Pollen fertility and meiotic behaviour in accessions and species of leucaena

TATIANA BOFF AND MARIA TERESA SCHIFINO-WITTMANN
Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Abstract

Pollen fertility and meiotic behaviour were analysed in some taxa of the Central American nitrogen-fixing multipurpose tree genus *Leucaena*. Pollen fertility exceeded 90% in most of the 26 accessions of 13 species [*L. confertiflora, L. diversifolia, L. involucrata, L. leucocephala, L. pallida, L. cuspidata, L. lanceolata, L. pulverulenta, L. retusa, L. salvadorensis, L. shannonii, L. trichandra*, (*L.* × *spontanea)*]. Chromosome associations at diakinesis and metaphase I were analysed in 14 accessions of *L. diversifolia, L. pallida, L. pulverulenta*, *L. retusa* and *L. trichandra* and in the *L. hybrid?* accession. The diploid species *L. pulverulenta, L. retusa* and *L. trichandra* presented regular chromosome pairing, mostly in bivalents, but 1 or 2 quadrivalents were observed in some cells. In the tetraploid *L. diversifolia* and *L. pallida,* the most common chromosome associations were also bivalents but quadrivalents were found in up to 67% of the cells of *L. pallida*. The *L. hybrid?* accession presented several irregularities, such as univalents and multivalents and chromosome stickiness, reflected in low pollen fertility. Further analysis of more species and accessions and a detailed study of meiotic behaviour are underway.

Correspondence: Dr M.T. Schifino-Wittmann, Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Caixa Postal 776, 91501-970 Porto Alegre, RS Brazil.
E-mail: mtschif@vortex.ufrgs.br

Introduction

The Central American nitrogen-fixing multipurpose tree genus *Leucaena* comprises 22 species, 4 subspecies, 2 varieties and 2 named hybrid taxa (Hughes 1998a; 1998b). Two species, *L. leucocephala* and *L. diversifolia*, are widely grown in the tropical regions of the world where they have great economic and social importance (Hughes 1993).

Despite some limitations, such as poor adaptation to acid soils and susceptibility to the psyllid *Heteropsylla cubana,* the broad ecological adaptation and genetic diversity of *Leucaena* species in their native range indicate that many may be successfully grown in different regions (Shelton and Jones 1995).

Many groups around the world are presently working with leucaena, as shown in the proceedings of the last two *Leucaena* workshops (Shelton *et al*. 1995; 1999). In Brazil, mainly *L. leucocephala* and selected hybrids with *L. diversifolia* are being used or tested for: their potential as forage or ornamental plants; use in culture rotation; control of soil erosion; and use in agroforestry systems generally. In southern Brazil, our group is working with a collection of growing plants of *Leucaena* species from the Oxford Forestry Institution germplasm collection. Our studies range from basic approaches, such as phenology and cytogenetics, to applied approaches, such as growth rate, forage yield and quality and winter-hardiness evaluations. (Kaminski *et al*. 2000; Schifino-Wittmann 2000).

Chromosome studies are difficult in leucaena, mainly because of the small size (*ca.* 1 μm) and high number of chromosomes. Only recently, the chromosome numbers for all species have been determined (Schifino-Wittmann *et al*. 2000; Cardoso *et al*. 2000). Most species are diploid, with 52 or 56 chromosomes and 5 are tetraploid, with 104 or 112 chromosomes in their somatic cells. Meiotic studies are available for only a few species and hybrid populations (Gonzalez *et al*. 1967; Pan and Brewbaker 1988; Freitas *et al*. 1988, 1991).

Basic data such as chromosome number, meiotic behaviour and pollen fertility estimations are important to determine the genetic variability of the species, for germplasm characterisation, to study biodiversity and to help in selection of plants to be included in a plant breeding program. The behaviour of chromosomes at meiosis will affect plant fertility; if meiosis is regular, *e.g.* chromosomes pair and separate regularly, then sterility of the gametes (the pollen grain nuclei and the egg cell) is not to be expected for cytological reasons. Almost all studies of meiosis are performed on pollen mother cells, located in the anthers, due to the high number of cells per anther and the easy accessibility of these cells. The female reproductive cells are much fewer in number and are located inside the ovary, making manipulation difficult. One technique that is simple to perform and cheap and requires little more equipment than a good light microscope, is the estimation of pollen fertility (viability) by staining. The assumption of these estimates is that pollen grains that are potentially fertile (meaning that they have no problem of sterility because of meiotic problems) will take up the stain and the sterile ones will appear empty on the microscope (Figure 1).

