

**FLORAL CHARACTERISTICS, CHROMOSOME NUMBER AND
MEIOTIC BEHAVIOR OF HYBRIDS BETWEEN
Leucaena leucocephala (2n = 104) AND TETRAPLOID
L. diversifolia (2n = 104) (LEGUMINOSAE)***

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ABSTRACT

Floral characteristics, chromosome number and meiotic behavior of F₂, F₃ and F₄ hybrids between *Leucaena leucocephala* and tetraploid *L. diversifolia* were analyzed. The hybrid population more closely resembled *L. leucocephala* in terms of number of flowers per inflorescence and inflorescence color but was intermediate between the two species for flower arrangement. Meiotic behavior was fairly regular, with predominance of bivalents at metaphase I and other associations occurring at variable frequencies. Meiotic index and pollen viability values were high.

INTRODUCTION

The genus *Leucaena* Benth. is native to Central America and some species, mainly *L. leucocephala*, are widely cultivated in the tropical and sub-tropical regions of the world. Due to the multiple uses of *L. leucocephala* for forage, green manure, wood production and erosion control and because of the great germplasm diversity,

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this quick-growing nitrogen-fixing tree has been extensively studied in recent years. Crosses have been made to select individuals and develop cultivars adapted to specific conditions such as acid-soil-tolerance, pest-resistance, frost-tolerance, etc...

L. leucocephala ($2n = 104$) has a tall-tree habit (4 to 18 m) with small white flowers (100 to 180 according to Takahashi and Ripperton (1949) and 200 to 250 according to Ganeshaiah *et al.* (1986), arranged in a contracted glomerulous inflorescence. *L. diversifolia* ssp. *diversifolia* ($2n = 104$), also has a tall-tree habit, and 40 to 55 pink flowers, loosely arranged in the inflorescence. (Pan, 1984; Brewbaker, 1985). Meiosis in both species is regular with a predominance of 52 II at metaphase I. (Pan, 1984; Hutton, 1985; Freitas *et al.*, 1988).

In an experiment designed to select cold and frost tolerant plants among *L. leucocephala* \times $2n = 104$ *L. diversifolia* hybrids, several morphological and cytogenetical characteristics were analyzed in the hybrid population. Floral characteristics, chromosome numbers and meiotic behavior results are presented here.

MATERIAL AND METHODS

Seeds from 51 F₂, F₃ and F₄ mother-plants divided into 18 selections, from crosses between *L. leucocephala* and tetraploid *L. diversifolia*, were supplied by Dr. E.M. Hutton, who made the crosses at CIAT, Colombia.

A hybrid population was established at the UFRGS Experimental Station with 5 repetitions (descendant individuals) per mother plant.

At least 20 adult inflorescences per individual were collected from 183 plants during the first flowering period (January-February, 1989). The number of flowers per inflorescence was counted and flower color and arrangement were recorded. The inflorescences were classified as white or pink, including all the varying pink tonalities. They were also classified as having a "contracted" or "loose" flower arrangement.

Chromosome number was determined from somatic root tip cells of young seedlings. Pretreatment was performed with a saturated solution of paradichlorobenzene for 18-20h at 4°C, fixation in 3:1 ethanol-acetic acid and storage in 70% ethanol in a freezer. Squash preparations were made following maceration in 4% pectinase for 1h, hydrolysis in 5N HCl for 30 min and staining with Feulgen or acetic carmine. At last 20 cells per individual were analyzed.

For the analysis of meiotic behavior, young inflorescences were fixed in ethanol-acetic acid-chloroform (6:3:1) for 24h and stored in 70% ethanol in a freezer. Slides were prepared by squashing and staining the anthers in propionic carmine. Meiosis in all 183 plants was analyzed, with an average of 100 cells per plant. Special attention was given to chromosome associations at metaphase I (average of 20 cells per individual). Meiotic indexes (number of normal quartets \times 100/total number of quartets) were determined from 300 quartets from three different inflorescences. Quartets with four equal-sized cells were considered as normal and those with different cell numbers or size were classified as abnormal.

Pollen viability was estimated by pollen stainability of 200 grains from two different inflorescences (number of well-stained 3-pored grains x 100/total number of grains). Abnormal-sized and 4-pored grains were recorded.

RESULTS AND DISCUSSION

Table I summarizes the results for number of flowers per inflorescence, inflorescence color and flower arrangement. Data for number of flowers among descendants of the same mother plant were pooled, those for inflorescence color and arrangement were grouped into selections.

Table I - Floral characteristics of the hybrid population.

