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Cryptic homoeology analysis in species and hybrids of genus *Zea*

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Abstract

Cryptic intergenomic pairing of genus *Zea* was induced by the use of a diluted colchicine solution in order to elucidate the phylogenetic relations and differentiation of the homoeologous genomes. Results indicate that in species and hybrids with 2n = 20, there was chromosome pairing between the homoeologous A and B genomes with a maximum of 5IV, with the exception of *Zea diploperennis* and their interspecific hybrids where cryptic homoeologous chromosome pairing was not induced. In almost all 2n = 30 hybrids, observed cryptic pairing increased to a maximum of 10III although *Z. mays* × *Z. mays* with 2n = 30 did not show significant differences between treated and untreated materials. Pairing was also observed in species and hybrids with 2n = 40, in which a maximum of 10IV was observed, with the exception of *Z. mays* with 2n = 40 where treated and untreated cells did not differ significantly.

Additional key words: colchicine, genome, maize, subgenome, teosinte.

Introduction

Zea is an important genus of the tribe *Maydeae*. According to Doebley and Iltis (1980), Iltis and Doebley (1980), and Iltis and Benz (2000), it is composed of two section: section Luxuriante which includes the annuals teosintes *Zea luxurians* and *Zea nicaraguensis* and the perennials *Zea diploperennis* and *Zea perennis.* Section *Zea* comprises only an annual species (*Z. mays*) which can be divided into three subspecies: *Z. mays* ssp. *mays* (maize), and the teosintes *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis.* All the species mentioned above have 2n = 20 chromosome except Z. *perennis* which has 2n = 40.

The maize genome is characterized by having a large number of duplicated genes (Wendel 2000). Three models can explain the large-scale duplications in the maize genome, that is, segmental duplication (multiple independent duplications within a genome), autotetraploidy (intraspecific genomic duplication), and allotetraploidy (interspecific genome hybridization).

Swigonová *et al.* (2004) support a theory in which maize has a tetraploid origin. This analysis also indicates a contemporaneous divergence of the ancestral sorghum genome and the two maize progenitor genomes about 11.9 million years ago (Mya). On the basis of a putative conversion event detected for one of the genes, tetraploidization must have occurred before 4.8 Mya and therefore, the major maize genome expansion.

According to Schnable *et al.* (2011) and Schnable and Freeling (2011), maize is a tetraploid species with two differentiated parental genomes, maize1 and maize2. Maize1 is the subgenome that experienced less gene losses followed by the whole genome duplication in

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Abbreviations: A - subgenome A *Zea;* B - subgenome B *Zea*; Bp1 - subgenome Bp1 *Zea perennis*; Bp2 - subgenome Bp2 *Zea perennis*; 2,4-D - 2,4 dichlorophenoxyacetic acid; I - chromosome monovalent; II - chromosome bivalent; III - chromosome trivalent; IV - chromosome quadrivalent; *pam1* - gene *plural abnormalities of meiosis*; *Ph* - gene *pairing homoeologous; Ph1 - pairing homoeologous 1.*

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maize lineage 5 - 12 Mya. Genes located on this subgenome tend to be expressed at a higher level in modern maize (Schnable and Freeling 2011).

Colchicine at concentration of 0.5×10^{-4} M induce homoeologous chromosome pairing (Poggio *et al.* 1990, Molina 2011) with the formation of heteromorphic bivalents or multivalents (Driscoll *et al* 1967, Feldman and Avivi 1988) which favors intergenomic pairing in species with homoeologous genomes (Dover and Riley 1973). Driscoll and Darvey (1970) observed that colchicine affects the spatial relationship of homoeologous chromosomes but not the formation of chiasmata. Additionally, they suggested that chromosome position is crucial in the meiotic pairing of homologous and homoeologous chromosomes, altering the arrangement of chromosomes in the nuclear membrane and allowing the expression of cryptic genomic homology.

