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Cytogenetic Study of a Tetraploid Hybrid *Zea diploperennis* × *Zea perennis*

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In 1979, Iltis *et al.* reported the discovery of a new species of the tribe Maydeae called *Zea diploperennis* Iltis, Doebley and Guzmán. *Z. diploperennis* is a diploid and perennial species being its chromosome number 2n=20. It easily hybridizes with *Z. mays* obtaining a fertile progeny.

Zea or *Euchlaena perennis* was discovered by Hitchoch in Jalisco, México in 1921. It is the only tetraploid species of the Maydeae tribe and it has chromosome number 2n=40.

Z. perennis hybridizes with great difficulty with the diploid *Z. mays*, being its progeny triploid and sterile (Mazoti and Rimieri 1978. Molina 1978). On the other hand when it crosses with the tetraploid *Z. mays* the descendants are tetraploid and fertile (Collins and Longley 1935, Emerson and Beadle 1930, Shaver 1962).

In 1980, *Z. diploperennis* was crossed with *Z. perennis*. It was easily hibridized when used as female parent. In that case the 60% of the seeds were apparently fertile and the 90% of them had germinated. On the other hand, in the reciprocal crossing, using *Z. perennis* as female the results were negative in most cases, since just one plant was obtained (Molina 1981).

The F_1 of *Z. diploperennis* and *Z. perennis* is both vigorous and perennial. It also has abundant tillering with an average of 45 tillers per plant, short rhizomes and slow propagation. The female inflorescence has the fragile rachis, so the seeds disperse when they mature.

It is very sterile and the chromosome number is 2n=30 because *Z. diploperennis* contributes 10 chromosmes and *Z. perennis* 20.

The meiotic configuration most frequently observed is 5III+5II+5I (Molina 1981, 1982).

From the set of the hybrids coming from the aforementioned crossing a plant stood out for its morphology more similar to *Z. diploperennis* than to the rest of hybrids which resembled to *Z. perennis*. But the main differences that were found were the chromosome number which was 2n=40, and the high fertility (80%) as opposed to the rest of the plants that were extremely sterile.

Due to the exceptional behaviour of the hybrid between *Z. diploperennis* and *Z. perennis* (with 2n=40 chromosomes) a cytogenetic study will be carry out in this work.

Material and methods

The tetraploid hybrid *Z. diploperennis* × *Z. perennis* was taken from the present investigation.

The chromosomes were stained with the Belling's aceto carmine squash technique and the fertility of the pollen was determined with Lugol solution considering as fertile those grains of which at least 3/4 were stained.

Results

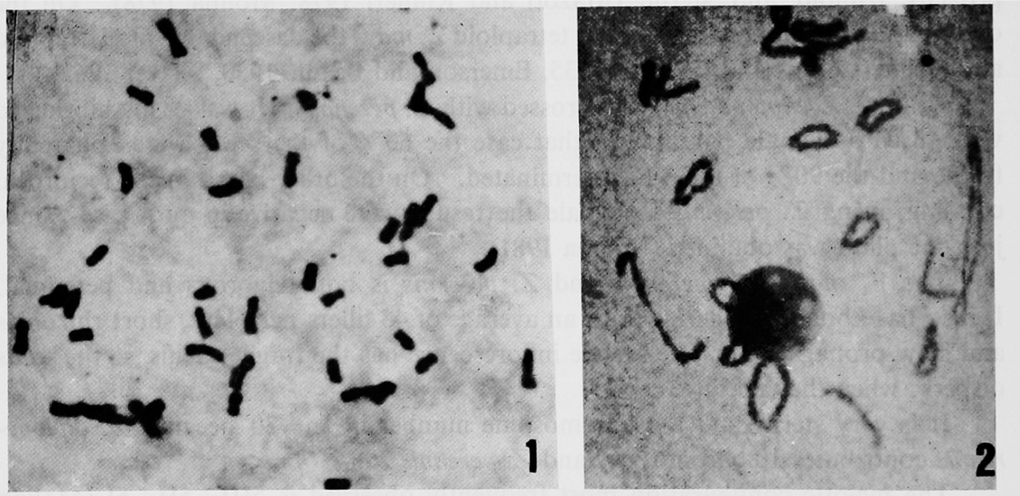
In April 1980, hybrids between *Z. diploperennis* (2n=20) and *Z. perennis* (2n=40) had been produced in a greenhouse.

The plants are perennial, vigorous, with abundant tillering, rhizomatous and sterile being its chromosome number 2n=30.

The meiotic configurations most frequently found was 5III+5II+5I.

The sterility (2% of fertile seeds) is due to the great quantity of univalents and trivalents which produce unbalanced and sterile gametes.

