INTERSPECIFIC CROSSING AMONG THE NEW WORLD LUPIN SPECIES FOR *LUPINUS MUTABILIS* CROP IMPROVEMENT

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ABSTRACT

There are as many as 300 New World lupin species, distributed from Alaska to the extreme south of South America. Only one species among these, *Lupinus mutabilis* has achieved crop status while several other species have been used as ornamentals or for land rehabilitation. *L. mutabilis* has considerable potential as a high protein and oil legume and it is phylogenetically related to the western North and South American lupins most of which share the chromosome number 2n = 48. The genetic variation in these species may provide useful traits for *L. mutabilis* breeding and a series of crosses was attempted to initiate an introgression program. We have obtained hybrids between *L. mutabilis* and each of *L. mexicanus* (both a germplasm accession and a horticultural accession listed as '*L. hartwegii*'), *L. arizonicus* and a South American species, *L. tomentosus*. Pod retention and immature seed growth after hand crossing and pollen viability data from F_1 hybrids indicated a moderate degree of compatibility between *L. mutabilis* and the other species. Embryo rescue was not required for the cross combinations: *L. hartwegii* × *L. mutabilis* and *L. mutabilis* × *L. tomentosus* however *L. mutabilis* × *L. mexicanus, L. hartwegii*, and *L. arizonicus* each required embryo rescue to produce F_1 plants. Additional species are being crossed to *L. mutabilis* and the results reported in this paper. Hybrids show intermediate morphological characters and reduced pollen viability from flowers produced on F_1 interspecific plants. Tripping and backcrossing is being carried out to improve hybrid fertility and BC_1 progeny will be advanced as potential parents in the crop improvement of *L. mutabilis*.

KEYWORDS

wide hybridisation, embryo rescue, introgression, Andean lupin, pearl lupin

INTRODUCTION

The genus *Lupinus* consists of two major groups, the Old World and New World species. The taxonomic classification of Gladstones (1974) for the Old World group of 12 species is generally accepted but that of the New World group, although recent efforts have reduced the complexity, is still being fully established. The taxonomy of the New World lupins has proven difficult due to the large number of apparent species and intermediates resulting from outcrossing and a high degree of phenotypic plasticity across their habitats (Plachuelo, 1994). The predominant chromosome number for the New World lupins is 2n = 48. Several studies lend support to the genetic separation of the western New World (western North and South America), the eastern New World (including east-central parts of South America and southeastern USA) and the Old World species (Ainouche and Bayer, 1999; Maciel and Schifino-Wittmann, 2002; Hughes and Eastwood, 2006). The western New World species are possibly closer to the Old World species than they are to the eastern New World species (Ainouche and Bayer, 1999). The central American species such as *L. mexicanus* and *L. elegans* (Mexican), and South American *L. mutabilis* (Andean) and *L. microcarpus* (N and S America), are grouped together with the western North American species (Ainouche and Bayer, 1999). The close relationship between *L. mutabilis* and the western North American species is also supported in serological, isozyme and DNA studies (Clements *et al.* 2005).

Several workers have attempted to interspecifically cross among the New World species. An early example of this is the ornamental Russell lupin which was bred by horticulturalist George Russell during the 1930s in England. With his aim of selecting brighter flower colours, Russell carried out crosses between north, central and southern American species, particularly *L. polyphyllus*, *L. laxiflorus*, *L. lepidus*, *L. hartwegii* and *L. mutabilis*.

As summarised by Clements *et al.* (2005), cross combinations that from published reports were apparently successful include *L. elegans* × *L. mutabilis*, viable seeds, *L. pubescens* × *L. mutablis,* F_1 seeds; *L. nanus* × *L. mutablis,* F_1 seeds; *L. polyphyllus* × *L. mutabilis*, viable plants, use of backcrossing to *L. mutabilis*; *L. hartwegii* × *L. mutabilis*, viable seeds; *L. mutabilis* × *L. hartwegii*, embryo rescue, hybrid F_1 plants, F_2 seed.