Jackson (1982) described a model that explains the chromosome pairing and chiasma formation in homologous and homoeologous genomes in which there is a genetic control of the specific binding site of chromosomes to the nuclear membrane under the regulation of the *Ph* gene. Jackson and Murray (1983) demonstrated that the application of diluted colchicine solution to meiotic cells can break the genetic control of these genes promoting the intergenomic pairing and thus revealing the cryptic homology in polyploids.

In maize, the *plural abnormalities of meiosis 1* (*pam1*) gene is associated with the formation of the bouquet by intervening in the telomere anchoring to the nuclear membrane and by facilitating homologous chromosome pairing. Golubovskaya *et al.* (2002) concluded that the *pam1* gene plays an important role in the formation of the bouquet and in the pairing of homologous chromosomes.

Several researchers have treated premeiotic cells of *Zea mays, Z. perennis*, *Z. diploperennis*, and their hybrids with diluted colchicine solutions (Poggio *et al.* 1990, Molina and García 1999, 2000, 2001, Molina *et al.* 2004, 2005, González and Poggio 2011, Molina 2011). Up to 5 quadrivalents have been observed in maize and hybrids with 2n = 20, whereas a higher number of quadrivalents has been found in *Zea perennis* when compared to untreated controls. Colchicine treatment has been observed to favor homoeologous chromosome pairing and more than one mechanism has been proposed to explain such effect: modification of the position of the chromosomes in the nuclear membrane, alteration in the formation of the bouquet (Bass *et al.* 2000), and annulment of the expression of a maize gene equivalent to the *Ph* gene of wheat (Poggio *et al.* 1990, Molina *et al.* 2004).

In the present study, cryptic homology in species and hybrids of the genus *Zea* was analyzed with the aim to explore the phylogenetic relationship among *Zea* species by using diluted colchicine solutions that induce intergenomic chromosome pairing.

Materials and methods

Parental species:
Zea mays ssp *mays* (2n = 20). Open pollinated population Colorado Klein. Inbred line knobless from the Maize Genetic Cooperation Stock Center (MGCSC), Urbana, Illinois, USA.
Zea mays ssp mays (2n = 40). Sugary inbred lines N103A, N104B, N107C, N107B, and 90-2189-2190, from MGCSC.
Zea mays ssp *mexicana* (2n = 20). From CIMMYT (Mexico).
Zea mays ssp *parviglumis* (2n = 20). From Dr. Bird, CIMMYT (Mexico).
Zea luxurians (2n = 20). From Guadalajara, Mexico.
Zea diploperennis (2n = 20). From Sierras Occidentales de Manjatlan, Jalisco, Mexico. Courtesy of Dr. Iltis.
Zea perennis (2n = 40). From Ciudad Guzmán, Jalisco, Mexico. Courtesy of Dr. Prywed, introduced in 1962 at the Instituto Fitotécnico de Santa Catalina, Llavallol, Argentina.
Hybrids:
Dihybrids (2n = 20)
Zea mays ssp *mays* × *Zea mays* ssp *mexicana*
Zea mays ssp *mays* × *Zea mays* ssp *parviglumis*
Zea mays ssp *mays* × *Zea luxurians*
Zea mays ssp *mays* × *Zea diploperennis*
Zea diploperennis × *Zea luxurians*.
Dihybrids (2n = 30)
Zea mays ssp *mays* (2n = 40) × *Zea mays* ssp *mays* (2n = 20)
Zea mays ssp *mays* (2n = 40) × *Zea parvig*lumis
Zea mays ssp *mays* × *Zea perennis*
Zea diploperennis × *Zea perennis*
Zea luxurians × *Zea perennis*
Zea perennis × *Zea mexicana*
Dihybrids (2n = 40)
Zea mays (2n = 40) × *Zea perennis*
Zea mays ssp *parviglumis* × *Zea diploperennis* (2n = 40) obtained by chromosome duplication of the (2n = 20) hybrid
Zea diploperennis × *Zea perennis* obtained by crossing a non-reduced gamete of *Zea diploperennis* with a normal gamete of *Zea perennis*
Trihybrids (2n = 20)
(*Zea mays* × *Zea diploperennis)* × *Zea luxurians* (2n = 40)
Zea mays ssp *mays* (2n = 40) × (*Zea diploperennis* × *Zea perennis*) (2n = 40).

