

MORPHOLOGICAL, CYTOLOGICAL AND MOLECULAR CHARACTERISTICS OF PARENTS AND INTERSPECIFIC HYBRID (*LUPINUS MUTABILIS* LM-13 X *LUPINUS ALBUS SENSU LATO*)

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ABSTRACT

The crossing between the Andean lupin (*L. mutabilis* LM-13; $2n = 48$) and white lupin (*L. albus* var. *graecus* – *L. graecus*; $2n = 50$) produced a hybrid, which was evaluated and compared to parents. Morphological features of hybrid and parental species were examined under field conditions in Experimental Agriculture Station – Swojec of Wrocław University of Environmental and Life Sciences. The cytological analysis of the somatic number of Primers were applied generating dominant (RAPD) and codominant (STS) markers. The hybrid was similar to the male parent with respect to rate of growth and development of generative organs. However plant growth habit and weight of seeds were like the female parent. The hybrid was much higher than both used species. The cytological analysis showed that hybrid and female parent had the same number of chromosomes ($2n = 48$). The primers that were applied in molecular analysis revealed a DNA polymorphism between parents and hybrid. On the basis of preliminary results it was confirmed, that the hybrid showed a significant range of variability in the measured traits.

KEY WORDS

Lupinus, morphological, cytological characteristic, molecular markers, RAPD, STS

INTRODUCTION

The genus *Lupinus* (Fabaceae) comprises about 250 species but only four of them are cultivated forms.

Representatives of this genus are spread over both American continents, around the Mediterranean and North Africa.

Lupin as a crop is used in food and fodder industry as a rich source of plant protein, fat and other macro- and microelements. Another advantage of the genus is its ability to fix atmospheric nitrogen resulting in more nitrogen in soil for better plant development.

The species of the genus *Lupinus* probably have a polyploid origin and they have different chromosomes

numbers (*L. angustifolius* $2n = 40$; *L. mutabilis* $2n = 48$; *L. albus* $2n = 50$; *L. luteus* $2n = 52$). Furthermore, the cytogenetical barriers which developed during the evolution process are responsible for the fact that interspecific hybrids in this genus are extremely difficult to obtain, and the cultivated species have been bred separately. Despite these difficulties, there is information published about successful interspecific hybridisation (Sholars, 1999; Swiecicki *et al.* 1999; Sawicka-Sienkiewicz *et al.* 1999; 2006; Clements *et al.* 2005).

Lupinus mutabilis is the only cultivated lupin species from South America. It is characterised by high protein and oil contents in the seeds (~40% and ~20%) and thus experiments have been started to adapt the Andean lupin to European temperate climatic conditions. Some investigations on the possibility of crossing this species with the white lupin (*Lupinus albus*) were successful (Augiewicz *et al.* 2001b; Sawicka-Sienkiewicz *et al.* 1999; 2006). The aim of the this work was to characterise an interspecific hybrid between the Andean lupin (*L. mutabilis* – LM-13) and white lupin *Lupinus albus* var. *graecus* – (*L. graecus*) with respect to morphological, cytological and molecular features.

MATERIALS AND METHODS

MORPHOLOGICAL RESEARCH

Both parental species and the interspecific hybrid were examined in field conditions at the Swojec Experimental Agriculture Station, Wrocław University of Environmental and Life Sciences. The female form (*L. mutabilis* Sweet – genotype LM-13) used in the crossing originated from France and the male form *L. albus* var. *graecus* (*L. graecus*) was from the Polish lupin collection of the Plant Breeding Station, Wiatrowo. The assessment of morphological characters, phenological phases and selected crop features concerned the last six years (Augiewicz *et al.* 2001b; Sawicka-Sienkiewicz *et al.* 2005).

CYTOLOGICAL METHODS

The cytological analysis pertained to the number of chromosomes in the hybrid's somatic cells. Seeds of the examined forms were germinated in Petri dishes on moistened filter paper. To induce development of

secondary roots, the tip of the primary root was removed. All young plants with secondary roots 1.5-2.0 cm long were treated with ice-cold water for 24 h, and after that the secondary roots were excised and finally fixed in the Carnoy mixture (ethanol 96%: glacial acetic acid – 3:1) and stored in a fridge.

Fixed roots were washed in distilled water and digested in 1N HCl for 6–7 min at 60°C. After repeated washing in distilled water, the meristems were digested in 1% water mixture of pectinase for 60 min in 37°C. Single root-tips were transferred on a microscope slide into a drop of DAPI stain (4'6-diamidyno-2-fenylindol) and squashed under glass coverslips.

The procedure of chromosomes spreads and preparations staining was based on the protocol presented by Wedzony (1996) and Rogalska *et al.* (2005) with minor modifications. The preparations were examined under a Nikon ECLIPSE (E200) microscope with an attached CCD camera interfaced to a PC running the CoolSNAP 1.2. software.

MOLECULAR ANALYSIS

Total genomic DNA from the parents and interspecific hybrid was extracted by a modified version of the method described by Murray and Thompson (1980).

The RAPD analysis based on PCR reactions was performed according to the original protocol of Williams *et al.* (1990) with some modifications. Thermocycler amplification followed the procedure by Gomez *et al.* (2001). The STS methodology was that described by Konovalov *et al.* (2005) with minor modifications. Six RAPD and 3 STS primer sequences were used in the amplifications of DNA (Table 1).

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERISTIC

The Andean lupin *L. mutabilis* is a tall plant with strong main stems with numerous lateral branches of I, II, III orders resulting in different times of pod and seed ripening.

The paternal form *L. albus* var. *graecus* (*L. graecus*) reaches a smaller size and has a limited number of lateral branches. Therefore pod formation and seed

ripening began earlier than was observed in *L. mutabilis*.

