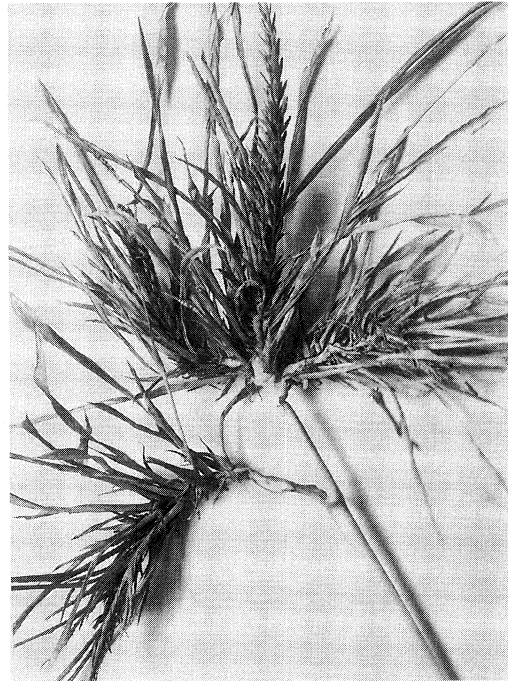


Fig. 2. Development of shoots from the male flowers (clorantia) of the hybrid MPa20.

were also observed (Molina and Naranjo 1987).

Zm40: The majority of the cells showed in this case 10IV (30%) or 9IV+2II (23.94%), with an average of 8.34IV+3.24II and 33.75 chiasmata/cell (Table 1, Fig. 3B).

Zpa: Cells revealed 10II (81.76%) or 9II+2I (13.25%) with an average of 9.76II+0.46I and 13.45 chiasmata/cell (Table 3, Fig. 3C). Secondary association ($\bar{x}=3.48/\text{cell}$) and separation of the chromosomes in 2 groups of 5 each were also observed in 53% of the cells studied.

MPa20: While most cells had 10II (73.45%), the average was of 0.40I+9.54II+0.05III+0.65IV and 15.42 chiasmata/cell (Table 1, Fig. 3D). During metaphase I, 45% of the cells showed chromosomes separated in 2 groups of 5 each. A 17% of the cells had one quadrivalent (Table 1, Fig. 3E) which could be originated from a reciprocal translocation. Lagging chromosomes (Fig. 3F) or 1-2 inversion bridges (Fig. 3G) could be observed in 10% of the anaphase cells.

MPa30: The most frequently observed patterns were of 5III+5II+5I (29.68%) and 4III+6II+6I (20.31%) with an overall average number/cell of 4.05III+5.93II+5.97I (Table 1, Fig. 3H, I). The average number of chiasmata/cell was 25.60. Laggard chromosomes were observed during anaphase I (Fig. 3I) and a different number of chromosomes migrating to each pole.

Colchicine effects on meiotic chromosomes

Zm20: Control plants only built bivalents, but the treated ones showed at least one quadrivalent in the 61% of the cells, with a maximum of 5IV in the 11% of the cells (Fig. 4a).

Zm40 (Fig. 4b): Treated plants showed a slight increase in the number of chiasmata and quadrivalents as compared to control plants, though there were not significant differences. Hexavalents or octovalents were not observed.

Zpa (Fig. 4c): Colchicine treatment induced the formation of quadrivalents in the 72% of the

Table 1. Meiotic configurations of *Zea mays* ssp. *mays* ($2n=20$ or 40), *Zea mays* ssp. *parviglumis* ($2n=20$) and their hybrids