Crosses and self-pollinated maize inbred lines and teosinte populations were made at field and greenhouse conditions. When teosinte was used as male, maize silks were cut to 3 or 4 cm since the maximum length of the pollinic tube in teosinte is about 6 to 7 cm, unlike maize, whose pollinic tube can reach more than 30 cm. In crosses between species whose hybrids have a chromosome number of 2n = 30, which are generally difficult to obtain due to a limited embryo growth, a solution of 0.45 mM of 2,4 dichlorophenoxyacetic acid (2,4-D; Furini and Jewel 1995) was applied to the ears two days after pollination in order to favor embryo development.

Immature embryos from 2n = 30 hybrids were rescued and cultured *in vitro*. About 12 to 30 d after fecundation, the embryo collapsed and died. The survival period depended on the environmental conditions. If the plants are grown under optimal field conditions for maize development, embryos can survive for 12 d, whereas under greenhouse conditions during winter, the development of embryos is generally delayed and survival is longer (21 to 30 d).

Ears were harvested when embryos had a maximum length of 1 mm. Caryopses were washed with a 2.5 % (m/v) sodium hypochlorite solution and then sown in a basic medium (García and Molina 1992, 2001) supplemented with 4.5 µM 2,4-D. Treated caryopses were incubated in a growth chamber with a 16-h photoperiod (irradiance of 250 µmol m^{-2} s^{-1}, day/night temperatures of 30/28 °C, and relative humidities of 70/80 %. They were subcultured every 30 d in a callus maintenance medium (García and Molina 1992). Regenerated plants were transplanted into a culture medium free of 2,4-D to develop roots. Subsequently, plants were transplanted and acclimated in a greenhouse.

Immature tassels from different *Zea* species and hybrids were cut and immersed in a 50 µM colchicine solution for 12 h and then stored in distilled water for 24 h (controls were stored in distilled water for 36 h). Then, the treated material and the controls were fixed in Farmer solution (3:1 ethanol/acetic acid) where they were conserved for 8 to 10 d and then stored at 4 °C in 70 % (v/v) ethanol.

To analyze the meiotic configurations in parental species and hybrids, anthers excised from male florets previously fixed in Farmer solution were squashed in a drop of 2 % (m/v) ferric hematoxylin using a micro-drop of ferric acid as mordant. It was analyzed between 130 and 224 cells in both control and treated plants.

Chromosomal notation: I (monovalent); II (bivalent); III (trivalent); IV (quadrivalent).

Significant differences between meiotic configurations (control *vs*. the corresponding treatment) were tested using the Mann-Whitney U test (Sokal and Rohlf 1978) at a 5 % probability level (*Statistica version 7*).

Results

To obtain the cryptic pairing of homoeologous chromosomes of different genomes from species and hybrids of the *Zea* complex, tassels were treated with a diluted colchicine solution. In *Z. mays* with 2n = 20, *Z. mexicana*, *Z. parviglumis*, and *Z. luxurians* (Table 1), colchicine induced the homoeologous chromosome pairing of the two relict genomes, designated as genomes A and B (Fig. 2), with a maximum of 5IV (Fig. 1*A*). The exception was *Z. diploperennis* in which no quadrivalents formation was observed using the colchicine concentration that showed quadrivalents in the other species.

Regarding *Zea* hybrids with 2n = 20 (Table 2), cryptic pairing was observed in *Z. mays* × *Z. mexicana*, *Z. mays* × *Z. parviglumis* with 2n = 20, *Z. mays* × *Z. luxurians*, and the trihybrid (*Z. mays* × *Z. diploperennis*) × *Z. luxurians* although in different proportions depending on the hybrid analyzed. The highest percentage of pairing was observed in *Z. mays* × *Z. parviglumis* with 2n = 20. On the other hand, cryptic pairing homoeologous chromosomes of the A and B genomes was not observed in *Z. mays* × *Z. diploperennis* with 2n = 20 and *Z. diploperennis* × *Z. luxurians* hybrids. In both cases, one of the parents was *Z. diploperennis* which also showed no induction of homoeologous chromosome pairing (Table 1).