The morphological observations revealed that the examined hybrid (*L. mutabilis* LM-13 x *L. graecus*) was similar to the female parent. These similarities were noticeable almost in every characters. More precise information of morphological characteristics has been presented by Augiewicz *et al.* (2001b).

CYTOLOGICAL RESEARCH

Traditional methods of staining preparations are not sufficient to research pursue into species of the genus *Lupinus* due to a large number of small chromosomes with similar morphology (Naganowska and Ladon, 2000; Augiewicz *et al.* 2001a; Naganowska and Zielinska, 2002; Hajdera *et al.* 2003). During this analysis the somatical number of chromosomes for the parental species and their hybrid was confirmed thanks to the techniques used (Augiewicz, 2001a; Sawicka-Sienkiewicz *et al.* 2004; 2005; 2006): *L. mutabilis* LM-13 - 2n = 48; *L. albus* var. *graecus* - 2n = 50; (*L. mutabilis* LM-3 x *L. graecus*) - 2n = 48.

Cytological observations, concerning the somatic number of chromosomes in the hybrid's cells, showed that the number was identical to that in *L. mutabilis* the female form. It suggests that chromosomes elimination took place during the process of hybrid's stabilisation (Sawicka-Sienkiewicz *et al.* 2004; 2005).

It is important to continue the cytological analysis of this and other hybrids obtained during similar crossings, as it may increase the knowledge of evolutionary relationships between the genomes of parental species originating from two such remote continents (Augiewicz *et al.* 2001a; Kalinska *et al.* 2001; Sawicka-Sienkiewicz and Augiewicz, 2004).

MOLECULAR ANALYSIS

Among the 9 primers tested, 5 amplified one or more PCR products (table 2). In the case of 4 primers the amplified DNA fragments were polymorphic. The remaining primer P393 resulted in monomorphic products. Four RAPD markers were generated using primers CS1525 and P3. The primer CS60 produced 3 markers. One marker of 600 bp generated by CS1525 was scored in the *L. mutabilis* LM-13 and hybrid.

Table 1. Primers and their sequence.

RAPD primers	Sequence (5' to 3')	STS primer	Sequence (5' to 3')
CS1525	GAACGACGCA	SG05_2537	5'AGGCCACTTCTCCTACTTACTTCAT
CS60	CCGCCTCCTT		5'CCATGATAAATCCTAACAAACCCTG
P3	GTCCGTTGGG	Dimin	5'AAGAGGAAGAAGATTTGGGT
P30	CAACTGGTAATG		5'TCAAAGCCTGCTTCTGGGTA
P6	TCGCCCAT	P393	5'CTGGTTGGTCCTTCCTTATTTTAC
P14	TGCCTCCAT		5'AACGGATAAAGAGTGACAAGAACC

Three polymorphic fragments, i.e. first designated as 300 bp, second as 800 bp for starter CS1525 and third designated as 500 bp for primer CS60, were observed in *L. albus* var. *graecus* and in the hybrid. The markers of 400 bp and 1600 bp for P3 were identified in both species used. To the contrary, the marker of 800 bp generated by above primer was detected only in the hybrid. For the same primer one marker designated as 750 bp was scored in *L. albus* var. *graecus*. With regard to the STS primers, the analysis revealed two markers for Dimin primer (Table 2). The marker designated as 1400 bp was observed in *L. mutabilis* LM-13, whereas the marker designated as 1700 bp was scored in *L. albus* var. *graecus*.

A PCR analysis of lupin DNA, using random 10-mer primers, revealed differences in the RAPD profiles in the hybrid derived from *L. mutabilis* LM-13 and *L. albus* var. *graecus*. The hybrid had more polymorphic fragments *L. albus* var. *graecus*. However, the hybrid was different from its parents in the absence of 400 bp and 1600 bp, and presence of 800 bp markers generated by P3. Therefore, these results suggest that the morphological differences between the hybrid and both parent species could be correlated with different polymorphic fragments revealed. The results obtained with the use of STS primers did not allow the establishment of the level.

The future studies should be performed for confirmation of hybrid characters in lupin lines originated from interspecific hybridisation. The main investigations could be concentrated on genomic in situ hybridisation (GISH) and numerous molecular analysis of DNA.

CONCLUSIONS

With respect to morphological features the hybrid (*L. mutabilis* LM-13 x *L. graecus*) analysis shows that the hybrid possesses the same number of chromosomes as the female species *L. mutabilis* LM-13 ($2n = 48$). The primers used in the molecular tests did not reveal the level of genetic differences between the hybrid and both parental forms used but some of them showed that the hybrid had more polymorphic fragments from the male parent.

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Table 2. Products of pcr reaction.

RAPD primers	Fragment length (bp)	Polymorphism		
		<i>L. mutabilis</i> LM-13	<i>L. mutabilis</i> LM-13 x <i>L. graecus</i>	<i>L. graecus</i>
CS1525	1250	+	-	-
	800	-	+	+
	600	+	+	-
	300	-	+	+
CS60	920	+	+	+
	620	+	+	+
	500	-	+	+
P3	1600	+	-	+
	800	-	+	-
	750	-	-	+
	400	+	-	+
P30	-	absence of amplification products		
P6	-	absence of amplification products		
P14	-	absence of amplification products		
STS primers	Fragment length (bp)	Polymorphism		
		<i>L. mutabilis</i> LM-13	<i>L. mutabilis</i> LM-13 x <i>L. graecus</i>	<i>L. graecus</i>
SG05_2537		absence of amplification products		
DIMIN	1700	-	-	+
	1400	+	-	-
P393	> 200	monomorphic products		

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