Species and hybrids	$2n$	Number of cells	Diakinesis-methaphase configurations				%
			I	II	III	IV	
Zm20	20	200		10			100
Zm40	40	142				10	30.28
				2		9	23.94
				4		8	16.90
				6		7	10.52
				8		6	7.75
				10		5	4.23
				12		4	0.70
				14		3	0.70
			1	6	1	6	1.40
			1	8	1	5	1.40
			1		1	9	1.40
			1	2	1	8	0.70
		\bar{x}	0.04	3.24	0.04	8.34	
Zpa	20	181		10			81.76
			2	9			13.25
			4	8			4.97
		\bar{x}	0.46	9.76			
MPa20	20	177		10			73.45
				8		1	14.69
			2	9			9.04
			2	7	1	2	1.13
			4	8			1.13
			1	6	1	1	0.56
		\bar{x}	0.40	9.54	0.05	0.65	
MPa30	30	128	5	5	5		29.68
			6	6	4		20.31
			7	7	3		15.62
			8	8	2		11.72
			4	4	6		7.81
			3	3	7		5.47
			9	9	1		3.91
			10	10			1.56
			2	2	8		0.78
			8	5	4		2.34
			7	10	1		0.78
		\bar{x}	5.97	5.93	4.05		

cells, ranging from 1 to 5IV/cell. Chiasmata number increased from 13.45 in control plants to 17.26 in treated ones.

MPa20: This hybrid (Fig. 4d) showed a remarkable increase in the number of quadrivalents as observed in treated plants (72%) as compared to control plants (16.38%).

MPa30 (Fig. 5): A significant increase of trivalents in treated plants ($\bar{x}=7.6$) was observed as compared to control plants ($\bar{x}=4.05$) and the number of chiasmata/cell also increased from 25.60 (control) to 36.70 (treated).

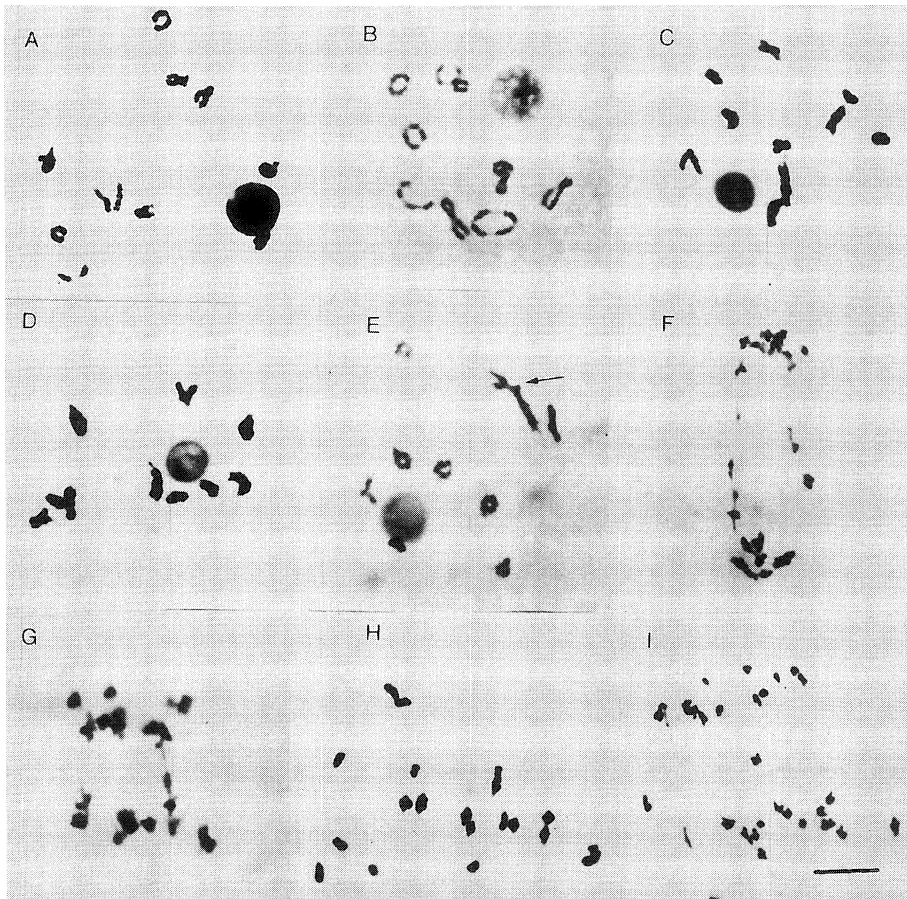


Fig. 3. Meiotic configurations in diakinesis-metaphase I in: A) Zm20 (10II), B) Zm40 (10IV), C) Zpa (10II), D) MPa20 (10II), E) MPa20 (8II+1IV), F) MPa20, anaphase showing lagging chromosomes, G) MPa20, anaphase showing one inversion bridge, H) MPa30 (5III+5II+5I), I) anaphase showing a different number of chromosomes migrating to each pole. Scale=10 μ m.