Table 1. Percentage of quadrivalents in parental species with 2n = 20 for plants treated with colchicine (50 µM) and untreated control plants. * - significant differences between control and treated plants at the probability level of 5 %.

Species	Treatment	IV/cell 0	1	2	3	4	5	Total number
Zea mays	control	100.0	0.0	0.0	0.0	0.0	0.0	200
	treated*	38.3	17.8	15.3	8.2	16.8	3.6	196
Zea mexicana	control	100.0	0.0	0.0	0.0	0.0	0.0	136
	treated*	37.7	25.7	18.0	7.8	6.6	4.2	167
Zea parviglumis	control	100.0	0.0	0.0	0.0	0.0	0.0	181
	treated*	34.3	20.0	17.8	12.0	13.1	2.8	175
Zea luxurians	control	100.0	0.0	0.0	0.0	0.0	0.0	162
	treated*	36.5	23.0	19.6	10.1	6.1	4.7	148
Zea diploperennis	control	100.0	0.0	0.0	0.0	0.0	0.0	164
	treated	100.0	0.0	0.0	0.0	0.0	0.0	196

Table 2. Percentage of quadrivalents in hybrids with 2n = 20 for plants treated with colchicine (50 µM) and untreated control plants. * - significant differences between control and treated plants at the probability level of 5 %.

Species	Treatment	IV/cell						Total number
		0	1	2	3	4	5	
Z. mays × *Z. mexicana*	control	100.0	0.0	0.0	0.0	0.0	0.0	127
	treated*	35.2	18.6	22.8	3.4	9.7	10.3	145
Z. mays × *Z. parviglumis*	control	100.0	0.0	0.0	0.0	0.0	0.0	177
	treated*	31.1	36.7	9.4	8.3	8.9	5.6	180
Z. mays × *Z. luxurians*	control	100.0	0.0	0.0	0.0	0.0	0.0	200
	treated*	59.0	15.4	13.4	4.4	4.9	2.9	203
Z. mays × *Z. diploperennis*	control	100.0	0.0	0.0	0.0	0.0	0.0	181
	treated	100.0	0.0	0.0	0.0	0.0	0.0	179
Z. diploperennis × *Z. luxurians*	control	100.0	0.0	0.0	0.0	0.0	0.0	154
	treated	100.0	0.0	0.0	0.0	0.0	0.0	157
(*Z. mays* × *Z. diploperennis*) × *Z. luxurians*	control	100.0	0.0	0.0	0.0	0.0	0.0	166
	treated*	62.0	8.8	11.1	9.9	5.3	2.9	171

Table 3. Percentage of trivalents in parental species with 2n = 30 for plants treated with colchicine (50 µM) and untreated control plants. * - significant differences between control and treated plants at the probability level of 5 %.

Species	Treatment	III/cell											Total number
		0	1	2	3	4	5	6	7	8	9	10	
Z. mays × *Z. mays* (2n = 30)	control	0.0	2.8	2.2	2.2	2.8	8.8	7.7	12.1	38.1	17.2	6.1	181
	treated	0.0	2.1	2.1	4.2	5.2	6.8	6.8	7.8	41.1	17.2	6.7	192
Z. mays × *Z. parviglumis*	control	5.1	7.2	11.6	13.8	19.6	29.0	5.7	3.6	2.2	0.0	2.2	138
	treated*	0.0	1.9	7.1	7.7	14.7	32.1	12.8	11.5	6.4	3.8	2.0	156
Z. mays × *Z. perennis*	control	0.0	0.0	0.0	2.8	30.0	59.3	5.7	2.2	0.0	0.0	0.0	140
	treated*	0.0	0.0	0.0	0.0	4.7	5.3	12.4	20.6	29.4	15.3	12.3	170
Z. perennis × *Z. mexicana*	control	0.0	0.0	3.8	5.0	26.1	58.4	6.7	0.0	0.0	0.0	0.0	180
	treated*	0.0	0.0	0.0	2.9	13.8	56.3	6.9	6.3	5.2	5.2	3.4	174
Z. luxurians × *Z. perennis*	control	3.1	4.4	8.1	6.9	23.1	26.2	11.9	8.1	5.0	1.9	1.3	160
	treated*	0.0	0.0	3.9	5.1	10.0	22.2	21.1	18.8	11.1	5.0	2.8	180
Z. diploperennis × *Z. perennis*	control	0.0	0.0	4.2	4.8	18.1	69.9	3.0	0.0	0.0	0.0	0.0	166
	treated*	0.0	0.0	0.0	0.0	9.1	44.0	21.2	8.0	5.7	6.9	5.1	175

