JJBS

Jordan Journal of Biological Sciences

Volume 11, Number 1,March 2018
ISSN 1995-6673
Pages 47 - 56

Evaluation of Six Imported Accessions of *Lupinus albus* for Nutritional and Molecular Characterizations under Egyptian Conditions

Sherin A. Mahfouze[1*], Heba A. Mahfouze[1], Dalia M. F. Mubarak[2] and Ramadan M. Esmail[1]

[1]*National Research Centre, Genetic Engineering and Biotechnology Research Division, Genetics and Cytology Department;*

[2]*Soil and Water Use Department, Agricultural and Biological Research Division, Dokki, 12622, Egypt*.

Received July 26, 2017; Revised August 28, 2017; Accepted September 21, 2017

Abstract

White lupin (*Lupinus albus* L.) is an annual grain-legume widely harvest and cultivated in Egypt and also worldwide. Lupin seeds are utilized as food for the human and livestock nutrition. The aim of the present study is to estimate the genetic diversity among the seven genotypes of *L. albus*. In addition, the field performance of these genotypes was evaluated to drought tolerant under Egyptian conditions. Also, the macro- and microelements in the seeds of the white lupin were determined. In the present investigation, high significant differences among seven white lupin genotypes were observed for field performance traits under water deficit. Two accessions *L. albus* CGN 10106 and CGN 10108 were tolerant of drought compared with other genotypes. Thus, these genotypes recorded the highest seed yield when exposed to water stress. The macro- and microelement contents of seven white lupin genotypes were found to be different based on the genotype. In the present study, slight differences were observed in the total protein bands among the seven genotypes, thus polymorphism is low (28.57% polymorphism). These genotypes varied in expression from strong to low both Polyphenol Oxidase (PPO) and Peroxidase (POX) isozymes. So, the highest expression of antioxidant enzymes was recorded in genotypes tolerant of drought CGN 10106 and CGN 10108. On the other hand, Random Amplified Polymorphism DNAs (RAPD); Inter Simple Sequence Repeats (ISSR) assays recorded the percentage of polymorphism 47.96% and 29.82%, respectively. The Nei genetic similarity index ranged from 0.74 to 0.88 using UPGMA. These results are important in the breeding programs for the selection process of parental strains that feasibility the prediction of crosses to generate hybrids with the best performance.

Keywords: White lupin, yield, isozyme, RAPD, ISSR.

1. Introduction

White lupin (*Lupinus albus* L.; 2n = 50) is a member of the family *Fabaceae* (El-Enany *et al*., 2013; EL-Harty *et al*., 2016; Prusinski, 2017). It is sown as a crucial rotational yield. In addition, white lupin is beneficial in the diseases and weeds controlling in a crop rotation in a mixed agriculture program; lupin fixes nitrogen ($N_{2)}$ gas of the *atmosphere* and improving of the soil fertility. *L. albus* is an efficient scavenger of phosphorus (P) ascribable the existence of proteoid roots which release organic acids and make P more available (Neumann and Martinoia, 2002). White lupin seeds are utilized for the human and livestock nutrition (Barnevelda, 1999).

The genetic variability of *Lupinus* species has been characterized by agronomical and morphological characters (Andres *et al*., 2007), biochemical (Vaz *et al*., 2004) and molecular markers, such as Random Amplified Polymorphism DNAs (RAPD), Inter Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism (AFLP) (Talhinhas *et al*., 2003). Estimation of genetic variability depending on the morphological properties is not very authoritative, as it may be affected by the environment and the number of traits with recognized inheritance is few. Molecular markers have the distinguished advantages of being independent of climatic changes.

