SEED COAT THICKNESS DIFFERENTIATION AND GENETIC POLYMORPHISM FOR *Lupinus mutabilis* SWEET BREEDING

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ABSTRACT

The paper investigates seed coat characteristics in *L. mutabilis*, a potential forage crop. In the study twelve genotypes, including three epigonal lines KW-5, KW-10, Pop.1 and lines with traditional type of growth XM.5, Pop.15 LM.13, LM.34, 'Potosi', Mut-45, Mut-160, Mut-136, Mut-628 were evaluated. The highest seed coat percentage was recorded in mutant line Mut-160 and the lowest was noted for mutant line Mut-45. The statistical significance relationship between seed coat thickness and 1000 seeds weight was not notice. It's gives capabilities for selection of favorable genotypes in breeding process. In order to illustrate genetic diversity among the genotypes tested, 24 ISSR primers and 6 pairs of AFLP primers were used. They generated a total of 685 polymorphic amplification products in 12 evaluated Andean lupin genotypes. The products characteristic only for the finest seed coat lines Mut-45 were detected as well as for the thickness seed coat line Mut-160.

Key words: AFLP markers, genetic diversity, ISSR markers, *Lupinus mutabilis*, seed coat proportion, seed coat ultrastructure.

INTRODUCTION

L. mutabilis cultivated population first appeared 3000 years ago as a result of natural selection and selection carried out by the early local farmers. *Lupinus mutabilis* (Andean or pearl lupin) is the only one cultivated species among more than 200 wild species of lupin originated from South America. It has been cultivated by indigenous people of Columbia, Ecuador, Peru and Bolivia. Seeds had been consumed as a traditional food after de-bittering process up to now (Falconí, 2012). L. mutabilis stands out as having the highest protein (up to 50%) and oil (up to 20%), compare well with the quality of soybean (Gross et al., 1988), it has been a focus of breeding efforts not only in South America (National Research Council, 1989; Falconí, 2012), but also in Australia (Clements et al., 2008) and in Europe (Römer, 1994; Römer et al., 1999).

In comparison with other cultivated lupins species, Andean lupin better tolerates drought and acidification of soil (Cowling et al., 1998). From breeding and agricultural point of view it has also got such advantages as softer and thinner seed coat in comparison to other, wild species of lupin, indehiscent and non-shattering pods, which is particularly important trait in industrial and automated type of harvesting. In addition *L. mutabilis* is a neutral photoperiodic plant and as a nematode - antagonistic species constitutes a good alternative in crop rotation with cereals and tubers (Cowling et al., 1998).

On the other hand, Andean lupin has many drawbacks, which have been identified during first studies in genetic and breeding in Europe over the acclimation of *L. mutabilis,* in the 'seventies' of last century (Römer et al., 1999). The international project (1993-1997) provided a lot of valuable information, especially on those characteristics that limit its full development and uses in crop production (Commission of the European Communities Directorate-General for Agriculture, 1997). It's for example: far too long and uncertain pod maturation, low and unstable yields (as the results of fall flowers and buds pods), high alkaloid content to 5%, and susceptibility to frost (von Baer and von Baer, 1988; Martins et al., 1992). Another drawback is a high percentage of pod walls and of seed coat as compared with other leguminous plants. The proportion of seed coat in seeds of the Andean lupin ranges between 11- 16%, the narrow-leaved lupin 17-24% and the white lupine 17-18%, while in soybean ~ 7%, and in pea ~ 9%. Seeds with lower percentage of seed coat are characterized by better digestibility, which is an important feature for feeding

The main purpose of presented paper was evaluation genotypes gathered in collection for searching forms with thin seed coat and also DNA polymorphism was done by AFLP and ISSR molecular markers usefulness in breeding programs.

MATERIALS AND METHODS

Plant material

For the purpose of these research 12 genotypes of *L. mutabilis* Sweet were chosen (Table 1). These genotypes were attributed with favorable agronomic traits such as: early flowering time, limited branching, earlier maturation and better harvest index.

