Differences between Diploid and Induced Tetraploid *Lotus glaber* Mill. (*Lotus tenuis* Waldst. & Kit.) Plants

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DIFFERENCES BETWEEN DIPLOID AND INDUCED TETRAPLOID *LOTUS GLABER* MILL. (*LOTUS TENUIS* WALDST. & KIT.) PLANTS

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Abstract

Narrowleaf birdsfoot trefoil (*Lotus glaber* Mill.) is an important forage legume which has spread widely and naturalized in the grasslands of the Depressed Pampas of the Province of Buenos Aires, Argentina. *L. glaber* is an alogamic diploid species with a low number of chromosomes (2n=2x= 12). These features show it as a species through which induced autopolyploids by colchicine-doubling of seedlings can be obtained. In this paper, we comparatively analyze fertility and size pollen grain, stomata length, central leaflet length and width and central leaflet ratio width/length in diploid plants and induced-autotetraploid plants of *L. glaber*. The results show that all the characters that were evaluated are useful to differentiate tetraploid plants in this species. Among these, the length of stomata would be the most profitable since it would allow for an early tetraploid level selection.

Keywords: *Lotus glaber* Mill., narrowleaf birdsfoot trefoil, induced autotetraploid, Colchicine doubling, forage legume, stomata

Introduction

Narrowleaf birdsfoot trefoil (*Lotus glaber* Mill.) diploid species (2n= 2x= 12), is an important forage legume that has spread and naturalized in the grasslands of the Depressed Pampas of the Province of Buenos Aires, Argentina. In the last years, a rising interest in this species is due mainly to the fact that it adapts better to drought conditions, flooding and soil restrictions than other perennial forage legumes of high productivity such as alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) in this region (García *et al*., 1994; Ayala Torales, 1997). On the other hand, narrowleaf birdsfoot trefoil has, theoretically, good characteristics to be used as a tetraploid forage (Barufaldi *et al*., 1999). The aim of this study was to comparatively analyze fertility and size of pollen grains, stomata length, central leaflet length and width and central leaflet ratio width/length in diploid plants and induced-autotetraploid plants in *L. glaber*. There is not much background data concerning asexual poliploidization in *L.glaber*. Tome and Johnson's studies, (1945) in *L. glaber* (2x) and induced autotetraploid showed a "gigas" effect in size of pollen, leaves and flowers in the 4x. They also found differences in the shape of pollen between diploid and autotetraploid plants.

Material and Methods

Induction of autotetraploids

In 1998, *L. glaber* seedlings were treated with an aqueous colchicine solution with concentrations varying from 0.1 to 0.4%. Different application techniques (immersion, dripping and immersion-dripping) and different treatment periods (from 3 to 24 h) were used. The treated plants (Co) that showed differential characteristics with respect to the control plants (2x) such as slow initial growth and thicker and bigger leaves were extracted as probable tetraploid material. Later, stomata length of these plants was measured and compared to the controls since this feature in different species shows a high correlation with

the ploidy level. After carrying out a statistical test, 11 genotypes that showed a size equal to or greater than 8 micro units were chosen (the significant value of the t test was 6.93 micro units).

Cytology

In autumn 1998, six genotypes were clonally propagated. From each genotype three to six ramets were obtained. Root tips were pretreated in cold water (4 º C) for 18-24h before fixation in ethanol and glacial acetic acid (3:1). Alcoholic-hydrochloric acid-carmine was used for staining the mitotic chromosomes (Snow, 1963). Chromosome number was determined from counts of three-five well- spread cells per root tip.

Stomatal guard cell measurements

The length of the stomata was determined in the inferior epidermis of central leaflets in leaves that had reached corresponding stage of development. They were taken at random from five stems of the upper half of each plant. Methods of preparing the epidermis were based on those of Clarke (1960) and Speckman *et al*.(1965).

Five measurements were made per central leaflet (25 from each plant) using an ocular-micrometer (magnification 400 x).

Length, width and ratio width/length of central leaflet

The central leaflets were taken at random from the stems of the upper half of each plant. Five measurements were taken for each character per plant.

Estimates of pollen fertility, equatorial and polar pollen grain diameter

Three inflorescences per plant and two buds per inflorescence were taken at random. Pollen fertility was determined by examining at least 200 pollen grains from each inflorescence (600 per genotype) according to Alexander (1969). On the same slide, pollen size was measured in 10 grains per inflorescence (30 grains/plant).

Statistical analysis

A Student's t test was used to locate differences between means.

Results and Discussion

Highly significant differences ($P<0.001$) were detected for most of the studied variables, with the exception of the width/length ratio character that only showed significant differences ($P<0.05$) (Table 1).

Regarding pollen shape, the autotetraploid is triangular or elliptical while the diploid is elliptical. Results concerning size and shape of pollen agree with those reported by Tome and Johnson (1945). As expected, fertility of pollen in individuals 4x was lower than in diploids due mainly to meiotic irregularities that lead to the formation of chromosomically unbalanced pollen. Nonetheless, this infertility does not compromise the production of enough seeds. With respect to stomata size, results show that it is an excellent characteristic to select colchicine treated autotetraploid plants during the vegetative stage. Since the colchicine technique may have produced stems with different ploidy level within the same plant, tetraploid stems may be detected by determining stomata length in Co plants. The width, length and width/length ratio of the central leaflet appear as traits to be taken into account because they show differences between the two ploidy levels, i.e. the central leaflet of plants 4x are bigger than those in plants 2x.

All the studied characters showed to be useful to differentiate tetraploid plants in this species. The length of stomata is probably the most advantageous character because it allows for an early selection of the level of tetraploid ploidy.

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Table 1 - Morphological measurements (mean ± SE), differences between means and *t* test results for the diploids (2x) and tetraploid (4x) plants.

TRAIT	LEVEL PLOIDY	MEANS	MEANS DIFFERENCES
POLLEN GRAIN SIZE:			
Equatorial diameter of pollen (MU)	2 x	3.18 ± 0.02	0.72***
	4 x	3.90 ± 0.06	
Polar diameter of pollen (MU)	2 x	4.04 ± 0.01	0.49***
	4 x	4.53 ± 0.05	
Pollen fertility (%)	2 x	88.91± 1.73	
	4 x	73.82 ± 2.17	- 15.09***
Stomata length (mu)	2 x	6.05 ± 0.06	
	4 x	8.13 ± 0.22	2.08***
Central leaflet width (cm)	2 x	0.59 ± 0.01	
	4 x	0.77 ± 0.03	0.18***
Central leaflet length (cm)	2 x	1.31± 0.03	
	4 x	1.53 ± 0.08	0.22 **
Central leaflet width/length ratio (cm)	2 x	0.46 ± 0.01	
	4 x	0.50 ± 0.01	0.04 *

*** P< 0.001 **P < 0.01 *P < 0.05

MU: Micro units