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An Investigation into the Mechanism for Reduced Seed Yield in *Lotus corniculatus*

S. Dobrofsky and W.F. Grant
Genetics Laboratory, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec (Canada)

Summary. A study of seed position in the pod of *Lotus corniculatus* L. cv. 'Mirabel' (Fabaceae) suggested that reduced seed set after self-pollination is not due to an inability of the self-pollen tube to reach the end of the ovary. As in other cultivars, it has been demonstrated that cultivar 'Mirabel' produced less seed per pod and shorter pods after self- than after cross-pollination. No differences were noted for percent germination of seeds produced by both types of pollination indicating that the number of seeds per pod is a reliable index of the ultimate productive potential of the pollination.

Key words: *Lotus corniculatus* – Birdsfoot trefoil – Seed set – Cross-pollination – Self-pollination – Self-discrimination

Introduction

Lotus corniculatus L. is a tetraploid (2n = 4x = 24) legume used for forage in many temperate regions of the world. Because of its complex genetic nature, it is a difficult species to use in conventional breeding programs (Dawson 1941). It is largely an outcrossing species and produces fewer seed per pod and smaller pods when self-pollinated than when cross-pollinated (Giles 1949; Brandenburg 1961; Wojciechowska 1963; Seaney 1964; Schaaf 1977).

No differences in pollen-tube growth rate between self- and cross-pollen tubes have been observed at the level of the stigma or the style (Giles 1949; Spiss 1969; Spiss and Paolillo 1969), therefore, diminished seed set in self-pollinated florets may be partially caused by reduced growth rate and shorter ultimate length of self-pollen tubes resulting in failure to reach the pedicellar end of the ovary. If this is occurring one would expect more aborted ovules and less viable seed at the pedicellar end of the mature pod than at the stylar end. This study investigates seed position in mature pods in order to compare indirectly the ultimate pollen-tube length after self- and cross-pollination.

Material and Methods

Plants of *L. corniculatus* cv. 'Mirabel', were grown in December 1976 from a single 1976 accession obtained from the A.E. Lods Research Station, Macdonald College. Cuttings were propagated when the plants were five months old. Mature pods were collected from six randomly selected clones (and parental plants) designated P, PY, PB, OB, Y and O. A temperature of 20° ± 1°C, a relative humidity of 75 ± 5%, and a day: night cycle of 18:6 hours were imposed to optimize flowering.

Florets were emasculated at the 'pointed bud' stage (Giles 1949) prior to cross-pollination. Clones P and PY were cross-pollinated with pollen of clone OB, clones OB and PB with pollen of clone P, clone Y with pollen of clone O and clone O with pollen of clone Y. Since some clones proved much more sensitive to emasculation and florets dropped prior to seed setting, only results from clones P and PY provided sufficient data to be treated statistically. To ascertain that self-discrimination was occurring in this cultivar, the number of seeds per pod and pod lengths were tabulated. Only mature pods, that is, with pods and sepals brown or straw colored, were used. Mature seeds were germinated on filter paper in petri dishes to compare seed viability in pods from self- and cross-pollinations.

Two measurements were used to indirectly compare ultimate pollen-tube length after self- and cross-pollination: a) the distance from the pedicellar end of the pod to the mature seed nearest that end as a percentage of total pod length (pedicellar seed position) and b) the number of mature seeds in the pedicellar vs. the stylar half of the pods (dividing the pods on the basis of ovule position rather than pod length). Seed number was analyzed using the Kolmogorov-Smirinov test (Siegel 1956), and for all clones the median and values one quintile above and below the median were computed. Pod length and pedicellar seed position were analyzed using the Student's t-test (Steel and Torrie 1960). Mean and standard deviation were computed for all clones.

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Results

1 Self-discrimination

Number of Seeds per Pod

The median and values 20% above and below the median for number of seeds per pod for self- and cross-pollinations in all six clones are given in Table 1. The medians for the self-pollinated samples were about half those for the cross-pollinated samples of the same clone. In none of the clones did the 20% limits above and below the medians overlap. For clones P and PY, there was a highly significant difference at the 0.0001 level. The maximum number of seeds was larger in the cross-pollinated sample than in the self-pollinated sample for all clones. However, there was considerable variation in the distribution of numbers of seeds per pod between the six clones. The largest seed set resulting from a cross-pollination was 39 seeds out of 42 ovules, and the largest seed set resulting from a self-pollination was 18 seeds out of 38 ovules. No pods contained more than 60 ovules (over 700 pods were examined) and most contained approximately 40 ovules.

Pod Lengths

Table 2 contains pod lengths and standard deviations resulting from self- and cross-pollinations from each of the six clones. In general, pods resulting from cross-pollinations were longer, but there was wide variation between the clones. The difference in pod length between self- and cross-pollinations for clones P and PY was significant at the 0.0001 level, whether variances were assumed to be equal or unequal. The F-test performed on the same material indicated that the variances were significantly different at the 0.04 level for clone P and at the 0.005 level for clone PY.

Mature Seed Viability

Percentage germination values for seeds resulting from self- vs. cross-pollinations did not differ significantly in any of the clones.