Table 1. Seed coat percentage and 1000 seed weight in analyzed genotypes of *Lupinus mutabilis*

No.	Genotypes	Origin	Seed coat (%)		Sd	2011		Sd	2012		Sd	1000 seed weight (g)		Sd	2011		Sd	2012		Sd
			2011-2012									2011-2012								
1	KW-10*	Germany	14,7	cd	0,6	13,0	ab	0,8	16,4	df	0,4	123,8	abc	1,2	118,5	def	1,7	129,0	abc	1,4
2	KW-5*	Germany	16,5	ef	0,7	14,3	b	0,3	18,8	g	0,4	113,1	ab	1,7	97,6	f	1,0	128,7	abc	1,4
3	LM.13	France	14,2	bc	0,4	13,6	b	0,4	14,8	bcd	0,5	130,1	cd	1,2	129,3	cde	2,0	130,8	abc	1,5
4	LM.34	France	14,4	bc	0,3	14,4	b	0,5	14,4	abcd	0,2	145,7	def	1,7	157,1	ab	1,4	134,3	abc	2,1
5	Mut-136**	Polish	14,7	bcd	0,3	14,2	b	0,3	15,2	cd	0,2	142,9	def	0,9	146,5	abc	1,5	139,4	ab	1,6
6	Mut-45**	Polish	11,9	a	0,4	11,4	a	0,4	12,4	a	0,4	129,3	bcd	1,4	144,7	abcd	1,9	113,9	cb	1,5
7	Mut-628***	Polish	14,3	bc	0,4	14,3	b	0,7	14,3	abc	0,5	133,8	cde	1,3	134,1	bcd	2,2	133,4	abc	1,3
8	Mut-160**	Polish	17,6	e	0,4	16,7	c	0,3	18,4	fg	0,4	140,3	cdef	1,6	131,1	bcde	1,1	149,5	a	0,6
9	Pop.1****	Polish	16,1	de	0,6	14,5	b	0,6	17,5	fg	0,4	109,2	a	1,1	105,4	ef	1,4	112,9	c	2,4
10	Pop.15	Polish	14,6	bc	0,3	14,2	b	0,3	14,9	bcd	0,4	140,8	def	1,0	141,1	abcd	1,4	140,4	a	1,5
11	Potosi	Portugal	13,5	bc	0,2	13,6	b	0,4	13,4	ab	0,2	148,6	ef	1,6	155,8	ab	1,4	141,4	a	1,7
12	XM-5	Germany	13,2	ab	0,3	13,4	ab	0,4	13,0	ab	0,9	152,9	f	2,1	163,4	a	1,7	142,4	a	1,3

*epigonal forms selected from epigonal mutant KW obtained from dr Römer

**own mutants of KGHRiN derived after inducing mutations (Sawicka 1993) from the line 21756 Polish Plant Breeding Station Wiatrowo,

***mutant derived from the line 21758/ Polish Plant Breeding Station Wiatrowo

****epigonal genotype Pop.1 derived from Polish Plant Breeding Station Przebędowo

Phenotypic evaluation of the seed coat thickness and 1000 seeds weight

Seed coat proportion was conducted according to Clements et al. (2002). The material originated from two years field experiments. A variance analysis was carried out in order to verify the hypothesis about lack of diversification of the means of the genotypes studied with respect to the weight of 1000 seeds and the percentage of seed coat. Two-way ANOVA Model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$
$$i = 1,...,a \qquad j = 1,...,b \qquad k = 1,...,n$$

where,

$Y_{ijk} =$ kth observed response value when level i of factor A and level j of factor B is used.

$\alpha_i =$ the genotype main effect

$\beta_j =$ the environmental main effect.

$(\alpha\beta)_{ij} =$ the genotype x environment interaction effect.

$\varepsilon_{ijk} =$ a random error.

The mean values of these two traits were grouped based on results of post hoc Tukey test. Correlation coefficient was calculated using MS Excel 2003 application.