Here we report preliminary data on pollen fertility and meiotic behaviour of some *Leucaena* taxa.

Materials and methods

The original seeds came from the Oxford Forestry Institute, UK, *Leucaena* seed collection. A collection of growing plants was established in November 1996 at the Experimental Station of the Universidade Federal do Rio Grande do Sul, Eldorado do Sul township, Rio Grande do Sul, Brazil (30°05′22″ S, 51°39′08″W; 46 m a.s.l.).

Flower buds were collected from each tree individually and were fixed in a mixture of 3 parts of alcohol:1 part of acetic acid. Slides for microscopic analysis were prepared by squashing the anthers in proprionic carmine. Meiotic behaviour was analysed by examining chromosome associations at diakinesis and metaphase I, since cells in other phases of meiosis were rarely found. Pollen viability was estimated by examining uptake of stain in 600 grains per individual (each tree is considered as an individual).

Results and discussion

Pollen fertility was analysed in a total of 110 individuals of 26 accessions of 13 species of

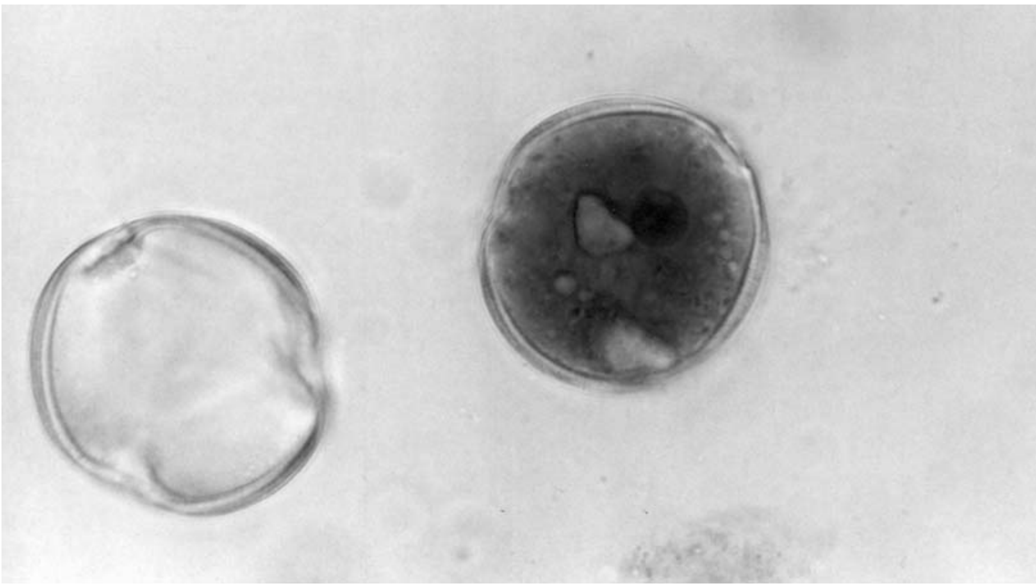

Figure 1. A full, fertile pollen grain, and an empty, sterile one (magnification 400x).

Leucaena [the tetraploid *L. confertiflora, L. diversifolia, L. involucrata, L. leucocephala, L. pallida;* the diploid *L. cuspidata, L. lanceolata, L. pulverulenta, L. retusa, L. salvadorensis, L. shannonii, L. trichandra*; the tetraploid hybrid taxa (*L.* × *spontanea)*] and one tetraploid hybrid of unknown parentage (*L. hybrid ?*) (Table 1). The average pollen fertility of most of the accessions was very high, in excess of 90%. Variation among trees in the same accession was sometimes high as in *L. lanceolata* 44/85, *L. salvadorensis* 17/82 and *L. hybrid?*. The lowest mean pollen fertility (49.1%) among the 26 accessions studied was found in the *L. hybrid ?*.

Chromosome associations at diakinesis and metaphase I were analysed in 14 accessions of 5 species (*L. diversifolia, L. pallida, L. pulverulenta*, *L. retusa*, *L. trichandra)* and in the *L. hybrid?* accession (Table 1). The diploid species *L. pulverulenta* (Figures 2 and 3), *L. retusa* and *L. trichandra* presented regular chromosome pairing, mostly in bivalents, but 1 or 2 quadrivalents were observed in some cells (up to 34% of the cells in *L. retusa* 23/86). In the tetraploid *L. diversifolia* and *L. pallida* the most common chromosome associations were also bivalents but quadrivalents were found in up to 67% of the cells of *L. pallida* 79/92. The *L. hybrid?* 52/87 presented several irregularities, such as univalents and multivalents, as well as chromosome stickiness, which were reflected in low pollen fertility.