IN J.A. Palta and J.B. Berger (eds). 2008. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

As a first step to broadening the genetic base available to a new *L. mutabilis* breeding program in Australia, we have attempted crosses between *L. mutabilis* and a number of New World species ranging in their relatedness to *L. mutabilis*.

MATERIALS AND METHODS

PLANT MATERIALS, CROSSING AND EMBRYO RESCUE

Seed of *Lupinus* species was sourced from The Australian Lupin Collection, Department of Agriculture and Food, Western Australia. Crosses were carried out in conditions suited to lupin in a glasshouse or screenhouse at The University of Western Australia with temperatures averaging 22ºC day and 13ºC night. Three flower buds per main stem or branch raceme were emasculated and stigmas were pollinated from 1 to 5 days afterward. Pods from cross combinations *L. mutabilis* with *L. hartwegii*, *L. mexicanus* and *L. aizonicus* were harvested after 14-35 days after pollination (DAP). Pods of *L. hartwegii* × *L. polyphyllus* hybrid (Russell A) crosses were collected on 18th and 25th DAP. In most cases, some pollinated flowers were allowed to develop on the plant and information collected on stage of abscission or ability to reach maturity. Otherwise, developing pods were cut at the pedicel and surface sterilised by immersion in 1% bleach and then rinsed with sterile distilled water.

Pods were opened inside a sterile laminar flow cabinet by cutting the abaxial pod suture and the embryos dissected from immature seeds under a stereomicroscope with a syringe needle. Embryos were placed into a 30 mL culture tube with a sloped solid medium. The basic medium was B5 (Gamborg *et al.* 1968) with 3% of sucrose and solidified with 0.6% agar. For subculture, a B5 medium with reduced sugar level was used (2% sucrose or no sugar). Embryos were incubated in darkness for one week and then maintained in a growth room with fluorescent tubes at 24ºC until shoots and roots developed. Slow developing embryos were subcultured in a fresh medium. In cases when callus was forming at the base of a rootless plantlet, the callus was removed and shoots were propagated in vitro.

TRANSFER OF *IN-VITRO* PLANTS TO GLASSHOUSE AND POLLEN VIABILITY

Developed plantlets were put into pots with potting mix, covered with plastic vials to ensure high humidity and kept at 15ºC under fluorescent light at 12 h photoperiod for 1-2 weeks. Shoots without roots were dipped into a commercial rooting hormone for softwood and semi-hardwood cuttings (Clonex, Yates) and kept in the same conditions as for plantlets with roots until well established. Pots were transferred first to a glasshouse, then to a shade house for development through flowering until harvesting.

Sterile F_1 plants are being propagated by shoot cuttings using rooting hormone to provide new material for backcrossing or selfing in attempts to restore fertility.

The cellophane method of pollen germination in vitro (Alexander 1980) was applied to evaluate pollen viability and tube growth of F_1 flower pollen.

RESULTS AND DISCUSSION

In this work we have attempted crosses with the Andean lupin, *L. mutabilis* to several other New World species with 2n = 48 ranging in phylogenetic relatedness. *L. tomentosus*, a perennial shrub, is distributed within Peru which is central to the distribution of *L. mutabilis* (Conterato and Schifino-Wittmann 2006). The Mexican (north and centrally distributed) annual species *L. mexicanus* (synonym *L. ehrenbergii*, commercially named *L. hartwegii*), has been known as an ornamental plant since the mid-twentieth century. Both *L. mexicanus* accession P28653 and an ornamental form (‘*L. hartwegii*’), were used for crossing to *L. mutabilis*. Both *L. exaltatus* and *L. arizonicus*, each annuals, are also distributed in Mexico with *L. arizonicus* also found in Arizona, California and Nevada. *L. succulentus* is also annual and is distributed in California and Arizona. The parentage of the Russell lupin was thought to be made up of *L. polyphyllus* and *L. arboreus* and possibly the Alaskan species *L. nootkatensis* (Gorer 1970) however it is likely that the perennial *L. polyphyllus* is the dominant component. The natural distribution of *L. polyphyllus* is on the west coast of the USA including Washington State and in north eastern states (USDA 2008).