Scanning electron microscopy - SEM

Four different genotypes (Mut-45, Mut-160, KW-5, KW-10) varying in seed coat thickness were chosen for the SEM analysis. They were evaluated in regard to the seed surface and cross-sections SEM in tree replications (seeds). Specimens were gold coated using the JEOL JFC 1200 ion coater and observed in the JEOL JSM – 5310 LV scanning electron microscope under 20kV. The dorsal surface layer of a cuticle (CU) (Fig. 1 A', B', C', D') and a ventral layer of cells of seed coat (Fig. 1A", B", C", D', the layer of palisade cells (MS – macrosclereids), the supportive layer (OS – osteosclereids) and the layer of parenchymal cells (PA) were distinguishable (Fig. 1 A, B, C, D).
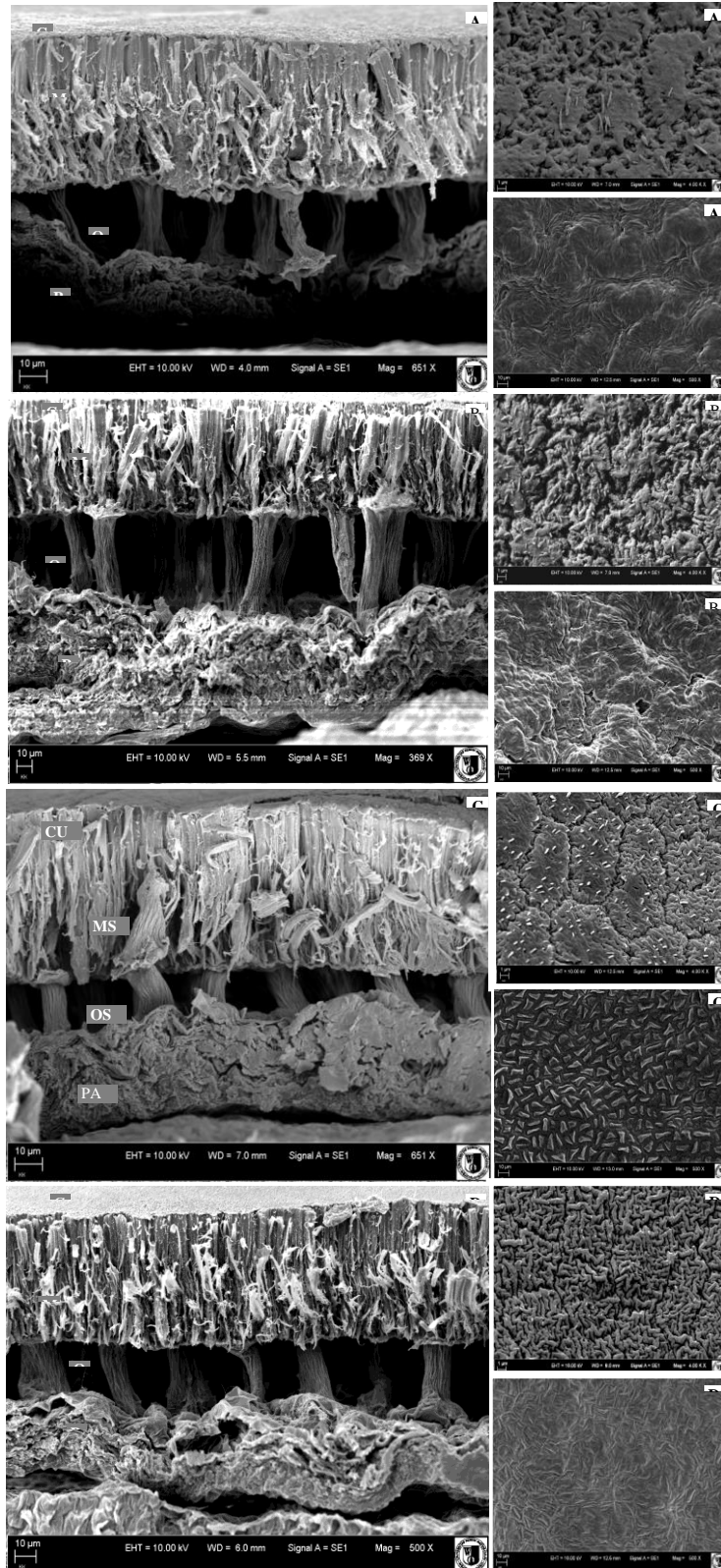
Figure 1. *Lupinus mutabilis* seed sculpture in Mut-45 (A, A',A''), Mut-160 (B, B', B''), KW-5 (C, C', C''), KW-10 (D, D', D''). SEM images and cross-section of the seed coat (A, B, C, D); CU – cuticule; MS – macrosclereid layer; OS – osteosclereid layer; PA – parenchal layer and its dorsal (A', B', C', D') and ventral surface (A'', B'', C'', D'')

DNA polymorphism characteristic

The DNA extraction

Genomic DNA was extracted from young leaf using the CTAB method (Doyle and Doyle, 1990) with minor modifications described by Clements et al. (2014).