White lupin seeds are beneficial source of macro- and microelement contents. Essential elements are divided to macronutrients [Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Manganese (Mg) and Sulfur (S)] and micronutrients [(Iron (Fe), Copper (Cu), Manganese (Mn), Zinc (Zn), Boron (B), Molybdenum (Mo), Nickel (Ni) and Chlorine (Cl)] and the classification depends on the relative abundance in the plants. A significance of mineral constitution is due to their nutritional characterizations and good health effects, also needed for a healthy diet (Kırbaşlar *et al*., 2012). Iron is required for haemoglobin (Hb) and myoglobin (Mb) synthesis (Saleh-e-in *et al*.,

* Corresponding author. e-mail: : sherinmahfouze@yahoo.com.

2008). Sodium (Na), K and Ca also impact heart functions (Rajurkar and Damame, 1997). Numerous metabolic processes need Mg, Fe and K. Zn plays main role in several immunological and biochemical functions (Özcan *et al*., 2013).

The aim of this study is to estimate the genetic diversity among the seven genotypes of *L. albus*. In addition, the field performance under water stress and the essential and non-essential elements of seven white lupine genotypes were determined.

2. Materials and Methods

2.1. Plant Materials

The six white lupin accessions imported from (Centre for Genetic Resources, The Netherlands) and one local cultivar Balady were used in this investigation. Names, pedigree and origin of lupin genotypes are presented in Table 1. These materials were evaluated in 2015/16 growing season in field experiment under water stress conditions in a Randomized complete block design with three replications. Plants received two only irrigations through the whole season. The plot size of one row was 0.60 m x 4 m. Lupin seeds were planted on 13th of November in hills with 0.25 m apart on one side of ridge in Delta region at Shebin El-Kom, Menofiya Governorate, Egypt. Yield components and the other related traits, plant height (cm), No. of pods/plant, No. of seed/pod, No. seeds/plant, 100 seed weight (g) and seed yield/plant (g) were measured at harvesting.

Table 1. Pedigree of genotypes used in this study.

No.	Accessions	Type	Name	Country
1	*Lupinus albus* CGN 10105	Research material	N106/50	Italy
2	*L. albus* CGN 10106	Research material	N107/50	Italy
3	*L. albus* CGN 10108	Research material	N121/50	Italy
4	*L. albus* CGN 10109	Research material	N122/50	Italy
5	*L. albus* CGN 10112	Land variety	Przehendowski Wezesnv	Poland
6	*L. albus* CGN 10113	Land variety	Kisordai Feheroiragu	Hungary
7	*L. albus* cv. Balady	Land variety	Balady	Egypt

2.2. Macro- and Micro-Nutrient Analyses

The seeds of seven white lupin genotypes were milled after being oven-dried at 40°C, and then kept in sealed vials for further analyses. A portion of the dried samples was dissolute in acids mixtures to be digested as described by Cottenie *et al*., (1982).

Macro- and micro-nutrients were determined in the digested aliquots. Magnesium, iron, manganese, zinc and copper were measured by atomic absorption spectroscopy (AAS, Unicam 939 AA Spectrometer); sodium, potassium and calcium by flame emission (Cottenie *et al*., 1982). Total nitrogen was determined by kjeldahl method and phosphorus was determined by ammonium-vanadate and molybdate method according to Motsara and Roy (2008).

2.3. Electrophoretic Analysis of Protein by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was done according to (Laemmli, 1970) as modified by (Studier, 1973).

2.4. Polyphenol Oxidase (PPO) and Peroxidase (POX) Isoforms.

For the assay of antioxidant enzymes Peroxidase (POX) and Polyphenol Oxidase (PPO) were extracted based on the method described in (Stagemann *et al*., 1985). PPO and POX isozymes were separated by Native-Polyacrylamide Gel Electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to (Baaziz *et al*., 1994).

2.5. Extraction of Genomic DNA

Young plant leaves of seven white lupin genotypes were soaked in liquid nitrogen for DNA extraction using 2% (CTAB) cetyl trimethyl ammonium bromide (Borsch *et al*., 2003).