Discussion

Effect of chromosome number

Meiosis and phenotype of *Z. mays* ssp. *mays* ($2n=20, 40$) and *Z. mays* ssp. *parviglumis* hybrid plants differed according to their chromosome number (Table 1, Figs. 1–3). MPa20 plants, with the same number of chromosomes contributed by each parent, showed characteristics more similar to Zpa, whilst MPa30, with 20 chromosomes from maize and 10 from *Z. mays* ssp. *parviglumis* were maize-like plants, except in the number of tillers per plant.

Similar results were observed in *Z. mays* ssp. *mays* ($2n=20, 40$) and *Z. perennis* crosses (Molina and García 1999b), which suggests that phenotypic expression of several traits depends upon the dose of genes contributed by each parent to the hybrid. This would explain the great variability observed in the progeny of the hybrids (Molina 1978).

Chromosome homoeology and genomic formulae

Genetic, cytogenetic and biochemical studies provided enough evidence to suggest that genus *Zea* has a basic chromosome number of $x=5$ and that *Zea mays* ssp. *mays* $2n=20$ is an allote-

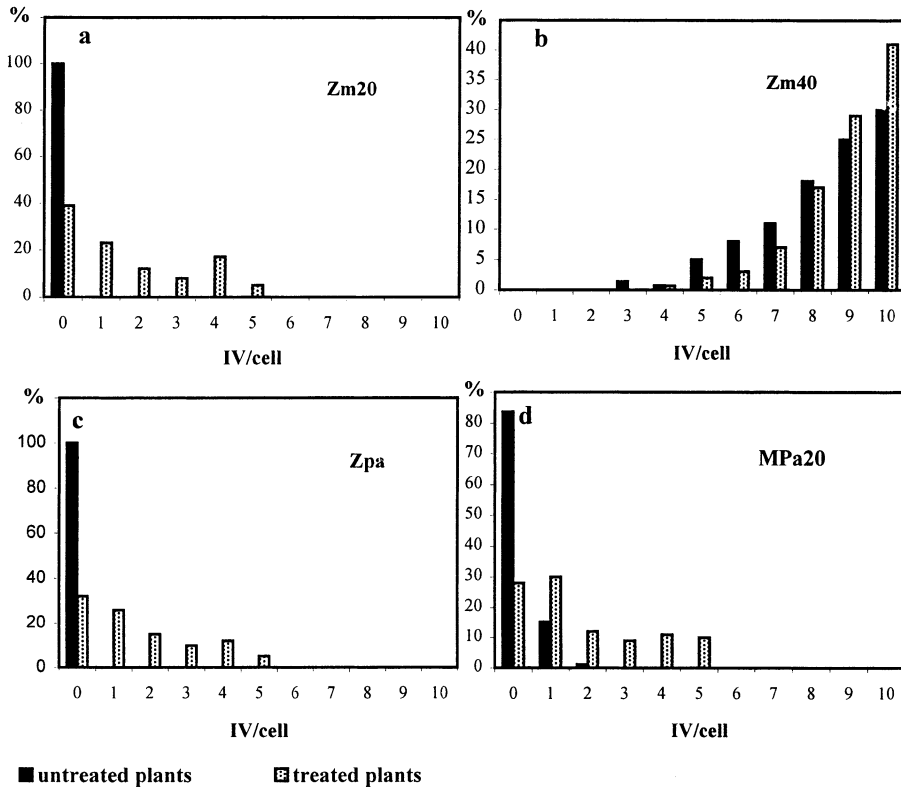


Fig. 4. Frequency of quadrivalents per cell in untreated (UT) and treated (T) plants with colchicine. a) Zm20 (UT: 200 cells, T: 184 cells), b) Zm40 (UT: 142 cells, T: 153 cells), c) Zpa (UT: 181 cells, T: 152 cells), d) MPa20 (UT: 177 cells, T: 143 cells).