Table 4. Percentage of quadrivalents in parental species and *Zea* hybrids with 2n = 40 for plants treated with colchicine (50 µM) and untreated control plants.* - significant differences between control and treated plants at the probability level of 5 %.

Species	Treatment	IV/cell											Total number
		0	1	2	3	4	5	6	7	8	9	10	
Z. mays	control	0.0	0.0	0.0	2.1	0.7	4.2	6.3	10.6	16.9	26.1	33.1	142
	treated	0.0	0.0	0.0	0.0	2.2	3.9	5.1	7.9	16.8	27.0	37.1	178
Z. perennis	control	0.0	2.3	2.8	6.7	28.1	52.8	7.3	0.0	0.0	0.0	0.0	178
	treated*	0.0	0.0	2.0	3.9	10.7	19.5	31.7	18.5	9.9	1.9	1.9	205
Z. mays × *Z. perennis*	control	3.1	5.8	9.4	20.6	21.4	34.8	4.9	0.0	0.0	0.0	0.0	224
	treated*	0.0	0.0	5.0	21.0	26.0	32.0	5.0	4.0	4.0	2.0	1.0	200
Z. diploperennis × *Z. perennis*	control	1.9	2.8	6.1	19.2	26.6	31.8	9.8	1.8	0.0	0.0	0.0	214
	treated*	0.0	0.0	2.0	4.9	5.9	27.9	26.3	15.3	10.8	3.9	3.0	204
Z. parviglumis × *Z. diploperennis*	control	15.1	26.9	29.0	17.7	11.3	0.0	0.0	0.0	0.0	0.0	0.0	186
	treated*	0.0	7.8	16.7	18.9	15.6	11.1	13.9	5.0	3.3	4.4	3.3	180
Z. mays × (*Z. diploperennis* × *Z. perennis*)	control	1.2	8.0	6.2	11.1	11.7	46.9	13.0	1.9	0.0	0.0	0.0	162
	treated*	0.0	0.0	1.9	5.0	6.9	30.0	28.1	11.2	8.1	5.0	3.8	160

In *Zea* hybrids with 2n = 30, no significant differences between treated material and controls were observed (Table 3). The most frequent configuration was 8III+2II+2I. In the hybrids *Z. mays* × *Z. parviglumis* with 2n = 30 and *Z. luxurians* × *Z. perennis* with 2n = 30, cryptic pairing was increased with a higher percentage of trivalents in the treated material in comparison with the controls. An increase in cryptic pairing was also observed in *Z. perennis* × *Z. mexicana* and *Z. diploperennis* × *Z. perennis* with 2n = 30. Although treated material showed up to 10III, a maximum of 6III was observed in the controls. The highest difference between treated genotypes and controls was observed in the 2n = 30 hybrid *Z. mays* × *Z. perennis*, where the meiotic configuration most frequently observed was 5III+5II+5I for the controls and 8III+2II+2I for the treated hybrids.

Table 5. Average number of chiasmata in species and hybrids of *Zea* in material treated with a diluted colchicine solution and untreated controls.