Among the set of these plants one of them stood out for its high fertility (80% of the seeds, fertile).



Figs. 1–2. 1, somatic chromosomes of hybrid *Z. diploperennis* × *Z. perennis* 2, a photomicrograph of meiotic chromosomes at metaphase I of hybrid *Z. diploperennis* × *Z. perennis* chromosomes. ×2000.

The chromosome counts gave 2n=40 (Fig. 1). It was determined that the hybrid has two chromosome sets of each parents.

On the contrary the rest of the hybrids between the same species have one chromosome set of *Z. diploperennis* and two of *Z. perennis*.

Cytological analysis of the hybrid

In the counts of 214 cells in diakinesis the following meiotic configurations were observed (Table 1).

From the results shown in Table 1 it can be deduced that the hybrid has a low percentage of univalents and trivalents being most of the chromosomes paired as bivalents and tetravalents preferentially open-ring (Fig. 2), with an average of 31.42 chiasmata per cell (15.96 in the bivalents, 0.10 in the trivalents and 15.30 in the tetravalents).

Comparing the meiotic configurations of the hybrids with those of its parents (Table 2) it can be concluded that they are very similar as well as the average of I, II, III and IV of *Z. perennis* and the hybrid.

In the hybrid, in pachytene it is extremely difficult to differentiate the chromosomes of *Z. perennis* from those of *Z. diploperennis* owing to the fact that in both species their chromosomes have small terminal knobs.

Table 1. Meiotic configurations of the tetraploid hybrid of *Z. diploperennis* × *Z. perennis.*

Number of cells	I	II	III	IV	%
68		10		5	31.78
43		12		4	20.09
33		14		3	15.42
24		8		6	11.22
7		16		2	3.27
5		18		1	2.33
2		6		7	0.93
8	1	10	1	4	3.74
7	2	11		4	3.27
6	2	13		3	2.80
5	2	9		5	2.33
3	1	12	1	3	1.40
2	1	14	1	2	0.93
1	4	10		4	0.47
214	0.24	11.29	0.06	4.20	

Table 2. Average of I, II, III and IV of the tetraploid hybrid of *Z. diploperennis* × *Z. perennis*

		I	II	III	IV	Chiasmata
Z.d. × *Z.p.*	(2n=40)	0.24	11.29	0.06	4.20	31.42
Z.p.	(2n=40)	0.16	11.06	0.01	4.44	34.79
Z.d.	(2n=20)	0.27	9.73			14

Z.d. = *Zea diploperennis*
Z.p. = *Zea perennis.*

There is only one chromosome set of *Z. diploperennis* that can be easily identified since it has a big terminal knob (Molina 1983).

In 78% of the cells analized at anaphase I 20 chromosomes migrate towards each pole and in the left 22% different number of chromosomes migrate towards each pole. Other abnormalities observed are lagging chromosomes and exceptionally chromatid bridges. The percentage of fertile pollen is 56 percent and the one of fertile seed is 80 percent.

Discussion

When *Z. diploperennis* (2n=20) was crossed with *Z. perennis* (2n=40) some extremely sterile hybrids had been obtained being their chromosome number 2n=30 with the exception of a fertile plant with 2n=40.

It has been considered doubtful if this plant would be a real hybrid or a false one; since it is known that in hybridizations of other species as *Tripsacum*×*Z. mays* and *Tripsacum*×*Z. mexicana* matroclinous plants have been produced as the result of the parthenogenetic development of the *Tripsacum* ovule.

In 1931, Mangelsdorf and Reeves had hybridized *Tripsacum* and *Z. perennis* obtaining a plant, very similar to *Z. perennis* by parthenogenetic development of pollen grain; this phenomenon is called patrogenesis.

In the hybridization of *Z. diploperennis* and *Z. perennis* the phenomenon of duplication and parthenogenetic multiplication of pollen grain (patrogenesis) has been discarded for two reasons:

1) The hybrid has morphological characteristics of both parents although it is more similar to *Z. diploperennis*.
2) From the cytological analysis it had been distinguished a chromosome with a large terminal knob which is characteristic of *Z. diploperennis*.

As the phenomenon of patrogenesis had been discarded it is important to analize the origin of the hybrid.

One of the suggested hypotheses was based on the fact that the hybrid would be obtained by the crossing of an unreduced gamete of *Z. diploperennis* (female parent) and a normal gamete of *Z. perennis*.