Amplification of the DNA by the ISSR markers

A set of 24 anchored microsatellite primers from the University of British Columbia list (UBC#9) was used (Table 2). Amplification of DNA was performed in 15 µl reaction mixture containing 10 mM Tris-HCl, 50mM KCl, 0.08 % (v/v) Nonidet-P40, 2 mM $MgCl_2$, 0.2 of each dNTP, 0.4 µM primers, and 1 U of Taq DNA polymerase enzyme (Fermentas, Canada) and 45 ng of DNA using a Biometra thermal cycler for 40 cycles. After initial 4 min denaturation at 94 °C, each following cycle comprised of 1 min denaturation process at 94 °C, 45 s annealing at 50 °C, 2 min extension at 72 °C with a final 5 min extension at 72 °C at the end of 40 cycles. Amplified products were then separated by electrophoresis in 2.0% agarose gels and visualized with ethidium bromide (1.0 µg*ml^{-1}). The electrophoretic patterns of the PCR products were photographed under UV light. Analyses were performed in two replications.

Table 2. Summary of genotyping studied lines with AFLP and ISSR marker

Name	Sequence (5'-3')	Number of products	Numbers of polymorphic products	Percentage of polymorphic products	PIC
		ISSR			
UBC_881	GGG TGG GGT GGG GTG	7	7	100%	0,27
UBC_890	VHV GTG TGT GTG TGT GT	4	2	50%	0,11
UBC_889	DBD ACA CAC ACA CAC AC	5	5	100%	0,31
UBC_855	ACA CAC ACA CAC ACA CYT	5	4	80%	0,24
UBC_825	ACA CAC ACA CAC ACA CT	7	7	100%	0,27
UBC_810	GAG AGA GAG AGA GAG AT	8	7	88%	0,14
UBC_808	AGA GAG AGA GAG AGA GC	5	5	100%	0,29
UBC_807	AGA GAG AGA GAG AGA GT	6	6	100%	0,26
UBC_814	CTC TCT CTC TCT CTC TA	4	4	100%	0,3
UBC_815	CTC TCT CTC TCT CTC TG	1	1	100%	0,14
UBC_820	GTG TGT GTG TGT GTG TC	2	2	100%	0,34
UBC_828	TGT GTG TGT GTG TGT GA	9	7	78%	0,15
UBC_841	GAG AGA GAG AGA GAG AYC	4	1	25%	0,04
UBC_868	GAA GAA GAA GAA GAA GAA	9	6	67%	0,16
UBC_876	GAT AGA TAG ACA GAC A	11	11	100%	0,28
UBC_886	VDV CTC TCT CTC TCT CT	11	4	36%	0,07
UBC_836	AGA GAG AGA GAG AGA GYA	8	5	63%	0,21
UBC_842	GAG AGA GAG AGA GAG AYG	8	5	63%	0,16
UBC_864	ATG ATG ATG ATG ATG ATG	2	0	0%	0
UBC_867	GGC GGC GGC GGC GGC GGC	5	2	40%	0,14
UBC_818	CAC ACA CAC ACA CAC AG	3	1	33%	0,08
UBC_827	ACA CAC ACA CAC ACA CG	3	1	33%	0,1
UBC_811	GAG AGA GAG AGA GAG AC	4	0	0%	0
UBC_817	CAC ACA CAC ACA CAC AA	7	1	14%	0,02
ISSR		138	94	68%	0,18
MEAN		6	4	65%	-
		AFLP			
ACG_CAG	GACTGCGTACCAATTC ACG GTAGAGTCCTGAGTAA CAG	121	89	74%	0,2
AAG_CAG	GACTGCGTACCAATTC AAG GTAGAGTCCTGAGTAA CAG	106	89	84%	0,2
ACC_CTA	GACTGCGTACCAATTC ACC GTAGAGTCCTGAGTAA CTA	90	56	62%	0,15
AGC_CTA	GACTGCGTACCAATTC AGC GTAGAGTCCTGAGTAA CTA	105	80	76%	0,19
AGC_CTT	GACTGCGTACCAATTC AGC GTAGAGTCCTGAGTAA CTT	91	69	76%	0,18
AAC_CTT	GACTGCGTACCAATTC AAC GTAGAGTCCTGAGTAA CTT	34	14	41%	0,11
AFLP		547	397	73%	0,18
MEAN		91	66	69%	-