traploid with 2 homoeologous genomes, Am and Bm (Rhoades 1951, Ghatnekar 1965, Vijendra Das 1970, Gottlieb 1982, Bennett 1983, 1984, Molina and Naranjo 1987). The 5 Am maize chromosomes pair with the homoeologous Bm chromosomes if their respective homologues are not present, as, for example, during diakinesis-metaphase I of haploid plants (McClintock 1933, Chaganti 1965, Ting 1966, 1969).

Lacadena (1996) and Yacobi *et al.* (1985a, b) demonstrated that in allopolyploid species and interspecific hybrids, chromosomes belonging to the same genome tend to be closer amongst themselves and at the same time, more distantly placed with respect to those of the other genome. This was also observed in Zm20, Zpa and in the MPa20 hybrid, whose chromosomes appeared as being arranged in 2 groups of 5 each in more than 50% of the cells. This is a strong indication of separation between genomes A and B.

Whilst all the homoeologous chromosomes of both species paired in MPa20, only the homoe-

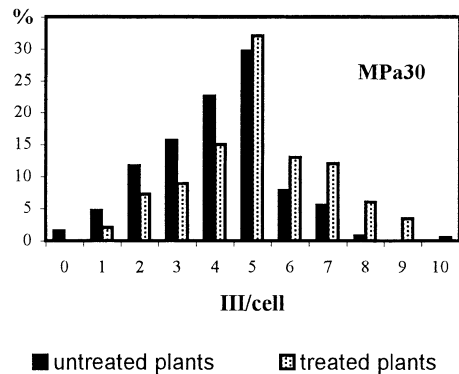


Fig. 5. Number of trivalents in MPa30 plants treated with colchicine (0.5 x 10⁻⁴ M) and control plants. Number of cells in untreated 128 and treated 135.

ologous chromosomes of genome A paired in MPa30. This suggests that genome A was more conserved, therefore pairing among homoeologous chromosomes was possible but genome B differentiated and its chromosomes only pair when their respective homologues are absent.

Based on the results of this work and previous observations (Bennet 1983, Molina 1978, Molina and García 1999a, Molina and Naranjo 1987, Moore *et al.* 1995, Naranjo *et al.* 1994, Poggio *et al.* 1990), the genomic formulae proposed are: AmAm BmBm for Zm20, AmAmAm BmBmBmBm for Zm40, ApaApa BpaBpa for Zpa, AmApa BmBpa for MPa20 and AmAmApa BmBm Bpa for MPa30.

Colchicine promoted pairing between homoeologous chromosomes from both genomes of *Zea mays* ssp. *mays* (AmAmBmBm) (Poggio *et al.* 1990). It was also observed an enlargement of the chiasmata average number, from 14 (control) to 20 (treated) per cell.

As was observed in Zm20 (Poggio *et al.* 1990), all A and B homoeologous chromosomes paired in cells from colchicine treated Zpa and MPa20 plants, which showed 5 quadrivalents each (Fig. 4a, c, d). Also, colchicine treatment notably increased the number of trivalents/cell in MPa30 (Fig. 5) because of pairing genome BmBm from maize with genome Bpa from Zpa, which points out that Bm and Bpa genomes are homoeologous. Similar results were reported for the hybrid *Z. mays* ssp. *mays* ($2n=20$) \times *Z. perennis* ($2n=40$) (Naranjo *et al.* 1994, Molina and García 1999b). On the contrary, colchicine treatment did not cause any significant increase in the number of quadrivalents per cell in Zm40 (Fig. 4b), because both homoeologous genomes were already paired.

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