Z. diploperennis × *Z. perennis*
unreduced gamete (2n=20) normal gamete (n=20)
$F_1=(2n=40)$.

The mentioned hypothesis is considered to be the most probable since the same phenomenon had already been reported in hybrids between *Z. mays* and *Z. perennis*. In this case some hybrids of 2n=40 were produced by the crossing of unreduced gametes of *Z. mays* with normal gametes of *Z. perennis* (Collins and Longley 1935, Emerson and Beadle 1930, Shaver 1962).

Chromosome pairing of the tetraploid hybrid

The cytological analysis indicated that the meiotic configuration most frequently found in *Z. perennis* was 10II+5IV so the one of the gamets was 10I+5II (Molina 1978).

In *Z. diploperennis* the meiotic configuration most frequently observed was 10II (Iltis *et al.* 1979, Molina 1981a, Pasupuleti and Galinat 1982).

The normal gamete had 10I and the unreduced gamete 10II.

In F_1 of *Z. diploperennis*×*Z. perennis* with 2n=30 the chromosomes of species paired obtaining the following meiotic configuration 5III+5II+5I.

Taking into account in the hybrid 2n=40 the chromosomes of both species paired totally the expected pairing could be as follows:

Z. diploperennis × *Z. perennis*
(unreduced gamete) (normal gamete)
10II 5II+10I

Consequently the predicted pairing in F_1 (2n=40) would be 5IV+5III+5I. But as the most frequent pairing was 10II+5IV (Table 1) and very few univalents and trivalents had been produced, the more probable mechanism would be the following:

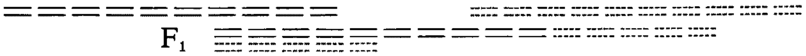
1) Autosyndesis of the chromosomes of *Z. perennis*.

The normal gamete of *Z. perennis* is 10I+5II but if the autosyndesis of the univalents takes place the gamete would have 10II and it would cross as follows:

Z. diploperennis × *Z. perennis*
(unreduced gamete) (autosyndesis of the chromosomes)
10II 10II
F_1 5IV+10II

Schematically

Z. diploperennis × *Z. perennis*
(unreduced gamete 10II) (autosyndesis of the chromosomes)



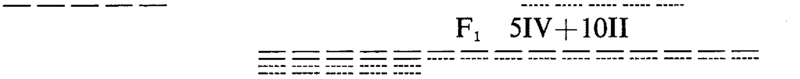
2) Pairing of the all chromosomes of the two species.

As it is known *Z. perennis* contributes with a gamete of 10I+5II, and *diploperennis* contributes with an unreduced gamete of 10II. When these gametes combined 5 of the bivalents of *Z. diploperennis* being paired with the chromosomes of *Z. perennis* (this is the opposite case that the one analized in item 1).

Z. diploperennis × *Z. perennis*
10II 5II+10I
F_1 5IV+10II

Schematically

Z. diploperennis × *Z. perennis*
(unreduced gametes 10II) 5II+10I
5 of the chromosomes separated
and paired with of *Z. perennis*



3) A combination of cases 1 and 2.

The results of the chromosome study of the hybrid between *Z. mays*, *Z. perennis* and *Z. diploperennis* could be interpreted as supporting the hypothesis that the 3 species have 5 homologous or homeologous chromosomes due to the fact that in all the hybridizations these chromosomes paired.

Consequently it could be considered that the 5 chromosomes in *Z. mays*, *Z. perennis* and *Z. diploperennis* have a common origin.

The chromosome number of the hybrid is an important fact to determine the fertility of the hybrids and the chromosome pairing.

Not only in the hybridizations between *Z. perennis* and *Z. diploperennis* but also in the crossing between *Z. perennis* and *Z. mays* when the hybrids have 2n=30 chromosomes, they are sterile with unbalanced gamete due to the great quantity of univalents and trivalents.

However in the hybrids (2n=40) between the same species the chromosomes paired as tetravalents and bivalents obtaining fertile progeny.

Summary

It had been obtained a tetraploid hybrid (2n=40), crossing *Z. diploperennis* (2n=20) with *Z. perennis* (2n=40) by non disjunction of the gametes of *Z. diploperennis.*

The cytological study indicated that there were no univalents and trivalents. Most of the chromosomes paired as bivalents and tetravalents preferently open-ring. The average of the counts in 214 cells in diakinesis was: 0.24I, 11.29II, 0.06III and 4.20IV. The average of chiasmata was 31.42 and at pachynema some translocations and inversions were observed.

In anaphase in the 78% of the cells the same number of chromosomes migrated to each pole and in the left 22% different number of chromosomes migrated towards each pole or remained as lagging chromosomes. Only exceptionally chromatid bridges were observed.

The fertility of the pollen is 56% with 80% of fertile seed being the progeny fertile and vigorous.

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