For further analysis DNA solutions were prepared in concentrations of 100 ng*μl^{-1}. Restriction digestion was carried out using restriction enzymes EcoRI and MseI (NewEngland BioLabs). The reaction 'mixture 1' consisted of 5 µl solution of DNA and 5 µl mixture of following compounds: H2O 3.8 µl buffer NE x 10 (NewEngland BioLabs), 1 µl BSA x 100 (NewEngland BioLabs), 0.1 µl EcoRI (20 u*μl^{-1}) 0.1 µl, MseI (10 u*μl^{-1}) 0.1 µl. Prepared mixture was incubated in 37 °C for 3 h. Then enzymes were deactivated by incubating the mixture for 5 min at 75 °C. For the ligation of adaptors to this mixture 6 µl of 'mixture 2' was added. 'Mixture 2' consisted of: 3.6 H2O µl, Ligase Buffer x 10 (Fermentas) 0.6 µl, MseI adapter (50 pmol*μl^{-1}) 0.6 µ l EcoRI adapter (5 pmol*μl^{-1}) 0.6 µl, T4 DNA ligase (1 u*μl^{-1}, Fermentas) 0.6 µl. The resultant mixture (called further 'mixture 3') was finally incubated at 37 °C for 16 h.

PCR was performed in the Biometra thermocycler using reaction 'mixture 4' of: H2O 2.7 µl, Taq buffer x 10 (Fermentas) 2 µl, MgCl2 (25 mM) 2 µl, dNTP mix (10 mM, Fermentas) 1µl 2.5 µl EcoRI-A primer (1 pmol*μl^{-1}), 2.5 µl primer MseI-C (5 pmol*μl^{-1}), BSA (Fermentas) 0.2 µl, DNA polymerase (5 U*μl^{-1} Fermentas) 0.1 and 4 µl 'mixture 3'. Thermal profile: initial denaturation at 94 °C for 2 min, 20 cycles of denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s, the amplification at 72 °C for 2 min and final amplification at 72 °C for 15 min. After the reaction has ended, 25 µl H2O was added to the 'mixture 4'.

Selective PCR was conducted with a combination of six pairs of primers (Table 2). The reaction mixture contained: H2O 3.55 µl, Taq buffer x 10 (Fermentas) 1 µl, MgCl2 (25mM) 1 µl, dNTP mix (10 mM, Fermentas) 0.25 µl, ANN EcoRI-primer (1 pmol*μl^{-1}) 1 µl, primer MseI-CNN (3 pmol*μl^{-1}) 0.5 µl, BSA (Fermentas) 0.8 µl, DNA polymerase (5 u*μl^{-1} Fermentas) 0.4 µl and 1.5 µl preselected mixture after PCR. Selective PCR thermal profile was as follows: initial denaturation at 94 °C for 2 min, 5 cycles of denaturation at 94 °C for 20 s, annealing from 66 °C to 58°C for 30 s. The temperature was lowered at 2 degrees each cycle, amplification at 72 °C for 2 min, 20 cycles of denaturation at 94 °C for 20 s, annealing at 56 °C for 30 s, the amplification at 72 °C for 2 min, final amplification at 72 °C for 15 min.

After selective PCR 2 µl of the product was added to the mixture of 24.5 µl of formamide and 0.5 µl of ROX500 (Lifetechnologies). Capillary electrophoresis after the selective PCR was performed on a ABI 310 under the following conditions: a module GS POP4 (1 ml), the filter F, the injection time of 12 s, separated for 30 min in temperature 60 °C.

Electropherograms obtained after separation of PCR products on the ABI 310 device were analyzed according to the method described by Rinehart, 2004 (modified). Obtained results were then verified manually. For each genotype, the whole analysis (restriction digestion, ligation of adapters, preselected and selective PCR) was performed in duplicate. Only peaks that were present in both repeats were scored.