The occurrence of quadrivalents in meiosis may be explained by heterozygosity for chromosome translocations or may reflect chromosomal homology due to polyploid origin (Stebbins 1971). The presence of some quadrivalents, as

Table 1. Chromosome associations and pollen fertility in some *Leucaena* species.

Species	Accession[1]	Meiosis		Pollen	
		Chromosome associations[2]	Number of individuals	Mean fertility[3] (%)	Range of variation
L. confertiflora	87/94			87.0 (1)	—
	119/92			69.0 (1)	—
L. diversifolia	105/94	52 II(32), multiple associations (3)	3	73.7 (5)	65.2–78.7
L. involucrata	87/92			90.4 (2)	88.7–92.0
L. leucocephala	133/92			95.2 (9)	81.3–99.2
	147/92			93.8 (2)	93.0–94.7
L. pallida	79/92	56 II(4), 1-2 IV(8)	2	91.5 (12)	84.0–97.7
	78/92	56 II(24), 1-2 IV(10)	2	93.8 (8)	85.8–98.2
L. cuspidata	83/94			97.8 (1)	—
L. lanceolata	43/85			92.5 (3)	87.3–97.7
	44/85			77.2 (2)	58.0–96.3
L. pulverulenta	84/87	28 II (35), 1-2 IV(9)	2	97.9 (10)	96.0–99,0
	83/87	28 II (69), 1-2 IV(11)	3	97.8 (10)	96.8–98.7
	22/86	28 II(8), 1 IV(1)	1	97.9 (3)	97.5–98.7
L. retusa	23/86	26 II(17), 1-2 IV(9)	1	98.8 (1)	—
L. salvadorensis	17/82			71.2 (2)	57.0–85.3
L. shannonii	141/92			96.0 (1)	—
L. trichandra	35/88	26 II(13)	1	92.7 (6)	88.8–97.7
	128/92	26 II(14)	2	98.2 (1)	—
	137/92	26 II(15)	2	82.1 (3)	75.3–89.0
	03/91	26 II(17), 1-2 IV(6)	2	90.6 (14)	77.3–96.5
	138/92	26 II (28)	2	93.9 (2)	92.7–95.5
	53/88	26 II(26), 1-2 IV(3)	2	82.8 (4)	72.8–90.2
	131/92	26 II(23)	3	91.8 (2)	90.8–92.8
L. × *spontanea*	98/94			97.3 (2)	96.3–98.2
L. hybrid ?	52/87	several irregularities	3	49.1 (3)	27.0–82.7

[1]OFI seed collection.
[2]Chromosome associations at diakinesis and metapahase I; II-bivalents; IV-quadrivalents; number of cells in brackets.
[3]Number of individuals in brackets.

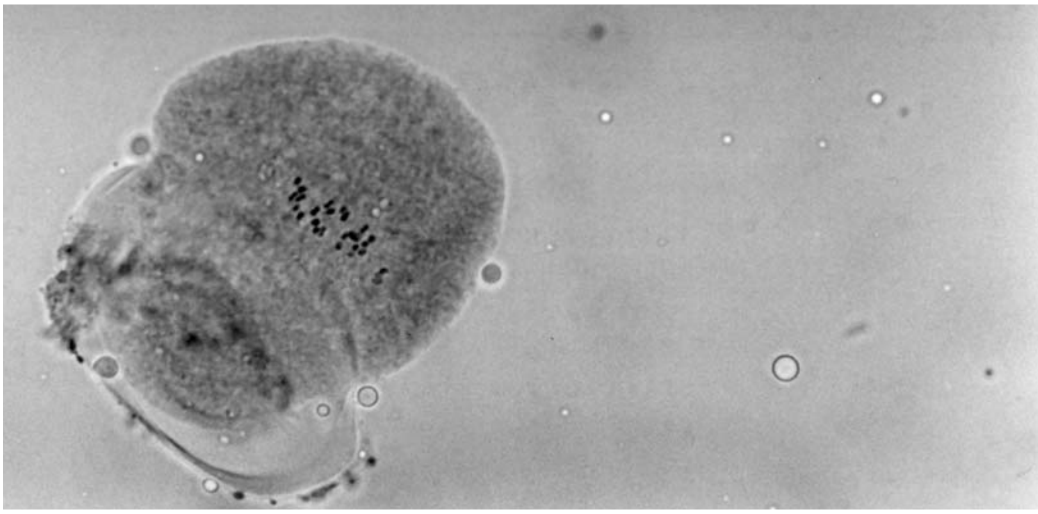

Figure 2. Normal chromosome pairing in *L. pulverulenta* 84/87 (magnification 400x).