Results of crossing success and F_1 pollen viability presented in Table 1 showed crossing success depended on direction for many of the crosses. For example, *L. mutabilis* × *L. hartwegii* could only be obtained through embryo rescue, while in the reciprocal, viable seed was produced on the plant. For *L. mutabilis* × *L. tomentosus*, viable seed was obtained on the plant but in the reciprocal direction, pods abscised within 14 days after pollination. No viable seed or embryos were obtained in crosses between *L. mutabilis* and *L. succulentus* in either direction. We report for the first time hybrids between *L. mutabilis* and both *L. tomentosus* and *L. arizonicus*. *Lupinus tomentosus*, the species with the closest geographic relationship to *L. mutabilis* crossed easily in one direction, resulting in partly fertile F_1 plants. Some male sterile plants were identified among the mostly fertile BC_1F_3 (*L. mutabilis*//*L. mutabilis*/*L. tomentosus*) population. It was noted that F_2 plants appeared to be mixed annual and perennials and flower racemes on some plants appeared to be developing whorls of up to 7 flowers (cf. 5 flowers per whorl in *L. mutabilis*).

Hybrids were obtained between *L. mutabilis* and *L. arizonicus* using embryo rescue, although F_1 plants are sterile and so far, backcrossing has not produced progeny.

Table 1. Crossability of Andean lupin (*L. mutabilis* Sweet) with other New World species and in selected additional cross combinations.

Female	Male	Pollinated flowers	Pods	Pods allowed to mature on plant	Seeds	Excised embryos	Result	Average F_1 pollen viability % for cross type
L. mutabilis ID8	*L. arizonicus* P27926 A	9	3	Yes	-	-	Pods abscised 50 DAP	
L. mutabilis ID8	*L. arizonicus* P27926 A	9	2	Embryo rescue	3	2	F_1 plants	1
L. mutabilis ID13	*L. arizonicus* P27926 A	9	1	Embryo rescue	1	1	F_1 plants	
L. arizonicus P27926 A	*L. mutabilis* ID13	9	2	Yes	-	-	Pods abscised 14 DAP	
L. mutabilis ID13	*L. hartwegii* parent D	18	7	Yes	15	-	Seeds shrivelled	
L. mutabilis ID13	*L. hartwegii* parent D	18	9	Embryo rescue	19	17	F_1 plants	63
L. mutabilis ID8	*L. hartwegii* parent D	15	5	Embryo rescue	16	12	F_1 plants	
L. hartwegii parent D	*L. mutabilis* ID13.2	6	1	Yes	3	-	Mature seeds	67
L. hartwegii parent B	*L. mutabilis* ID8	9	2	Yes	8	-	Mature seeds	
L. mutabilis ID8	*L. mexicanus* P28653 A	6	2	Yes	6		Seeds shrivelled	
L. mutabilis ID8	*L. mexicanus* P28653 A	9	5	Embryo rescue	21	20	F_1 plants	80
L. mutabilis ID8	*L. mexicanus* P28653 B	6	3	Embryo rescue	17	16	F_1 plants	
L. mutabilis 06M57 MS	*L. mexicanus* parent C	12	9	Embryo rescue	18	18	F_1 plants	
L. mutabilis ID8.9	*L. succulentus*	15	5	Yes	-	-	Pods abscised 40 DAP	
L. mutabilis ID8.9	*L. succulentus*	12	2	Embryo rescue	2	0	Dead embryos	
L. succulentus	*L. mutabilis* ID8.9	3	0	-	-	-	Emasc. flowers abscised	
L. mutabilis (23-4, -11, -17, -28)	*L. tomentosus*	27	6	Yes	15	-	F_1 , F_2, BC_1F_2 plants	60
L. tomentosus	*L. mutabilis* (23-4)	12	0	Yes	-	-	Pollinated flowers abscised	
L. hartwegii parent B	*L. tomentosus*	15	3	Yes	6	-	Seeds shrivelled	
L. mutabilis ID13	*L. exaltatus* P28648	6	1	Yes	1	-	Seeds empty	
L. mutabilis ID8.9	*L. exaltatus* P28648	9	1	Embryo rescue	0	0	Dead embryos	
L. exaltatus P28648	*L. mutabilis* ID8.9	9	0	Yes	-	-	Pods abscised 14 DAP	
L. hartwegii parent E	*L. exaltatus* P28648	6	0	Yes	-	-	Pods abscised 30 DAP	
L. mutabilis P26956	*L. polyphyllusX (*Russell A*)*	9	2	Yes	5	-	Seeds shrivelled	
L. mutabilis ID8.9	*L. polyphyllusX (*Russell A*)*	9	1	Embryo rescue	2	0	Dead embryos	
L. hartwegii parent B	*L. polyphyllusX (*Russell A*)*	18	12	Yes	53	-	Mostly empty seeds	
L. hartwegii parent B	*L. polyphyllusX (*Russell A*)*	6	4	Embryo rescue	18	15	F1 seedlings growing	