Molecular data analysis

Each fragment that had been amplified was treated as a unit character and scored in terms of a binary code (1/0=+/-). The basic genetic diversity parameters were calculated: the polymorphism of amplification products (P – %), the mean number of observed alleles (na), and the PIC (Polymorphism Information Content), using the PowerMarker Software (Nei and Li, 1979; Liu and Muse, 2005). A matrix of Nei genetic distance (Ds) was calculated (Nei and Li, 1979). For calculation of the Ds the NTsys_pc2.2 software was used (Rohlf, 2009). For objects grouping, based on the genetic distances, the unweighted pair-group method with arithmetic mean (UPGMA) was used. The dendrogram was created with the Treecon ver. 1.34b software based on the Nei genetic distance matrix (Van de Peer and De Wachter, 1994).

RESULTS AND DISCUSSION

Phenotypic characteristics of the seed coat thickness

Performed variance analysis revealed significant differentiation of the genotypes studied with regard to proportion of the seed coat to the 1000 seed weight. Moreover the climatic conditions were found to significantly affect analyzed traits (Table 3). The thickest seed coat ranged from 11.9% to 17.6%, respectively for Mut-45 and the mutant line Mut-160 (Table1). Based on the Tukey test, these genotypes were classified to different groups. Described variation of the features in other studies which differentiate between wild accessions and the breeding lines of L. mutabilis is within 10.6 - 16% range, which is similar to our results. Line Mut-45 is in a lower range of the observed average properties values, making it useful in breeding programs focused on improvement of these species as a crop plant.

Table 3. Mean squares from variance analysis for seed coat percentage and 1000 seed weight

Source of variability	Degrees of freedom	Seed coat (%)	1000 seed weight (g)
Genotypes (G)	11	0,001365*	1128,50*
Years (Y)	1	0,003183*	101,11
Interaction G x Y	11	0,000367*	487,76*
Error	48	0,000049	69,99

*significance at the level α =0.05

The genotype Pop.1 was distinguished by the smallest seeds of the whole group under analysis, whilst line XM-5 was characterized by the largest ones (Table 1). Linear regression between the seed size and the percentage of seed coat was not significant (r=-0.39, p>0.05) between these traits (Fig. 2). The results could be interpreted as beneficial, because our goal was to break those undesired correlation. Moreover, the correlation coefficient indicates a tendency where larger seed genotypes have a thinner seed coat. Also, similar results were reported by Sawicka-Sienkiewicz et al. (2006) in collection of *L. angustifolius*. Such tendency was not found in results of same tests ran on the Mut-45 line, which points to the possibility that genotypes of fairly small seeds with a thinner seed coat are also available. Obtained results suggested that genetic-breeding investigations should include other significant factors, i.e. exposure of the genotype over the year's interaction which affects the expression of analyzed traits depending on the variations in climatic conditions within a given year (Table 3). It is also confirmed by the results of similar studies (Mera et al., 2004; Sawicka-Sienkiewicz et al., 2006; Clements et al., 2014; Mut et al. 2012).
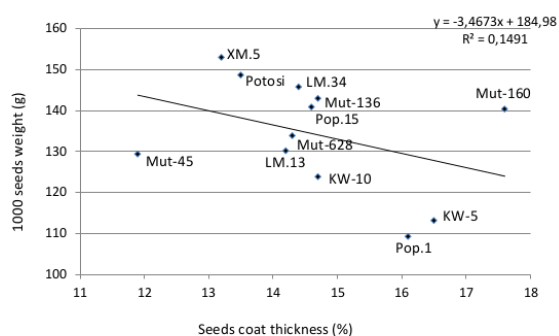

Figure 2. The linear regression between 1000 seed and seed coat proportion for analyzed genotypes *L. mutabilis*

Due to variation in the seed coat thickness in various tested types of lupin of low and high 1000 seeds weight, seed coat microscopic structure observations were carried out. In analyzed four of chosen lupine genotypes, the seed coat was composed of the following layers depicted in figures 1A-D (Fig. 1): cuticule, macrosclereids layer, osteosclereids layer and parenchymal cells. The seed coat thickness, seen in the photographs taken by SEM, ranged from 170 μm to 200 μm in particular cultivars and breeding lines of the Andean lupin (Fig. 1). The macrosclereids layer was the thickest (100 μm) in all seeds, while the layer of osteosclereids was the thinnest (≥30 μm). Figures 1A'A''–D'D'' show that in most of the investigated genotypes the seed coat surface was cristate-papillate, formed by elongated polygonal cells, as described by Planchuelo and Perisse (2006), but KW-5 characterized specific celss (Fig. 1C''). The shape of cells which build the surface layer of the seed coat in lupins is species specific and can be treated as a diagnostic feature in taxonomy of this group (Fig. 1) after expanding our study on next *L. mutabilis* accessions.