Species and hybrids	2n	Chiasmata control	treated
Z. mays	20	14.00	20.00
Z. mexicana	20	16.48	21.70
Z. parviglumis	20	15.36	20.08
Z. luxurians	20	15.34	21.34
Z. diploperennis	20	14.00	14.64
Z. mays × *Z. mexicana*	20	16.75	22.72
Z. mays × *Z. parviglumis*	20	15.42	21.06
Z. mays × *Z. luxurians*	20	15.48	20.36
Z. mays × *Z. diploperennis*	20	15.00	15.35
Z. diploperennis × *Z. luxurians*	20	15.26	16.24
Z. mays × *Z. diploperennis* × *Z. luxurians*	20	14.57	19.38
Z. mays × *Z. mays*	30	28.12	29.03
Z. mays × *Z. parviglumis*	30	25.60	36.70
Z. mays × *Z. perennis*	30	23.06	37.09
Z. perennis × *Z. mexicana*	30	24.02	32.50
Z. luxurians × *Z. perennis*	30	21.36	29.60
Z. diploperennis × *Z. perennis*	30	17.25	26.34
Z. mays	40	33.75	34.25
Z. perennis	40	34.56	41.80
Z. mays × *Z. perennis*	40	33.59	42.50
Z. diploperennis × *Z. perennis*	40	31.42	40.36
Z. parviglumis × *Z. diploperennis*	40	28.20	37.25
Z. mays × *Z. diploperennis* × *Z. perennis*	40	33.81	42.25

In the 2n = 40 maize, only a slight increase in the meiotic configuration of 9IV+2II or 10IV, and a minimal decrease in the configurations with less than 7IV was observed (Table 4). The most frequent meiotic configuration in *Z. perennis* controls was 5IV+10II and less frequently 6IV+8II. In the treated material, the percentage of quadrivalents increased to a maximum of 10 with an average of 7IV+6II.

In *Z. mays* × *Z. perennis* hybrids with 2n = 40 (Fig. 1*D*), *Z. diploperennis* × *Z. perennis*, and the trihybrid *Z. mays* × (*Z. diploperennis* × *Z. perennis*), there was an increase in the number of IV treated material reaching a maximum of 10IV. In all the cases, the controls showed a very low proportion of pairing among homoeologous chromosomes especially between *Z. diploperennis* and *Z. perennis* (Table 4).

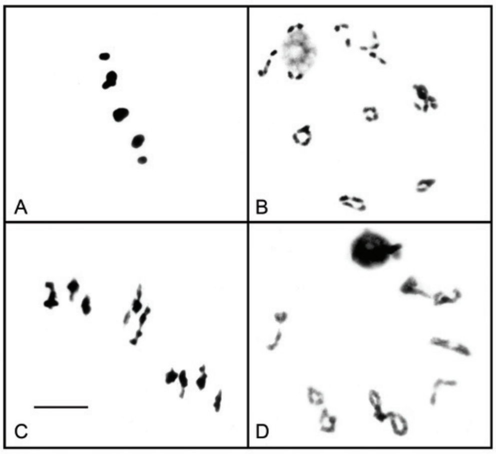

Fig. 1. Meiotic configurations induced by colchicine treatment in: *A* - *Z. mays* (2n = 20; 5IV), *B* - *Z. perennis* (81V + 4II), *C* - *Z. mays* × *Z. perennis* (2n = 30; 10III), *D* - *Z. mays* × *Z. perennis* (2n = 40; 9IV + 2II). *Scale bar* = 10 µm.

The behavior of the hybrid *Z. parviglumis* × *Z. diploperennis* with 2n = 40, obtained by chromosome duplication of *Z. parviglumis* × *Z. diploperennis* with 2n = 20, was different from that of the rest of the hybrids with the same chromosome number (Table 4). The most frequent meiotic configuration in the control was 2IV+16II. Apparently, pairing identical chromosomes in this hybrid was favored, reducing the homoeologous pairing between the A and B genomes which could explain the low frequency of IV observed. The treated material showed results similar to the rest of the hybrids studied.

