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Further studies with interspecific hybridization among mediterranean/African lupin species

N. N. Roy and J. S. Gladstones

Department of Agriculture, South Perth, Western Australia 6151, Australia

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Summary. Interspecific hybridization has a role to play in the domestication of wild species through the introgression of desirable genes from related domesticated species. Almost complete genetic isolation among the Mediterranean-African rough-seeded lupin species has hitherto ruled out introgression within that group. Recent work in Western Australia with *L. atlanticus, L. cosentinii, L. pilosus* and *L. digitatus* has nevertheless suggested that hybrid sterility may be overcome if specially selected lines of each are used in crossing. We have now selected F_2-F_5 plants and backcrosses (Bc_1, Bc_2) from *L. atlanticus/L. cosentinii*, some with improvement in seed fertility combined with domestication genes from either species. Relatively better F_1-F_2 plant fertility in subsequent crosses of *L. atlanticus/L. digitatus* than in *L. atlanticus/L. cosentinii* or *L. digitatus/L. cosentinii* indicate closer relationship between *L. atlanticus* and *L. digitatus* than in the other cross combinations. Use of embryo culture may help to overcome *L. pilosus/L. atlanticus* F_1 plant sterility. Some of the interspecific selections could act as genetic bridges between *L. cosentinii* and *L. pilosus.*

Key words: Wild lupin species – Interspecific cross – Hybrid sterility – Embryo culture – Genetic bridge

Introduction

Among the rough-seeded group of Mediterranean-African wild lupins (Gladstones 1974), *L. pilosus, L. atlanticus* and *L. cosentinii* have evident potential for domestication. Limited trials and nursery observations, together with their natural distributions, have suggested that the first two tolerate low temperatures and heavy, possibly wet soils, while *L. cosentinii* grows on very poor sands but needs mild temperatures. Two other species of the group, *L. digitatus* and *L. palaestinus,* are desert or semi-desert plants and may be able to contribute valuable hardiness genes. All the species have large to very large seeds, while "domestication" genes for low alkaloid content, non-shattering pods, earliness, white flower and seed colour as a marker, and possibly soft-seededness, are available in *L. cosentinii* and, in part, *L. atlanticus.* Transfer of these genes to the other species would be desirable as a possible alternative to separate mutation breeding in each species.

However, genetic isolation has hitherto precluded successful introgression within the group (Gladstones 1974, 1984; Williams 1984). Apart from strong similarities of morphology, these species have genetic compatibility to the extent that certain parental combinations can give viable seeds and vigorous F_1 plants on crossing (Roy and Gladstones 1985). But in no past case has a fertile F_1 plant been reported, other than between *L. palaestinus* and *L. pilosus* (Pazy et al. 1981), which have the same chromosome number and showed partial fertility when *L. palaestinus* was used as the mother plant. Recent work in Western Australia by Roy and Gladstones (1985), using selected lines of *L. atlanticus* and *L. cosentinii* in crosses, has nevertheless shown some potential for overcoming hybrid sterility between members of the group with different chromosome numbers. Further progress with this material is reported here, together with initial results from other crossing combinations within the group.

Materials and methods

Five *Lupinus* species were used in the hybridization programme. Their more important characteristics are listed below:

L. palaestinus (2n=42): Spreading habit, slow growing, a possible source of drought resistance, wild.
L. pilosus (2n=42): Very large pods and seeds, good erect plant type, early vigour, slow maturation, wild.
L. atlanticus (2n=38): High yield, many pod-bearing branches, slow early growth, late maturity but rapid maturation, all domestication genes available in mutants except soft-seededness and reduced pod-shattering.
L. digitatus (2n=36): Drought resistance, many pod-bearing banches, wild.
L. cosentinii (2n=32): Moderate yield, early vigour and maturity, all domestication genes combined in cv. Erregulla-s.

Details of the crosses made earlier (1982) between *L. atlanticus* and *L. cosentinii*, and of F_1–F_2 plants and the first backcrosses (Bc_1F_1), have already been reported (Roy and Gladstones 1985). Further studies were made during 1983–1986 with the F_3–F_5 progenies of three surviving crosses, and with their backcrosses (Bc_1F_2, Bc_1F_3 and Bc_2F_2) as shown in Table 2.

In 1985 we attempted a large number of interspecific crosses involving 10 selected lines of *L. pilosus* (mainly as female), a number of *L. atlanticus* mutant lines with low alkaloid content and/or white flowers and seeds, and some *L. atlanticus* wild selections with large, white seeds and their crosses with mutant types. Also used for crossing were two crop-type selections of *L. cosentinii:* cv. Erregulla and Erregulla soft-seeded selection (Erregulla-s). Five lines of *L. palaestinus* and one of *L. digitatus* were used as parents in some crosses. All crossing was done in the greenhouse.