2 Mature Seed Position

Pedicellar Seed Position

In Table 3 the mean pedicellar seed position and the standard deviation, respectively, are given for each of the six clones. Pedicellar seed position compared for self- and cross-pollinations was significantly different at the 0.001 level for clone P and at the 0.0018 level for clone PY assuming unequal variances, since F-tests to test the null hypothesis that the variances of the two samples for each clone were equal was rejected at the 0.01 level for clone P and at the 0.0001 level for clone PY. Except for clones OB and Y, more space remained at the distal end of the pod after self-pollination than after cross-pollination. This was especially obvious in clones P and PY where the pedicellar seed position values for pods resulting from self-pollinations were almost double those resulting from cross-pollinations.

Comparison of Number of Seeds in Pedicellar and Stylar Halves of the Pod

The number of seeds in pedicellar and stylar halves of the pods after self- or cross-pollinations tended to be equal. No significant differences were observed.

Discussion and Conclusion

1 Self-discrimination

Self-discrimination has been demonstrated in *Lotus corniculatus* cv. 'Mirabel', since cross-pollinated florets were shown to produce significantly more seeds per pod and longer pods than self-pollinated florets. The median number of seeds after cross-pollination was approximately double that after self-pollination. Reduced seed set and pod size after self-pollination have been demonstrated for different cultivars of this species as noted in the Introduction. It is obvious that the self-incompatibility and/or the self-sterility mechanism is incomplete since production of self seed is not a rare event, confirming the observations of Wojciechowska (1963).

To determine whether seeds produced from self-pollinations were of lower viability than those produced from cross-pollinations, the percent germination was recorded for both seed types. No significant differences were observed, thus seed yield values alone are a realistic assessment of the productivity of both types of pollination.

This study does not distinguish between self-incompatibility and self-sterility since aborted unfertilized ovules were not discriminated from seeds that died shortly after fertilization.

2 Mature Seed Position

The pedicellar seed position, that is, the distance from the pedicellar end of the mature pod to the nearest mature

Table 1. Comparisons of number of seeds per pod after cross- and self-pollinations in all six clones. Median values are given, plus one quintile above and below the median. Range and sample size are indicated

	Self-pollination				Cross-pollination			
Clone	Median	± 20%	Range	Sample size	Median ± 20%		Range	Sample size
P	3	3-5	2-8	24	11	9-14	2-26	50
PY	6	4-7	1-14	94	11	8-16	2-25	58
OB	4	3-6	1-11	45	13	9-13	1-19	7
O	8	6-9	1-18	124	17	12-17	7-28	11
Y	4	3-4	1-7	14	7	5-7	1-11	8
PB	6	5-8	3-10	9	20	17-35	6-39	11

Table 2. Comparisons of pod lengths after cross- and self-pollination in all six clones. Mean, standard deviation, and sample size are indicated. Mean and standard deviation are in mm

	Self-pollination			Cross-pollination		
Clone	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size
P	18.1	3.1	24	25.9	4.63	50
PY	17.4	3.53	95	22.7	4.87	58
OB	17.3	4.06	45	20.0	6.35	7
O	22.0	7.05	109	27.5	7.03	11
PB	19.1	2.85	11	28.1	5.94	8
Y	13.7	2.57	14	19.4	4.14	8

Table 3. Comparisons of the distance of the most distal seed, to the distal end of the pod (relative to the style), expressed as a percent of the total length of the pod, after cross- and self-pollination in all six clones. Mean, standard deviation, and sample size are indicated. Measurements in mm

	Self-pollination			Cross-pollination		
Clone	Mean	Standard deviation	Sample size	Mean	Standard deviation	Sample size
P	22.8	15.5	24	10.2	10.0	46
PY	12.6	13.6	88	6.9	7.7	52
OB	19.6	16.3	44	23.3	11.0	7
O	12.1	12.6	108	10.6	10.0	10
PB	12.0	11.4	10	8.7	13.0	8
Y	17.5	17.1	14	18.6	13.7	6

seed, expressed as a percentage of total pod length, was significantly greater for pods that had been cross-pollinated than for pods that had been self-pollinated for pollinations within the same clone. This suggests two possible explanations: 1) that self-pollen tubes tend not to travel as far into the ovary as cross-pollen tubes resulting in lower seed set, or 2) that because self-pods tend to contain fewer mature seeds, the distances between individual mature seeds anywhere in the pod tend to be larger, including the distance from the pedicellar end of the pod to the nearest mature seed.

To distinguish between these two possibilities the position of successfully vs. unsuccessfully fertilized or unfertilized ovules were noted by dividing the mature pods into pedicellar and stylar halves on the basis of original ovule position and counting the number of mature seeds in both

halves. No significant difference in numbers of mature seeds was found between the two halves of pods of both self- and cross-pollinated florets, thus the difference in the distances from the pedicellar end of the pod to the nearest seed, between self- and cross-pollinations, is probably due to an overall reduction in seed set rather than to a shorter ultimate pollen-tube length after an incompatible pollination. Perhaps self-pollen tubes grow as far into the ovary as cross-pollen tubes, but fail to fertilize ovules as efficiently throughout the length of the pod.

Further studies are required to determine the exact mechanism of self-incompatibility or self-sterility in *Lotus corniculatus*. Since the ovary is most likely the region where discrimination between self- and cross-pollination occurs, it is suggested that an analysis of biochemical discrimination of the pollen tubes by the ovary, rather than of pollen-tube length or growth rates, may provide solutions to this problem (Dobrofsky and Grant 1980).

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Miss S. Dobrofsky
Prof. Dr. W.F. Grant
Genetics Laboratory
Macdonald Campus of McGill University
Ste. Anne de Bellevue
Quebec H9X 1CO (Canada)