In all the cases, the number of chiasmata increased in the treated material, excepting *Z. diploperennis, Z. mays* with 2n = 40, the hybrid *Z. mays* × *Z. mays* 2n = 30, *Z. mays* × *Z. diploperennis*, and *Z. diploperennis* × *Z. luxurians* with 2n = 20 (Table 5).

Discussion

Cryptic pairing among homoeologous chromosomes of the genus *Zea*: The analysis of the meiotic behavior of *Zea* species with 2n = 20 after treatment with a diluted colchicine solution (Table 1) showed homoeologous chromosome pairing in all species (with a maximum of 5IV) with the exception of *Zea diploperennis* which did not show any significant differences when compared treated and control plants (Table 1). More than one possible reason can be mentioned to explain the lack of pairing in *Zea diploperennis.* First, the expected alteration of the spatial relation of chromosomes (Driscoll and Darvey 1970) was not observed, hence, they were able to maintain their ordering (Feldman and Avivi 1988, Feldman *et al.* 1997) and anchor to the nuclear membrane (Bass *et al.* 2000, Chikashige *et al.* 2010) favoring pairing among homologous chromosomes. Second, the *pam1* gene, which intervenes in the presynaptic mechanisms (Felman and Avivi 1988, Bozza and Pawlowski 2008) and in the chromosome reorganization related to the formation of the bouquet (Zickler and Kleckner 1998, Bass *et al.* 2000), may have a different effect on *Z. diploperennis* than on the other *Zea* species. Third, the action of an equivalent *Ph* gene in *Z. diploperennis* is not inhibited by the diluted colchicine solution used. Fourth, *Z. diploperennis* chromosomes are not homoeologous which is possible if *Z. diploperennis* was obtained from a haploid *Z. perennis*. Independently of the mechanism that resulted in the observed lack of pairing, it would be interesting to analyze the last hypothesis in future research.

Cryptic pairing of homoeologous chromosomes in 2n = 20 hybrids (Table 2) varied according to the cross analyzed but was higher in the hybrids between more closely related species (*Z. mays, Z. mexicana*, and *Z. parviglumis*) than in more distant species (*Z. luxurians* and *Z. diploperennis*). The hybrids of *Z. mays* × *Z. luxurians* and (*Z. mays* × *Z. diploperennis*) × *Z. luxurians* showed a similar cryptic pairing but lower than in species with higher chromosome affinity. The cryptic pairing observed in the hybrid *Z. mays* × *Z. luxurians* may be attributable to a higher differentiation among their homoeologous chromosomes, since parental species are evolutionarily more distant.

The meiotic behavior of *Zea* in the species and hybrids with 2n = 30 after treatment with colchicine showed no significant differences between treated and untreated material in the hybrids between species of *Z. mays* (Table 3). A possible explanation is the fact that this hybrid is a cross between genotypes of the same species with different ploidy level and the preferential pairing is between homologous chromosomes. The preferential pairing of homologous chromosomes was observed by Santos and Orellana 1983, Santos *et al.* 1984, Jenkins and Chatterjee 1994, Jenczewski and Alix 2004. However, it is not clear why 2n = 20 maize showed cryptic pairing between the A and B genomes (Table 1, Fig. 1*A*) which was inhibited with a higher ploidy level (Table 4).

A high level of homoeologous chromosome pairing, with a maximum of 10III, was detected in untreated *Z. mays* × *Z. parviglumis* with 2n = 30 and *Z. luxurians* × *Z. perennis* (Table 3). Colchicine increased the percentage of III only slightly.

The highest response to colchicine treatment, with a maximum of 10III, was obtained in the 2n = 30 hybrid *Z. mays* × *Z. perennis* (Table 3, Fig. 1*C*) suggesting that chromosomes of *Z. mays* and *Z. perennis* are homoeologous even though both species are evolutionarily differentiated.

Different results were found in 2n = 40 species and hybrids. Treated and untreated *Z. mays* showed pairing between A and B homologous chromosomes with a maximum of 10IV (Table 4). Colchicine treatment did not induce homoeologous pairing and no hexavalents or octovalents chromosomes were observed (Table 4).

