Research on 2n pollen production in *Lotus tenuis* at I.M.G.V of Perugia University.

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Introduction

Lotus tenuis (2n=2x=12) can be crossed to *L. corniculatus* (2n=2x=24) in seminatural conditions; the cross results in a high fertile, tetraploid progeny morphologically resembling birdsfoot trefoil. This suggests that the former species should have contributed to the *L. corniculatus* gene pool through unreduced (2n) gametes (Negri and Veronesi, 1989). Screening the frequency of big pollen production in twelve natural populations of *L. tenuis* (Negri, 1992), several 2n gamete producing genotypes were found (Table 1). Crosses among 2x (*L. tenuis*) x 4x (*L. corniculatus*) detected a 2n female gamete producer (1770/16).

Table 1: Interesting populations, frequency of plants producing more than 1% of big pollen in initial population, plants showing the highest percentage of big pollen and their percentage of big pollen production.

Populations	Frequency of big pollen producing plants in initial pop. %	Interesting plants	% of big pollen found
Abbadia S. Salvatore	5%	1321/8	6.1
"	"	1321/8-23	17.4
"	"	1321/8-28	8.3
"	"	1321/8-44	10.1
"	"	1321/46	12.5
Roseto degli Abruzzi	9%	1170/73	10.0
Ferro Monte Urano	11%	1322/147	100.0
Ancona	2%	0937/21	18.3
Monte Franco	1%	1160/51-35	8.4

Cytological analysis

Cytological analysis revealed that different mechanisms are involved in big pollen production. In two mutants (1321/8 and 1321/46) parallel and tripolar spindles in metaphases II were observed. As a consequence of parallel spindles, at the end of telophases II, being the four sets of chromosomes localized in one plane, dyads of 2n microspores were obtained. As for tripolar spindles, after telophase II, two cleavage furrows was formed and a triad of two n and one 2n microspores were obtained (Negri et al., 1994).

Use of *L. tenuis* mutants in trasferring useful character to *L. corniculatus.*

Since both the above mentioned mechanisms produce first division restitution (FDR) type microspores, the examined genotypes are presently used in transferring powdery mildew resistance and ability to vegetate during the winter from *L. tenuis* to *L. corniculatus*. In a first experiment 23 plants of the 1321/8 genotype and 9 plants of the 1321/46 genotype were planted under two isolation cages with honey bees with a male sterile clone of *L. corniculatus* (1766/81) for interspecific crosses. Seeds from the male sterile plants were harvested separately; 9 mature tetraploids plants (2 from 1766/81 x 1321/8 and 7 from 1766/81 x 1321/46), morphologically resembling *L. corniculatus*, were obtained and cloned; parent plants were also cloned. For powdery mildew resistance evaluation, each plant within a clone was scored from 1=maximum resistance to 9=minimum resistance (susceptibility) on August 8th, 1994 following artificial inoculation as described in Veronesi et al. (1986). For winter growth evaluation, plants were scored from 1=minimum to 9=maximum growth on February the 2nd, 1994. Clonal evaluation showed that only progenies from 1766/81 x 1321/46 have intermediate characters (Table 2); among them two genotypes showed high resistance to powdery mildew infection (x= 2.2 and 1.8, respectively) and good winter growth (x= 6.1 and 7.3, respectively).

Table 2: Average values relative to clonal evaluation, of powdery mildew susceptibility (1=minimum, 9=maximum, August, 1994) and winter growth (1=minimum, 9=maximum, February, 1994) in *L. corniculatus* female parent, *L. tenuis* pollen parents and their progenies, (in brackets the number of clones evaluated).

	Parents			Progenies	
	1766/81	1321/46	1321/8	1766/81x 1321/46 (7)	1766/81x 1321/8 (2)
Powdery mildew susceptibility	8.0	1.0	1.1	5.3	8.2
Winter growth	4.0	6.7	7.8	5.1	2.2

Selection for increasing 2n gamete production

Pair hand crosses under isolation cages among nine 2n gamete producing genotypes were conducted in 1993, in order to increase frequency of 2n gametes production. We were not able to obtain an experimental population with increased frequency of 2n gamete producing genotypes since only 7 plants on 361 observed (2%), resulting from the crosses 1321/8-23 x 1321/8-28, 1321/8-28 x 1321/8-44 and 1170/73 x 1770/16, produced big pollen; but it is interesting to note that these plants produced a much higher percentage of big pollen (over 75%) than their parents (Table 1). Besides, 7 plants were found to be male sterile probably as a consequence of accumulation of different mutations at different steps of the meiotic process. Cytological analysis of mutants found is in progress. Detection of 2n gametes producers might be influenced by variable expressivity in relation to environment. Some clones of 2n pollen producers are actually growing under two controlled environments (20 hs photoperiod and 20°C and 30°C, respectively), to verify the effect of temperature on 2n gamete production.

References

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