2.6. RAPD Analysis

A total of five primers were used to amplify DNA (Mahfouze *et al*., 2012) (manufactured by Bioneer, New technology certification from ATS Korea). The total reaction mixture was 25 µl contained 10X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 *p*mol primer, 1.25 U *Taq* polymerase and about 150 ng genomic DNA. RAPD-PCR amplification was performed in thermal cycler (Biometra Inc., Germany). The temperature profile was as follows: An initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation temperature 94°C for 5 min; annealing temperature 37°C for 1 min and extension temperature 72°C for 1 min, final extension at 72ºC for 5 min.

2.7. ISSR Profiles

A total of four anchored ISSR primers were used to amplify DNA (Life Technologies, Gaithersburg, Md.). Each 25-µl amplification reaction consisted of 2.5 µl 10X PCR buffer, 2.5 µl 25 mM $MgCl_2$; 0.5 µl 40 mM dNTPs; 1 µl *Taq* DNA polymerase (1 unit/µl); 2 µl 0.4 µM primer. Amplification was carried out in DNA thermocycler (Biometra, Germany) under the following conditions: one cycle of 94°C at 3 min, followed by 28 cycles of denaturation temperature 94°C/45 sec, annealing temperature 52°C/30 sec and extension temperature 72°C/2 min at; a final extension 72°C/6 min.

Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and 0.5 µg/ml ethidium bromide at 90 V. Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

2.8. Data Analysis

A matrix for SDS-PAGE, POX, PPO, RAPD and ISSR combined was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the genotype. Genetic similarity coefficients were computed according to (Nei and Li, 1979). The data were subsequently used to construct a dendrogram using the un-

weighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 (Rohlf, 2000). Correlation coefficients were calculated using similarity coefficients obtained from combined SDS-PAGE, POX, PPO, RAPD and ISSR analysis.

2.9. Statistical Analysis

The data were analyzed by ANOVA procedure of program SPSS (1995) statistical procedures version 21 (Chicago, USA) (www.spss.com).

3. Results

3.1. Field Performance

The analysis of variance of all traits studied is presented in Table 2. Highly significant differences among seven white lupin genotypes were recorded for all the traits studied, indicating the presence of a considerable genetic diversity among the tested lupin genotypes. Also, these variations among genotypes might partially reflect their different genetic backgrounds.

Mean performance of seed yield and its componenets for the seven lupin genotypes are presented in Table 3. All the six forign genotypes exhibited exceeded the plant height of the local landraces Balady. With regard to the number of pods/plant means ranged from 3.67 pods for the genotype CGN 10105 to 12 pods per plant for the genotype CGN 10108. Significant differences were found among genotypes for number of seeds/pod and the highest number found in genotype CGN 10106 (5.67 seeds/pod). Concerning the number of seeds/plant, genotype CGN 10106 had a larger number of seeds/plant (63.67), followed by CGN 10108 (58.67 seeds/plant). Also, results showed that the genotypes CGN 10106 and CGN 10108 had the highest values of seed yield. Moreover, these lines had the highest values for 100 seed weight.

Table 2. Mean square values for all studied characters among seven lupin genotypes evaluated in the growing season 2015/16.

S.O.V	D.F	Plant height.(cm)	No. of Pods/ plant	No. of seeds/pod	No. of seeds/plant	100 seed weight (g)	Seed yield /plant (g)
Reps	2	1.286	0.190	0.190	22.333	0.136	2.086
Genotypes	6	200.603**	28.857**	1.937**	974.429**	38.69**	146.17**
Error	12	26.175	1.857	0.746	22.667	1.069	2.451

** Significant at $p = 0.01$; * significant at $p = 0.05$.

Table 3. Mean performance and standard error for all studied characters in seven white lupin genotypes evaluated in the growing season 2015/16.