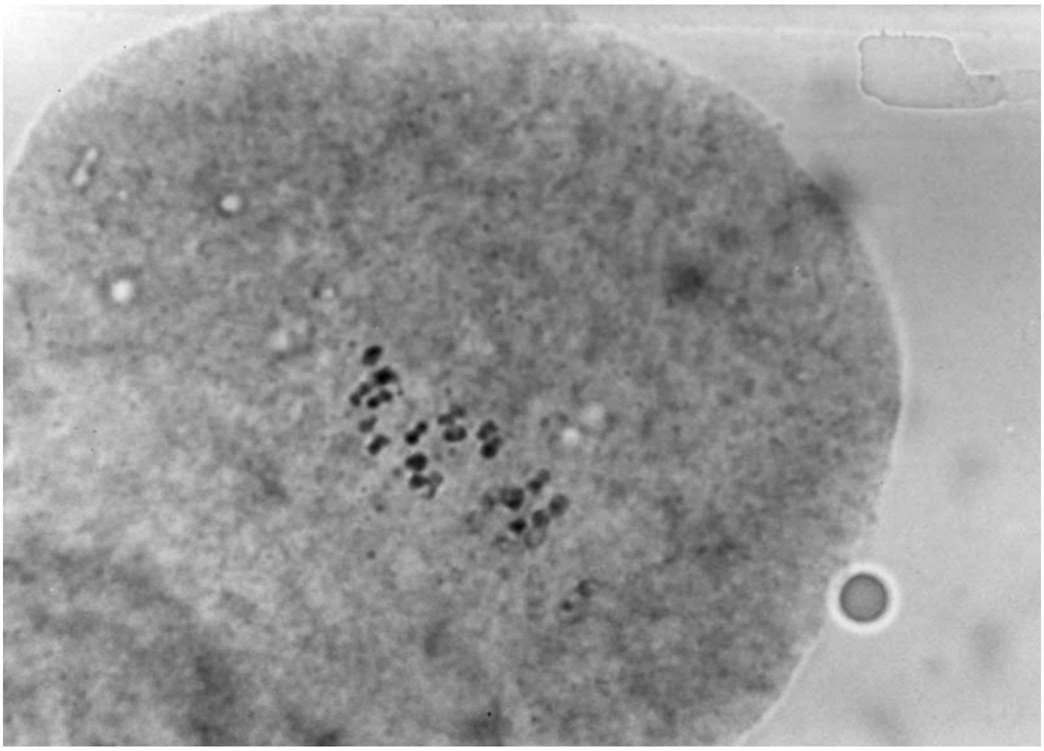

Figure 3. Detail of normal chromosome pairing in *L. pulverulenta* 84/87 (magnification 1000x).

found in *L. diversifolia* and *L. pallida*, may be expected even in allopolyploids originating from different species which share some genetic similarity (segmental allopolyploids). Gonzalez *et al.* (1967), Pan and Brewbaker (1988) and Freitas *et al*. (1988) reported meiotic pairing in bivalents for some diploid and tetraploid *Leucaena* species. Multivalents have been observed in tetraploid hybrids between *L. leucocephala* and *L. diversifolia* (Freitas *et al.* 1991). In the present work, quadrivalents have been found in tetraploid species as well as in the diploid *L. pulverulenta, L. retusa* and *L. trichandra* which could support the paleopolyploid origin of these taxa. However, much more data are necessary to allow reliable conclusions.

Further analysis of more species and accessions and a detailed study of meiotic behaviour are underway and will help to improve knowledge of *Leucaena* cytogenetics as well as providing useful information for plant breeding.

Although these results do not have an immediate impact for leucaena cultivation, they provide a better understanding of these species and are important to help to select fertile plants to be used as male parents in programmed crosses.

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(Received for publication July 6, 2001; accepted November 10, 2001)