Treatment of *Z. perennis* induced only pairing homoeologous chromosomes of the B genome (Table 4, Fig. 1*B*) with a maximum of 10IV. Genome B chromosomes paired as bivalents although genome A chromosomes paired as quadrivalents. There was no pairing between chromosomes of the A and B genomes.

Considering *Zea mays* with 2n = 40, *Z. diploperennis* × *Z. perennis*, *Z. mays* × *Z. perennis* (Fig.1*D*), and *Z. mays* × (*Z. diploperennis* × *Z. perennis)*, chromosomes of the A genome from species that form the hybrids paired similarly in both treated and untreated material. Colchicine induced the cryptic pairing homologous chromosomes of the B genome with a maximum of 10IV (Table 4).

Possible evolution of the genomes of *Zea*: Previous results (Molina and Naranjo 1987, Naranjo *et al.* 1989, 2004, Poggio *et al.* 1990, Molina and García 2001, Molina *et al.* 2004, Molina 2011, González and Poggio 2011) support the theory that consider *Zea* as an autotetraploid with two subgenome with different level of conservation (Swigonová *et al.* 2004, Schnable and Freeling 2011, Schnable *et al.* 2011).

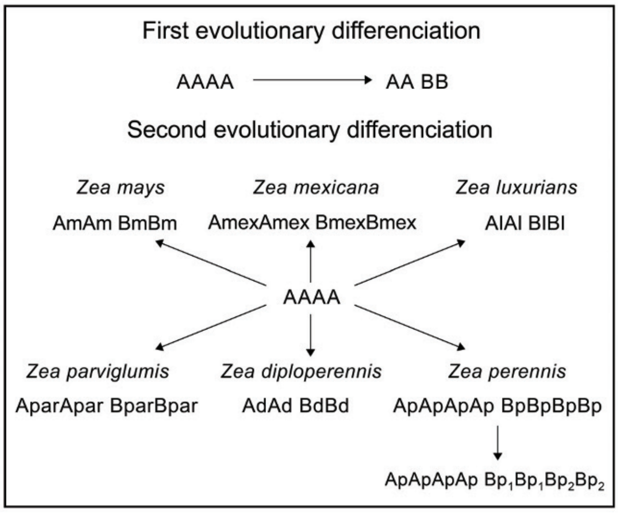

Fig. 2. Possible mechanisms of chromosome differentiation in the genus *Zea*. First differentiation: Diploid species with A genome would have differentiated into A and B genomes. Second differentiation: B genomes would have mutated or differentiated between them resulting in different *Zea* species with 2n = 20. Particularly in the case of *Zea perennis*, chromosomes of the B genome could have undergone further differentiation which resulted in Bp_1 and Bp_2 homoeologous chromosomes.

A possible mechanism of differentiation of subgenomes which could have led to different *Zea* species would consist in a duplication of the A genome of a diploid species followed by a differentiation in the homoeologous genomes A and B (Fig. 2). Another possible mechanism that resulted in the genotypic constitution of AA BB individuals is an ancestral cross between two diploid species (AA and BB). Our results support the theory of a chromosome duplication followed by a differentiation of two genomes, since in the hybrid *Zea mays* ssp. *parviglumis* × *Zea diploperennis* with 2n = 40 (obtained by crossing two *Zea* species and then duplicated), chromosomes of each species paired preferentially with the homologous instead of homoeologous chromosomes resulting in a more frequent configuration of 2IV+16II although in most hybrids, the configuration was 5IV+10II. The A genome would have remained undifferentiated allowing the current pairing of homoeologous chromosomes of interspecific hybrids as if they were homologous. The differentiation of B genomes would be the evolutionary process (Fig. 2) which would have resulted in different species of *Zea* probably favoring their geographic isolation (Ruiz *et al.* 2001, Fukunaga *et al.* 2005) or domestication (Swanson-Wagner *et al.* 2012).

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