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Effect of induced polyploidy on some characteristics of seed production and quality in *Lotus glaber* Mill.

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Introduction

In 1998 in the Faculty of Agronomy in Azul, UNCPBA, a research project was initiated aimed at the production of artificially induced tetraploids in *L. glaber*, using colchicine as the polyploidising antimitotic agent. In this way, tetraploid germplasm was obtained that derived from the chromosomal duplication of seedlings belonging to four naturalised populations found in the Province of Buenos Aires (Barufaldi *et al*., 2000). Chromosomal counts have been carried out in this tetraploid population, and they continue to be made in order to verify the stability of the ploidy level of different random-crossing cycles derived from it.

The induced polyploids have been used in some forage species such as *Dactylis glomerata* ssp. *lusitanica*, *Lolium perenne*, *Lolium multiflorum*, *Trifolium pratense*, *Trifolium hybridum*, *Lotus pedunculatus* and *Secale cereale*, with the purpose of obtaining improved autotetraploid cultivars or to generate interspecific hybrids. In general, the induced autotetraploids in ryegrass, rye, clovers and *Lotus pedunculatus* show better establishment, higher *in vitro* digestibility and forage production, and better performance in response to such adverse factors as disease, frost and drought, than the corresponding diploids (Evans, 1955; Ahloowalia, 1971; Armstrong, 1974; Simonsen, 1976; Luchini, 1979; Dennis, 1980; Pérez, 1980; Herrera *et al*., 1988; Carámbula *et al*., 1994).

However, gamete fertility of induced autotetraploid plants is reduced compared with that of the diploid forms. The cause of this decline and, therefore, of the reduction in seed production, is mainly due to meiotic irregularities that lead to the formation of chromosomally unbalanced gametes. These gametes are commonly sterile or, on those occasions where their fertility permits zygote formation, produce viable abnormal zygotes that give origin to aneuploids of reduced fertility. In the induced tetraploid forms of species like red clover and rye, seed yields increased after the first generations, due to a both natural and artificial selection. Nevertheless, seed production was considerably lower than in similar diploid forms (Dennis, 1980; Funes *et al*., 2001).

The present work analyses the effect that chromosomal duplication in *Lotus glaber* produces on the following characteristics related to seed production and quality:

- Number of flowers per umbel (size of the umbel).

- Number of pods per umbel.
- Number of seeds per pod.
- 1000 seed weight.
- Percentage of normal seedlings (germinative power)
- Percentage of hard seeds.
- Percentage of ungerminated soft seeds.
- Percentage of abnormal seedlings.
- Percentage of dead seeds.

Material and methods

The research was carried out in the experimental field of the Faculty of Agronomy of Azul, using spaced planted. The evaluations were carried out on diploid plants of a naturalised population denominated "Azul" and plants of the tetraploid germplasm under study.

Determination of the number of flowers per umbel and the number of pods per umbel.
Starting from 20th January 2002, samples were taken at random:
- 284 and 254 umbels in the tetraploid and the diploid populations, respectively, to determine the number of flowers per umbel.
- The same number of umbels with mature pods were harvested at random the following week for the pod count.

Determination of the number of seeds per pod, 1000 seed weight and seed quality.
The parameters of seed quality were evaluated in both cytotypes on the samples harvested previously, in the following way:
- 20 pods were taken at random from each population and the number of seeds per pod counted.
- 1000 seed weight was determined taking the average of eight repetitions of 100 seeds each, according to the rules of the International Seed Testing Association (ISTA, 2002).

Samples were taken at random for the determination of seed quality for each cytotype. After mechanical scarification, 100 seeds per tray were laid out with 4 repetitions (400 seeds) and were pre-chilled during 7 days at 8º C. Later on, they were located in a germination camera during 12 days at 20º C (according to ISTA norms). The percentage of normal and abnormal seedlings, as well as the percentage of hard, fresh and dead seeds, were recorded at the end of this period.

Statistical Analysis
Pearson chi-square analysis was performed for testing the independence between the number of flowers per umbel and cytotype, as well as between the number of pods per umbel and cytotype. A t-test was carried out to compare the means of the variables: number of seeds per pod and the characteristics of seed quality in both cytotypes.

Results and discussion

The existence of a dependence relationship between the number of flowers per umbel and the diploid and tetraploid cytotypes was detected by means of the chi-square test.

The highest frequency for both cytotypes corresponded to 5 flowers per umbel (Table 1). However, the diploid cytotype showed a distribution closer to normal than did the tetraploid cytotype, with the latter presenting a higher concentration of umbels with low numbers of flowers. Also, 2.4% of the umbels of the diploid had 8 -10 flowers, while the tetraploid did not produce umbels that had more than 7 flowers.