DNA polymorphism characteristic

With 24 ISSR primers used in the studied 22 generated amplification products diversified tested genotypes of Andean lupin. Those 22 primers allowed obtaining 138 bands, of which 94 (68%) were polymorphic. Two primers - UBC 876 and UBC 886 amplified the highest numbers of products – 11. First primers pair created all 11 polymorphic bands and the second one 4 out of 11 (Table 2). Primers UBC 814 and UBC 890 amplified product characteristic for line Mut-45 with lowest percentage of wall in the seed. Those characteristic bands were 830 and 500 bp in size respectively.

Based on genetic distances calculated for ISSR data the most similar genotypes are the epigonal line KW-5 and KW-10 (Ds 0.063). The highest value of genetic distance was observed between lines 'Potosi' and Pop.15 (DS 0.268). Distance between all analyzed genotypes illustrates UPGMA dendrogram (Fig. 3). The most distant from other analyzed genotypes was line LM.13. The largest cluster consisted of six lines: Mut-628, Mut-160, XM.5, Mut-136, KW-5 and KW-10.

After analyzing 547 amplification products generated by 6 AFLP primers pairs, it was found that differentiating products represented 66,8% (384). Primers pair AAG/CAG generated the highest number of products (124), where 91 of them were polymorphic. Combination AGC/CTA was characterized by the highest percentage of polymorphic product (Table2). Combinations: ACC/CTA(315), ACG/CAG(320), ACG/CAG(264) delivered characteristic products for thin-walled line Mut-45. Furthermore, combinations ACG/CAG(271), ACG/CAG(350), AGC/CTA(206), AGC/CTT(343) delivered products specific to line with the highest percentage of wall in the seed, the Mut-160 line.

Based on UPGMA (Fig. 3) analyzed together with AFLP data similarly as for ISSR data it was found that epigonal lines of KW make one cluster. The most distance objects were lines 'Potosi' and Pop.1. Values of Ds coefficients for 'Potosi' and both KW lines were highest (Ds 0.335 for 'Potosi' and KW-5 and 0.325 for 'Potosi' and KW-10).

Dendrogram created based on data obtained from the UPGMA method together with data from ISSR and AFLP in the best way illustrate diversity of investigated genotypes (Fig. 3). Analogously as it was with ISSR and AFLP dendrogram separately analyzed Portugal line 'Potosi' was the most distant from other genotypes, especially from the KW-5, LM.34 and KW-10 genotypes (Ds 0.327, 0.321, 0.317 respectively). The most similar genotypes were KW-5 and KW-10 (Ds 0.145), KW-5 and LM.34 (Ds 0.193) and KW-10 with LM.34 (Ds 0.177), Mut-628 and Mut-160 (Ds 0.175). Created dendrogram didn't reflect perfectly origin of analyzed genotypes. Genetically similar lines Mut-628 and Mut-160 were obtained in process of mutagenesis (400 Gy) from lines 21758 and 21756 respectively. What's more selection focused on early maturation conducted over several years could have played essential role in observed diversity of analyzed material.
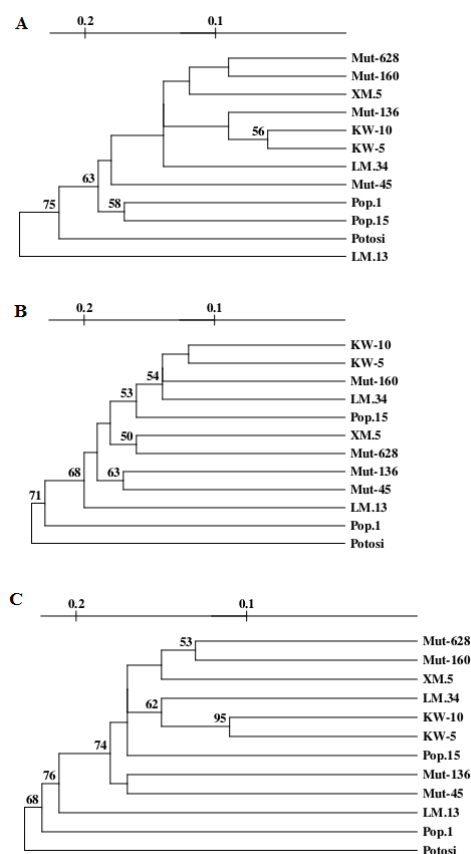
Figure 3. A - Dendrogram of 12 *Lupinus mutabilis* genotypes based on ISSR data using the Nei genetic distance matrix of similarity and UPGMA clustering method B - Dendrogram of 12 *Lupinus mutabilis* genotypes based on AFLP data using the Nei genetic distance matrix of similarity and UPGMA clustering method C - Derndrogram of 12 Lupinus mutabilis genotypes based on ISSR and AFLP data using the Nei genetic distance matrix of similarity and UPGMA clustering method