A list of crosses from which some F_1 seeds were obtained is given in Table 1. Three cross combinations, *L. pilosus/L. palaestinus, L. digitatus/L. pilosus* and *L. atlanticus/L. palaestinus,* were attempted in at least 3 to 5 combinations of genotypes, but did not set any F_1 seeds.

In the 1985/1986 summer, all the F_1 seeds then available were sown in a controlled-temperature greenhouse; the remainder were mostly grown in the winter of 1986. The *L. atlanticus/L. cosentinii* crosses and backcrosses were grown both in the field (winter) and greenhouse (summer and winter). Whenever any cross appeared to be failing due to F_1 sterility, attempts were always made to rescue it by backcrossing to desirable parents.

Results

L. atlanticus/L. cosentinii

As reported earlier (Roy and Gladstones 1985), the successful crosses used *L. atlanticus* wild selections as female parents in crosses with Erregulla-s, a soft-seeded selection from *L. cosentinii* cv. Erregulla with all the domestication genes either artificially induced (low alkaloid, white flower/seed, early flowering) or naturally occurring (reduced pod-shattering, soft-seededness).

Of the three successful crosses 82E13, 82E30 and 82E75, 82E13 was found to be F_2-plant sterile. It was perpetuated through backcrosses to *L. atlanticus* (mutant) and *L. cosentinii* (Erregulla-s). Even through backcrossing it was not always possible to restore fertility; one of the backcrosses, 82E13-1/*L. cosentinii* (Erregulla-s), gave 8 Bc_1F_2 seeds, but the other backcrossed plants were sterile (Table 2).

The other two *L. atlanticus/L. cosentinii* crosses, 82E30 and 82E75, gave F_2–F_4 progenies which were poor in vitality or seed fertility under both field and greenhouse conditions. The best of the surviving plants gave some seeds (F_3: 3–22; F_4: 24–30). Ultimately 82E75 was lost after the F_4 generation, through poor growth or seed sterility. Using F_2 plants from this cross as female parents, a number of backcrossed seeds were obtained, but these gave sterile plants. However one F_3 plant, when used as pollen parent, gave at least eight backcrosses to mutant and wild *L. atlanticus* which resulted in plants with improved growth, vigour and seed fertility (Table 2). Some of these backcross progenies segregated for domestication genes.

Table 1. List of successful interspecific crosses (1985)

Inter-specific cross	No. of successful crosses	No. of F_1 seeds/ cross
L. pilosus/L. atlanticus	27	1–10
L. pilosus/L. cosentinii	3	1
L. cosentinii/L. digitatus	3	3–12
L. atlanticus/L. digitatus	1	2

Table 2. Seed fertility in three *L. atlanticus/L. cosentinii* (Eregulla-s) crosses and their backcrosses grown in the glasshouse

Crosses	No. of seeds and generation					
	F_3	F_4	F_5	Bc_1F_2	Bc_1F_3	Bc_2F_2
82E 13-1 (F_2)	Nil	–	–	–	–	–
82E 13-1 (F_2)/Atl (M)	–	–	–	Nil (2)[a]	–	–
82E 13-1 (F_2)/Cos (Erg)	–	–	–	8 (1)[a]	–	–
82E 75-2 (F_2)	22	24	Nil	–	–	–
82E 75-2 (F_2)/Atl (M & W)	–	–	–	Nil (5)	–	–
82E 75-2 (F_2)/Cos (Erg)	–	–	–	Nil (1)	–	–
82E 75-2 (F_3)/Atl (M)	–	–	–	22 (1)	67	–
Atl (M & W)/82E 75-2 (F_3)	–	–	–	70–322 (8)	3–129	–
82E 30-1 (F_2)	3	30	255	–	–	–
82E 30-1 (F_2)/Atl (M)	–	–	–	210 (1)	143	1
82E 30-1 (F_2)/Atl (W)	–	–	–	47 (1)	44–167	197
82E 30-1 (F_2)/Cos (Erg)	–	–	–	1 (1)	Nil	162

[a] Figure within bracket indicates number of crosses
M=mutant; W=wild

Fig. 1. A fertile progeny (F5) of 82E30 (*L. atlanticus*/*L. cosentinii*) grown in summer 1986/1987 in greenhouse

Table 3. Seed fertility in interspecific crosses with *L. digitatus*

Crosses	Number of seeds				
	F_1	F_2	F_3	Bc_1 (F_1)	Bc_1 (F_2)
cosentinii (Erg)/ *digitatus*	12	1	1	2	6
cosentinii (Erg-s)/ *digitatus*	3	Nil	–	–	–
digitatus/*cosentinii* (Erg-s)	10	2	3	2	Nil
atlanticus/*digitatus*	2	24			

Among this group of crosses, 82E30 appeared to be the most promising. It also improved in growth and seed fertility through two successive backcrosses (Table 2). One such backcross (Bc_2), viz. 82E30(F_2)/Erg-s//Erg-s, showed good vigour and segregated for sweetness and white flower/seed type. From 82E30 it was also possible to select directly a fertile F_4 plant combining low alkaloid content, white flower/seed and possibly soft-seededness. F_5 progenies from this selection were grown during the 1986/1987 summer in a temperature-controlled greenhouse. They were still segregating for seed-fertility (very high to sterile), but the domestication genes appeared to be stable (Fig. 1).