Table 1. Number of flowers per umbel in the tetraploid and diploid cytotypes of *L. glaber* Mill.

Cytotype	Number of flowers per umbel										
	1	2	3	4	5	6	7	8	9	10	Total
Tetraploid											
Absolute frequency	6	23	45	86	90	31	3	0	0	0	284
Relative frequency (%)	2.1	8.1	15.8	30.3	31.7	10.9	1.1	-	-	-	100
Accumulated relative frequency (%)	2.1	10.2	26.0	56.3	88.0	98.9	100				
Diploid											
Absolute frequency	3	12	27	54	78	59	15	3	2	1	254
Relative frequency (%)	1.2	4.7	10.6	21.3	30.7	23.2	5.9	1.2	0.8	0.4	100
Accumulated relative frequency (%)	1.2	5.9	16.5	37.8	68.5	91.7	97.6	98.8	99.6	100	

The chi-square test confirmed the existence of a dependence relationship between the number of pods per umbel and the diploide and tetraploide cytotypes. The highest frequency in the number of pods corresponded to 1 pod per umbel in the tetraploid, while it corresponded to 4 pods in the diploid (Table 2). The diploid cytotype concentrated 50% of the umbels with 4 and 5 pods, whereas 80% of the umbels had between 1 and 2 pods in the tetraploid. From the analysis of these results it could be concluded that, although a reduction existed in the number of pods compared to the number of flowers per umbel in both cytotypes, this reduction was significantly higher in the tetraploid than in the diploid.

Table 2. Number of pods per umbel in the tetraploid and diploid cytotypes of *L. glaber* Mill.

Cytotype	*Number of pods per umbel*							
	1	2	3	4	5	6	7	Total
Tetraploid								
Absolute frequency	132	95	40	9	7	1	0	284
Relative frequency (%)	46.5	33.5	14	3.1	2.5	0.4	-	100
Accumulated relative frequency (%)	46.5	80	94	97.1	99.6	100		
Diploid								
Absolute frequency	16	37	46	68	58	28	1	254
Relative frequency (%)	6.3	14.6	18.1	26.8	22.8	11	0.4	100
Acumulated relative frequency (%)	6.3	20.9	39	65.8	88.6	99.6	100	

With regard to the number of seeds per pod and 1000 seed weight, significant differences were detected (P <0.001) between the cytotypes. The tetraploid produced half the seeds per pod with approximately double the seed weight, compared with the diploid cytotype (Table 3).

Table 3. Comparison of characters between the diploid and tetraploid cytotype of *L. glaber* Mill.

Character	*Diploid Cytotype*§	*Tetraploid Cytotype*§	*Differences between means*
Number of seeds per pod	17 ± 7.25	8 ± 4.6	***
1000 seed weight (g)	0.94 ± 0.06	1.60 ± 0.02	***
Normal seedlings (%)	76 ± 2.29	74 ± 1.70	ns
Hard seeds (%)	13 ± 2.54	11 ± 0.96	ns
Soft seeds (%)	5 ± 1.03	3 ± 2.22	ns
Abnormal seedlings (%)	2 ± 0.92	3 ± 1.15	ns
Dead seedlings (%)	4 ± 0.75	9 ± 0.96	***

§ Mean values ± standard deviation
ns: differences non significant P <0.05
*** significant differences P <0.001

With reference to the characters that define the quality of the seeds: for the percentage of normal and abnormal seedlings, and for the percentage of hard and soft seeds, significant differences were not observed; however, for the percentage of dead seeds, a significant difference was obtained (Table 3), with the tetraploid population presenting a higher percentage of dead seeds (9%) compared with the diploid population (4%). The high percentage of dead seeds of the germplasm under study was also obtained when it was compared with the diploid cultivar Chajá Tresur (Barufaldi *et a*l., 2003). Therefore, an effect of the induced polyploidy would appear to be an increase in the number of non-viable seeds produced. This suggests the need for future study of the causes that affect seed viability, by means of the topographical test with tetrazolium.

Conclusions

Among the components of seed production analysed in this work, it was observed that polyploidy had a negative effect on the number of flowers per umbel, on the number of pods per umbel and on the number of seeds per pod, which would appear to affect seed production markedly.

Due to the induced polyploidy, the seed weight was superior in the tetraploid cytotype. Detrimental effects were not detected in the majority of characteristics related to seed quality, such as the percentage of normal and abnormal seedlings, and the percentage of hard and soft seeds, although seed viability was affected.

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