Genotypes	Plant height (cm)	No. of Pods/ plant	No. of seeds / Pod	No. of seeds / plant	100 seed weight (g)	Seed yield /plant(g)
L.albus CGN 10105	58.67±1.85	3.67±0.33	5.67±0.32	21.00±3.00	33.85±0.25	7.09±0.98
L. albus CGN 10106	70.00±2.88	11.33±0.88	5.67±0.33	63.67±1.8	34.78±0.57	22.13±0.56
L. albus CGN 10108	69.00±2.08	12.00±0.88	5.0±0.57	58.67±1.33	36.58±0.26	21.43±0.36
L. albus CGN 10109	64.33±2.60	9.0±0.57	5.30±0.33	48.0±4.16	34.37±0.51	16.50±1.43
L. albus CGN 10112	62.33±0.65	6.0±0.58	5.33±0.67	31.33±2.40	33.20±0.09	10.39±0.76
L. albus CGN 10113	52.67±1.45	6.66±0.60	3.66±0.33	24.0±0.01	25.71±1.17	6.17±0.81
L. albus cv. Balady	48.00±1.53	5.67±0.66	4.0±0.58	22.66±3.92	30.53±0.24	6.92±1.21

3.2. Macro- and Micro-Elements of White Lupin Seeds

Studying the nutrients' content in the seeds of *L. albus* are important from a human nutritional point of view as well as from the preferences of which genotype is best for agriculture. Generally, content of macro- and micro-nutrients in white lupin seeds based on the genotype (Tables 4 and 5). The highest content of N was recorded for genotype CGN 10106; however, the differences in this element's content, among the studied genotypes, cannot be statistically considered. CGN 10113 seeds showed the best genotype in K, P and Na contents reached 1.78, 0.334 and 0.289 g 100 g^{-1} dried material, respectively; followed by the seeds of Balady cultivar. The influence of the genotypes on the contents of Ca and Mg were not statistically significant. Nevertheless, the data showed that the CGN 10105 and CGN 10112 genotypes were the highest content of Mg and Ca, respectively, compared with the other genotypes (Table 5). On the other hand, the results revealed that the reduction of the K contents was depicted in CGN 10105 and CGN 10108 genotypes. In addition, the lowest contents of Na were found in CGN 10112 compared with the other genotypes (Table 5).

Among all the examined genotypes, CGN 10112 and CGN 10113 accessions and Balady cultivar had the superior content of the micronutrients (Tables 6 and 7). Wherein the nutrients increased as follow: Fe by (0.14 – 0.35), Mn by (0.7 – 1.5), Zn by (0.3 – 0.7), and Cu by (0.11 – 0.34) fold, compared with the other studied genotypes. Furthermore, the minimum contents of Fe and Cu nutrients were exhibited in the CGN 10106 genotype. According to, Mn and Zn were recorded in CGN 10108 and CGN 10105 genotypes, respectively. The results indicated the significant influence of the genotypes on the content of some nutritional elements in the white lupin seeds (Table 7).

Table 4. Analysis of variance of macronutrients element contents (%) in white lupin genotypes

S.O.V	D.F	N	P	K	Ca	Mg	Na
Reps	2	0.886	0.043	0.056	0.001	0.003	0.001
Genotypes	6	0.450**	0.005**	0.122**	0.027ns	0.005ns	0.003**
Error	12	0.131	0.001	0.009	0.030	0.004	0.001

** Significant at $p = 0.01$; * significant at $p = 0.05$., ns = not significant.

Table 5. Mean values ± SE for macronutrients element (%) in seven white lupin genotypes studied (in milligrams per 100 gram)

Genotypes	N	K	P	Mg	Ca	Na
L. albus CGN 10105	4.13±0.35	1.28±0.068^{b}	0.26±0.004^{c}	0.22	1.09	0.24±0.012^{b}
L. albus CGN 10106	4.30±0.32	1.32±0.083^{b}	0.23±0.014^{c}	0.11	1.16	0.23±0.003^{b}
L. albus CGN 10108	3.65±0.19	1.28±0.021^{b}	0.24±0.015^{c}	0.11	1.28	0.24±0.009^{b}
L. albus CGN 10109	4.03±0.362	1.29±0.014^{b}	0.27±0.002^{c}	0.14	1.09	0.218 ± 0.006^{b}
L. albus CGN 10112	3.67±0.098	1.38±0.067^{b}	0.297±0.007^{b}	0.12	1.34	0.211±0.012^{b}
L. albus CGN 10113	3.27±0.372	1.78±0.140^{a}	0.334±0.013^{a}	0.111	1.26	0.289±0.003^{a}
L. albus cv. Balady	3.38±0.142	1.64±0.027^{a}	0.319±0.008^{b}	0.114	1.23	0.286±0.011^{a}

Values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$).