Selections/ mother-plants	Number of flowers per inflorescence			Inflorescence arrangement number of plants		Inflorescence color number of plants	
	Min.	Max.	Mean \pm S	contracted	loose	white	pink ^a
235A-2 (1-2-3-4)	68	150	105.1 \pm 24.9	5	10	6	9
235A-3 (5-6)	80	110	95.0 \pm 8.5	-	5	4	1
235A-5 (7-8-9)	82	156	103.9 \pm 28.0	6	5	6	5
235A-6 (10-11-12-13)	79	132	103.7 \pm 15.5	4	3	5	2
235A-9 (14-15)	62	105	87.3 \pm 18.5	4	1	5	-
235B-2 (16-17-18)	73	206	111.3 \pm 48.3	3	7	4	6
235B-3 (19-20-21)	56	132	99.7 \pm 29.1	11	2	11	2
235B-7 (22-23-24)	72	150	104.0 \pm 30.9	3	8	5	6
235B-10 (25-26-27-28)	76	134	100.7 \pm 22.8	9	10	9	10
235B-11 (29-30-31-32)	76	114	98.3 \pm 14.8	3	12	2	13
235C-4 (33-34-35-36)	90	198	122.9 \pm 35.1	9	6	9	6
235C-3 (37-38-39-40)	80	146	110.0 \pm 19.2	4	10	5	9
235C-9 (41-42-43)	70	150	94.3 \pm 29.4	9	4	4	9
235C-11 (44-45-46-47)	68	152	103.2 \pm 31.6	9	6	4	11
54-1 (48)	106	121	113.1 \pm 0.0	1	2	3	-
54-5 (49)	96	164	136.7 \pm 0.0	4	-	4	-
54-8 (50)	90	168	131.3 \pm 0.0	2	2	4	-
54-9 (51)	138	148	143.3 \pm 0.0	4	-	2	2
				90	93	92	91
Total				(49.2%)	(50.8%)	(50.3%)	(49.7%)

^a Those with mix-colored flowers are included here.

There was a tendency in the hybrid population towards a high number of flowers per inflorescence, near that of *L. leucocephala* or intermediate between the parent species despite some intra and interindividual variation.

Flower production is genetically controlled but may also be extremely influenced by environmental conditions (Armstrong and Robertson, 1960). In *L. leucocephala*, Mamiepic and Empig (1980) observed an average of 145 flowers per inflorescence and a high percentage of non-persistent flowers.

The proportion of individuals with contracted and loose inflorescences was 1:1 (49.2% to 50.8%). (Table I).

50.3% of the individuals presented white and 49.7% had pink flowers (Table I), including those with intermediate pink tonalities. Most of the plants with pink flowers also had pink pigments in the stem. "Typical" mature *L. leucocephala* inflorescences are yellowish-white, during anthesis. In the field, the inflorescences were classified as white or pink. However, under a stereomicroscope it was clearly seen that those inflorescences with lighter pink tonalities presented flowers with some floral parts (anthers, filaments, stigma, style, sepals and petals) white and others pink. This mixture of white and pink floral parts shows that *L. leucocephala* genes for flower color are being expressed in more than 50% of the population. In crosses between *L. diversifolia* and *L. shannoni* some descendants presented light pink inflorescences (Hutton, 1981).

Leucaena is not good material for cytogenetic studies due to the small chromosome size (0.7 to 1.6 μm according to Tijo (1948)), chromosome clumping and difficulty in obtaining good contrast between chromosomes and cytoplasm (Gonzales *et al.*, 1967; Pan, 1984). However, analysis of a large number of cells can lessen these problems.

Cytogenetic results are presented in Table II and Figure 1. Chromosome number was almost always $2n = 104$, as expected. Some aneuploids with $2n$ varying from 86 to 103 were also detected and the data were confirmed by meiotic counts.

Meiotic behavior was fairly regular, with a predominance of cells having bivalents only. Other associations were present at varied frequencies. (Figure 1A).

Freitas *et al.* (1988) analyzing F_1 , F_2 and F_3 hybrids between *L. leucocephala* and di and tetraploid *L. diversifolia*, selected for acid soil tolerance, found regular meiotic behavior with a predominance of pollen mother cells having only bivalents at metaphase I, suggesting preferential intraspecific homologue pairing.

Regularity of meiosis was reflected in high meiotic indexes. 70.5% of the mother plants and descendants had meiotic indexes equal to or greater than 90%, showing meiotic stability (according to Love's (1949) concept).

Table II - Chromosome number, meiotic behavior and pollen viability in the hybrid population.