L. cosentinii/*L. digitatus*

L. digitatus as a female or male parent crossed easily with *L. cosentinii* (cv. Erregulla or Erregulla-s), giving 3–12 seeds per cross (Table 3). F_1 plants showed hybrid vigour, with the *L. digitatus* plant type dominant. However, all the F_1, F_2 and Bc_1F_1 plants were more or less sterile, giving only a few seeds when grown in the greenhouse. F_1 plants grown in field were completely sterile.

L. atlanticus/*L. digitatus*

Only one cross combination of *L. atlanticus* (wild)/*L. digitatus* was successful, giving two F_1 seeds (Table 3). The two F_1 plants grown in the greenhouse showed predominantly *L. atlanticus* plant type and were more fertile than any other interspecific F_1 studied. One F_2 plant was grown in summer (1986/1987) in the temperature-controlled greenhouse (Fig. 2). The plant and the flowers looked almost *digitatus* type and were highly fertile. Some backcrosses to *L. atlanticus* selections with domestication genes were successful.

L. pilosus/*L. atlanticus*

Twenty-seven crosses, including two reciprocals, were successful (F_1 seeds 1–10) between selected lines of *L. pilosus* (wild) and *L. atlanticus* (mutant or wild). The F_1 plants showed hybrid vigour (particularly in the greenhouse), and were intermediate in plant type, with large *pilosus*-type blue flowers, but were poor in pollen development. In spite of numerous attempts at pollination (selfing and backcrossing) there was no seed set. A few cross combinations, however, showed some initiation of pod/seed development in the F_1, which at maturity gave small, undeveloped F_2 seeds.

L. pilosus/*L. cosentinii*

Three crosses produced one seed per cross. When grown, only two F_1 plants survived. They were poor in growth and sterile.

Discussion

The results indicate a real possibility of interspecific crossing within this group of lupins for transfer of domestication genes from one species to another. In the interspecific cross 82E30 (*L. atlanticus* – wild/*L. cosentinii* cv. Erregulla-s), it was possible to select an F_4

Fig. 2. One F_2 plant of 85E48 (*L. atlanticus/L. digitatus*) showing almost normal fertility

plant homozygous for three of the domestication traits from Erregulla-s, viz. low alkaloid, white flower/white seed/green stem, and soft-seededness, in combination with good fertility. It remains to be seen whether full transfer from one species to the other can be achieved, and if so, whether deletereous genetic imbalances can be avoided or eliminated completely.

Most of the interspecific crosses attempted here have not been reported before, and are of considerable interest in understanding the species relationships. The relative success of crosses between *L. atlanticus/L. digitatus, L. atlanticus/L. cosentinii* and *L. digitatus/L. cosentinii* indicates fairly close relationships among these three species. *L. atlanticus/L. digitatus* appears to be the most F_1-F_2 fertile combination, indicating that *L. atlanticus* ($2n = 38$) and *L. digitatus* ($2n = 36$) are closer to each other than to *L. cosentinii* ($2n = 32$). Thus crossing behaviour confirms the evidence of chromosome numbers. Cytological analysis of the crossbreds is a logical next step towards better understanding of the genetic relationships within the group.

The high F_1 seed set in crosses between *L. pilosus* (female)/*L. atlanticus,* but complete F_1 plant sterility, could be due to lack of embryo – endosperm cooperation. This was indicated by the initiation of pod/F_1 seed development in some cross-combinations, followed by poor development at later stages of F_1 seed growth. In such cases, embryo culture can sometimes help to overcome the hybrid plant sterility, as reported with interspecific crosses of *Brassica* (Sacristan and Gerdemann 1986).

The success in obtaining only one F_1 seed for each of three *L. pilosus/L. cosentinii* crosses, and F_1 plants showing poor growth and complete sterility, indicates a more distant relationship between *L. pilosus* ($2n = 42$) and *L. cosentinii* ($2n = 32$). But if through embryo culture it is possible to overcome the hybrid plant sterility barrier between *L. pilosus* and *L. atlanticus, L. atlanticus* might then become a usable genetic bridge between *L. cosentinii* and *L. pilosus.*

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