Table 6. Analysis of variance of micronutrients element contents (%) in white lupin genotypes.

S.O.V	D.F	Fe	Mn	Zn	Cu
Reps	2	0.001	78.619	7.620	0.147
Genotypes	6	260.475**	17414.52**	9.982**	1.989**
error	12	18.329	201.76	3.237	0.116

** Significant at $p = 0.01$; * significant at $p = 0.05$.

Table 7. Mean values ± SE for micronutrients element (%) in seven white lupin genotypes studied (in milligrams per 100 gram).

Genotypes	Fe	Mn	Zn	Cu
L. albus CGN 10105	78.34±1.17	194.33±2.96^{c}	7.77±0.296	6.15±0.406^{b}
L. albus CGN 10106	66.53±2.84	194.67±2.60^{c}	8.65±1.16	6.00±0.037^{b}
L. albus CGN 10108	70.40±0.58	153.33±10.98^{d}	9.01±2.39	6.22±0.243^{b}
L. albus CGN 10109	76.53±0.267	222.67±4.05^{c}	9.54±0.427	7.25±0.017^{a}
L. albus CGN 10112	87.86±1.07	268.67±10.08^{b}	12.87±0.712	7.63±0.070^{a}
L. albus CGN 10113	89.7±0.91	384.33±10.34^{a}	11.29±0.442	7.41±0.151^{a}
L. albus cv. Balady	88.83±1.93	275.33±8.25^{b}	11.36±0.981	8.02±0.163^{a}

Values followed by the same letter are not significantly different according to *Duncan's* multiple range test ($p < 0.05$).

3.3. SDS-PAGE

The electrophoresis of the total protein extracted from the leaves of seven white lupin genotypes determined by SDS-PAGE as shown in Figure 1. SDS-PAGE revealed that, seven *L. albus* genotypes were rich with protein content depending on number of bands on the gel. The electrophoregrams were estimated depending on their molecular masses. A total of 14 polypeptide chains were recorded ranging from 4.5 to 250 kDa; ten of these were monomorphic (71.43%), while four were polymorphic (28.57% polymorphism). The highest number of polypeptides scored in accessions CGN 10106 and CGN 10113 (14 polypeptides), followed Balady cultivar (13 bands) and CGN 10109 (12 subunits). However, the lowest number of subunits detected in accessions CGN 10105, CGN 10108 and CGN 10112 (ten subunits). On the other hand, one unique band with molecular weight 170 kDa scored in two accessions CGN 10106 and CGN 10113 (Figure 1).

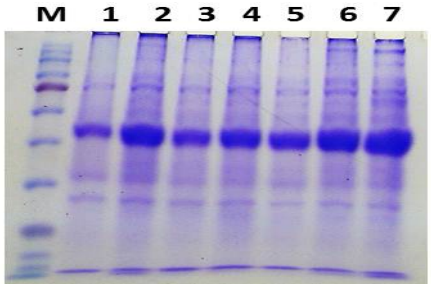

Figure 1. SDS-PAGE banding patterns of leaf protein extracted from seven genotypes *Lupinus albus*. Lane M: Marker protein. Lane 1: CGN 10105; lane 2: CGN 10106, lane 3: CGN 10108; lane 4: CGN 10109; lane 5: CGN 10112; lane 6: CGN 10113 and lane 7: Balady cultivar.