Mother-plant	2n ^a	Chromosome associations ^a	Meiotic Index ^b		Pollen viability ^b	
			%		%	
1	104	0-1IV, 0-2III, 47-52II	92.0		84.2	
2	104	0-1IV, 0-2III, 46-52II, 0-2I	94.4		93.8	
3	104	0-1IV, 0-2III, 49-52II, 0-2I	87.6		90.0	
	96	0-1IV, 0-1III, 43-45II, 0-3I				
4	104	0-2IV, 0-2III, 48-52II	93.0		80.3	
	96	46-49II, 0-4I				
5	104	0-2IV, 48-52II	91.6		79.0	
	100	0-2IV, 45-49II, 0-2I				
6	104	0-2IV, 0-2III, 45-52II	94.5		72.5	
	100	1IV, 46II, 4I				
7	104	0-3IV, 0-2III, 43-52II, 0-3I	92.2		79.6	
	100	0-1III, 47-49II, 0-3I				
8	104	0-2IV, 0-3III, 43-52II, 0-1I	78.3		71.2	
	100	0-1IV, 0-1III, 48II, 0-1I				
9	104	0-1IV, 0-1III, 48-52II, 0-1I	85.7		88.8	
	96	48II				
10	104	0-2IV, 0-1III, 46-52II, 0-1I	75.4		95.0	
11	104	0-1IV, 0-2III, 46-52II, 0-2I	94.9		85.6	
	94	1IV, 2III, 40II, 4I				
12	104	0-2IV, 48-52II	31.4		14.8	
13	104	0-2IV, 48-52II	91.5		91.7	
	96	1IV, 46II				
14	104	52II	93.0		97.5	
15	104	0-2IV, 48-52II	93.0		97.5	
16	104	0-2IV, 48-52II	91.2		78.5	
	96	1III, 46II, 1I				
17	90	0-2III, 41-45II, 0-2I	66.5		17.2	
	86	42II, 2I				
18	104	0-2IV, 48-52II,	90.4		80.4	
	98	0-2III, 46-49II				
19	104	0-2IV, 48-52II	95.0		86.3	
	98	0-2III, 46-49II				
20	104	0-2IV, 0-1III, 46-52II, 0-3I	92.1		90.8	
21	104	0-2IV, 0-2III, 46-52II, 0-2I	58.7		83.5	
22	104	0-2IV, 0-2III, 45-52II	96.4		85.5	

Continued

Table II - Continued.

Mother-plant	2n ^a	Chromosome associations ^a	Meiotic Index ^b %	Pollen viability ^b %
	96	1-III, 46II, 1I		
23	104	0-2IV, 0-2III, 46-52II, 0-2I	93.5	78.3
	103	2IV, 46III, 3I		
24	104	0-3IV, 45-52II, 0-2I	79.3	89.4
25	104	0-1IV, 0-3III, 45-52II, 0-3I	53.0	84.5
26	104	0-2IV, 46-52II, 0-4I	80.2	39.2
	103	0-3IV, 43-48II, 0-4I		
27	104	0-3IV, 0-1III, 46-52II, 0-2I	93.1	89.5
28	104	0-2IV, 0-1III, 46-52II, 0-4I	88.9	80.0
29	104	0-2IV, 0-2III, 45-50II	94.4	96.2
30	104	0-2IV, 0-3III, 46-48II, 0-2I	93.5	64.5
31	104	0-2IV, 48-52II	85.3	49.4
	96	0-2IV, 44-48II		
32	104	0-2IV, 0-2III, 46-52II, 0-2I	91.5	88.4
33	104	52II	55.3	54.1
	96	1III, 45II, 3I		
34	104	0-2IV, 48-52II	93.3	85.9
	96	2IV, 2III, 41II		
35	104	0-2VI, 48-52II, 0-4I	90.4	84.7
36	104	0-2IV, 46-52II, 0-4I	92.0	93.0
	100	3IV, 44II		
37	104	51-52II, 0-2I	93.5	79.6
	96	46II, 4I		
38	104	0-2IV, 46-52II, 0-2I	84.0	88.0
	103	1IV, 48II, 2I		
39	104	0-2IV, 46-52II, 0-2I	93.0	97.4
40	104	0-2IV, 46-52II, 0-4I	92.7	89.2
41	104	0-2IV, 46-52II, 0-4I	95.0	91.8
	102	2IV, 46II, 2I		
42	104	0-2IV, 0-2III, 46-52II, 0-2I	90.1	76.0
	100	1III, 48II, 1I		
43	104	0-2IV, 48-52II	90.0	82.2
44	104	0-1IV, 0-2III, 46-52II, 0-2I	86.6	74.8
45	104	52II	92.1	40.4
	96	3III, 42II, 3I		

Continued

Table II - Continued.