MS = male sterile line.
*L. polyphyllusX (*Russell A*)* = ornamental Russell lupin genotype, considered an *L. polyphyllus* hybrid.
Parent pollen viability across all species averaged 85%.

Partially fertile hybrids were achieved between *L. mutabilis* and both *L. mexicanus* and *L. hartwegii* although F_1 plants of the latter combination showed a generally lower pollen viability (Table 1). There were a number of plant morphological differences between *L. mexicanus* and *L. hartwegii*, possibly as a result of ornamental breeding. Hybrids were relatively easily obtained for the direction *L. hartwegii* × *L. mutabilis* as has been reported by other workers (e.g. Schäfer-Menuhr *et al*. 1988; Clements *et al*. 2005). We did not obtain hybrids in limited attempts between *L. mutabilis* and each of *L. exaltus*, *L. succulentus* and *L. polyphyllus* hybrid (Russell). Hybrids have been reported by several workers for the combination *L. mutabilis* × *L. polyphyllus* (Clements *et al*. 2005). We did however, succeed in crossing *L. hartwegii* with *L. polyphyllus* hybrid (Russell) using embryo rescue. This provides one avenue of introgressing annual growth habit into the perennial Russell lupin. Hybrid status of leaf tissue from F_1 plants is being assessed using RGAP (Yan *et al*. 2003).

Embryo rescue was carried out on embryos from 17 to 35 DAP. In the case of *L. mutabilis* x *L. hartwegii*, it was found that higher success of embryo survival was found for rescuing from 28-35 DAP compared with 17-24 DAP. In all cases, embryos less than the early heart stage of development (i.e. globular) never survived and this is consistent with other work for *Lupinus* interspecific hybridisation (e.g. Schäfer-Menuhr *et al*. 1988).

Interspecific hybridisation between the Andean lupin and other New World species and among the other New World species will greatly broaden the genetic base available to breeders particularly of *L. mutabilis* but can also provide a potentially exciting diversity of new plant types for agricultural, horticultural and ornamental use. It should allow the introgression of traits such as increased hardiness and drought tolerance into *L. mutabilis* possibly from some dry climate lupin species distributed in regions such as Mexico, Arizona and California. Many New World species are highly outcrossing, and this provides the opportunity through wide crossing to manipulate the outcrossing rate in *L. mutabilis* if required. Pollen viability of F_1 plants provides a useful assessment of relatedness between species and this may compliment phylogenetic studies based on DNA data.

ACKNOWLEDGEMENTS

We wish to thank the GRDC for funding this work as part of projects involving interspecific hybridisation in lupin and pearl lupin pre-breeding. CLIMA internal funding is also acknowledged.

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