Values of correlation coefficient between obtained dendrograms based on ISSR, AFLP and ISSR and AFLP combined data were: 0.52 (ISSR vs. AFLP), 0.74 (ISSR vs. ISSR+AFLP) and 0.96 (AFLP vs. AFLP+ISSR) respectively. Dendrograms obtained based on ISSR and AFLP data were different from each other as suggested by low correlation coefficient value (r=0.52). Different dendrograms based on these two marker systems in Lupins diversity research were reported by Sbabou et al., (2010). Similarity clusters were obtained with AFLP and joined AFLP and ISSR data (r=0.96).

ISSR primers were generated much less products (mean 6 per one primer) in comparison to AFLP primers (mean 91 per primers pair). These results confirmed findings reported by other authors who also used those two genotyping methods (Hodkinson et al., 2002; Talhinhas et al., 2003; Carlier et al., 2004; Sarwat et al., 2008). Percentage of polymorphic products obtained by used both techniques used in our study were similar (73% for AFLP, 68% for ISSR). Information value (PIC) of both types of markers was identical and equaled 0.18. It suggests that effectiveness of AFLP and ISSR techniques in diversity determination of Andean Lupin is approximate. Observed values of genetic diversity among tested genotypes suggest relatively low diversity in

domesticated material - selected from those cultivated under similar conditions. Both used techniques grouped two epigonal lines KW-5 and KW-10 in one close related clad. This suggests different genetic background of those genotypes compared to lines with traditional type of growth. However, lines with epigonal type of growth (Pop.1, KW-5, KW-10) and common origin were selected under slightly different conditions and placed in different clad.

CONCLUSIONS

The research on lupins and also that on other leguminous plants such as pea, bean or soybean, involve studies which are aimed, amongst other, at acquisition of thin-walled pods and the thinnest possible seed coat (Clements et al., 2002). Lack of linear correlation in our study between analysed traits gives breeders an opportunity to select genotypes with medium seed weight and low seed coat proportion. The Mut-45 line seems to show potential to improve these properties. Moreover, this genotype was under environmental stress of Western Poland climatic conditions over number of years which led to selective development of its many current genetic traits of favorable agronomic values.

In the nearest future it will be necessary to undertake study of our *L. mutabilis* collection focused on proportionally lower share of wall content in a pod. The Andean lupin is characterized by the proportion of pod wall ranged from 29 to 47%, the white lupin at an approximate value of 27% and the narrow-leaved lupine varying from 32% to 35%. This factor approaches 13% in the pea and reaches as high as 24% in the bean (Clements et al., 2002). If lupin has to compete with other legumes, needs to be improved.

The ISSR and the AFLP markers allowed us to reveal an important polymorphism within the lupin plants. Moreover, products normally attributed to the thin-walled line of Mut-45 and those of the thick-walled Mut-160 line could yet prove to be advantageous in inventing markers which would be applied in further selection of thin-walled lines. The identified up till now markers AFLP and ISSR, specific to the Mut-45 and the Mut-160 respectively, should be yet subjected to, in the course of further research, the BLAST sequencing and analysis. Developed markers in near future could become useful in selecting genotypes with desired seed coat proportion. In particular, it can be applied to the Polish lupins collection.

LITERATURE CITED

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