Mother-plant	2n ^a	Chromosome associations ^a			Meiotic Index ^b	Pollen viability ^b
					%	%
46	104	0-2IV,	46-52II	96.1	87.2	
47	104	0-2IV,	46-52II, 0-4I	95.0	84.2	
48	104	0-2IV,	48,52II	92.8	78.6	
49	104	0-2IV, 0-2III,	47-52II, 0-4I	92.1	78.6	
50	104	0-2IV, 0-2III,	45-52II	96.6	96.0	
	98	1IV,	47II			
51	104	0-2IV,	48-52II	93.8	89.0	

^a In the descendant individuals.

^b Averages of values in the descendant individuals.

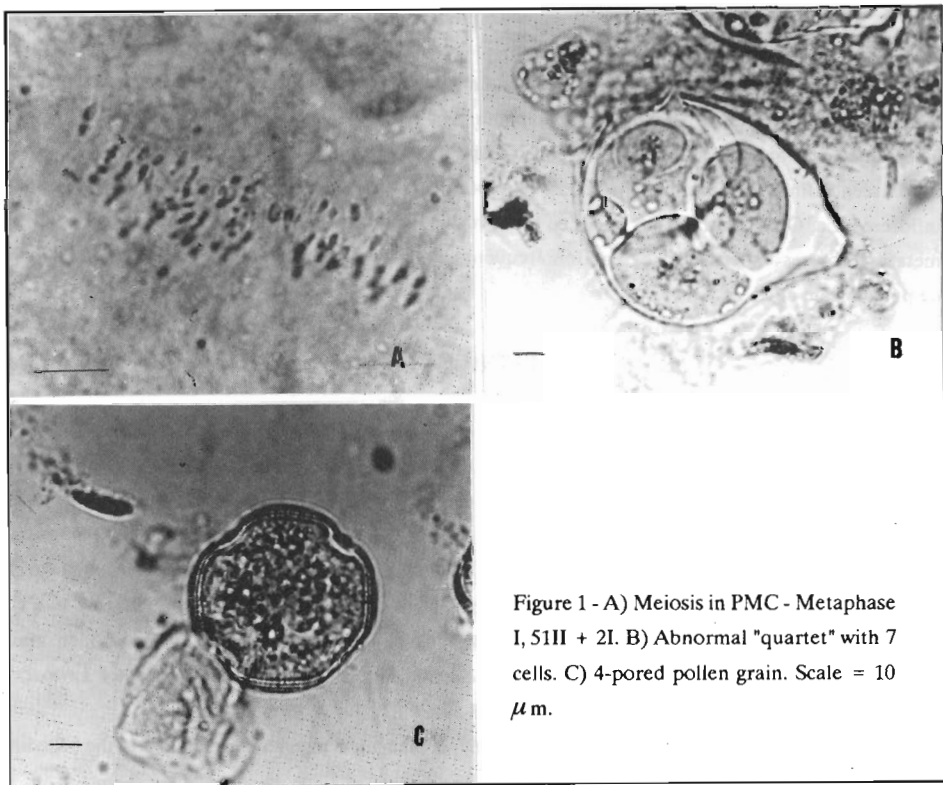


Figure 1 - A) Meiosis in PMC - Metaphase I, 51II + 2I. B) Abnormal "quartet" with 7 cells. C) 4-pored pollen grain. Scale = 10 μ m.

Some very abnormal tetrads (Figure 1B) were seen. Estimated pollen viability showed great variability: 14.8% to 97.5%. However, 65% of the plants had more than 80% viable pollen. "Macro" as well as 4-pored grains were observed (Figure 1C). From data in Table II, a general but not absolute relation between meiotic index and pollen viability can be observed.

In conclusion, the floral data as well as those on other morphological characters, not yet published, point to a more pronounced expression of the *L. leucocephala* genome. The predominance of bivalents at meiosis also gives ground for future studies on chromosome pairing control in *Leucaena*.

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RESUMO

Características florais, número cromossômico e comportamento meiótico foram estudados em híbridos F₂, F₃ e F₄ entre *Leucaena leucocephala* e *L. diversifolia* tetraplóide. A população híbrida assemelhava-se mais a *L. leucocephala* considerando número de flores por inflorescência e cor da inflorescência mas era intermediária entre as espécies parentais em relação a arranjo das flores na inflorescência. O comportamento meiótico foi bastante regular, com predominância de bivalentes em metafase I, outras associações ocorrendo em frequência variáveis. Valores de índice meiótico e viabilidade de pólen foram altos.

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