3.4. POX and PPO Isozymes

Isozyme spectra of two tested isoforms (PPO and POX) were determined by native-PAGE in leaves of seven white lupin genotypes as shown in Figure 2. POX recorded three isoforms with *Rf* value ranging of 0.293 to 0.693. The highest expression was scored in CGN 10105, CGN 10106, CGN 10108 and CGN 10109 (three alleles), followed by CGN 10113 (two isoforms). However, the lowest expression was found in two genotypes CGN 10112 and Balady cultivar (one isoform) (Figure 2).

The Isozyme spectra of PPO of all studied *L. albus* genotypes, composed of four detectable bands with *Rf* value, ranged from 0.320 to 0.620 (Figure 2). Accessions CGN 10108 and CGN 10109 had a strong expression (four alleles). On the contrary, accession CGN 10106 gave a weak expression (two isoenzymes). However, the other genotypes recorded a moderate expression (three isoforms) (Figure 2).

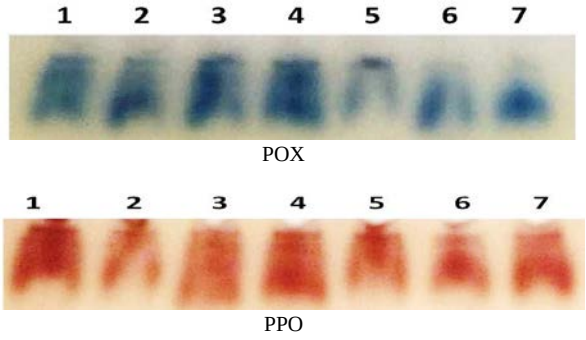

Figure 2. POX and PPO isozyme profiles of seven genotypes Lupinus albus.. Lane 1: CGN 10105; lane 2: CGN 10106, lane 3: CGN 10108; lane 4: CGN 10109; lane 5: CGN 10112; lane 6: CGN 10113 and lane 7: Balady cultivar.

3.5. RAPD Analysis

Five decamer RAPD primers (10 nucleotide length) from arbitrary nucleotide sequences were used to amplify seven *L. albus* genotypes (Figure 3 and Table 8). A total number of 98 scorable bands were amplified by five RAPD primers (19.6 bands per primer), ranging from 90 to 1730 bp (RAPD-2) (Figure 3 and Table 8). Fifty-one out of 98 fragments were common bands (52.04%), 47 loci were polymorphic (47.96%). The number of fragments per primer varied from 16 (RAPD-1) to 26 (RAPD-2). Primer RAPD-1 scored the highest number of polymorphism (75%), followed by primer RAPD-5 (58.82%). However, Primer RAPD-3 recorded the lowest number of polymorphism (29.41%). On the other hand, 14 out of the 98 were unique markers (14.29%) (Table 8). CGN 10105 appeared the maximum number of positive and negative markers (six) with molecular sizes (+180; +191 and +500 bp) and (-700; -703 and -1510 bp), respectively. However, CGN 10108, CGN 10109 and Balady cultivar scored two bands of (+1730 and -185 bp); (+160 and +382); and (+730 and +781) bp, respectively. In contrast, CGN 10106 revealed the minimum number of specific bands (one) of +133 bp (Table 8).

Table 8. RAPD-PCR analysis, a total number of loci, monomorphic, polymorphic, unique loci of seven *L. albus* genotypes

Primer Code No.	Primer sequences	Size range of the scorable loci (bp)	Total loci	No. of monomorphic loci	No. of polymorphic loci	% Polymorphism	Unique loci	Molecular size of markers (bp)
RAPD-1	GTTTCGCTCC	160 -1600	16	4	12	75	3	+160; +730; -703
RAPD-2	AACGCGCAAC	90-1730	26	16	10	38.46	2	+1730; -482
RAPD-3	CCCGTCAGCA	133-805	17	12	5	29.41	3	+133;+382;+500
RAPD-4	GGACGGCGTT	100-1510	22	12	10	45.45	2	-185; -1510
RAPD-5	AAGCCCGAGG	180-1500	17	7	10	58.82	4	+180; +191;+ 781; -700
Total		**90-1730**	**98**	**51(52.04%)**	**47**	**47.96%**	**14**	**14.29%**

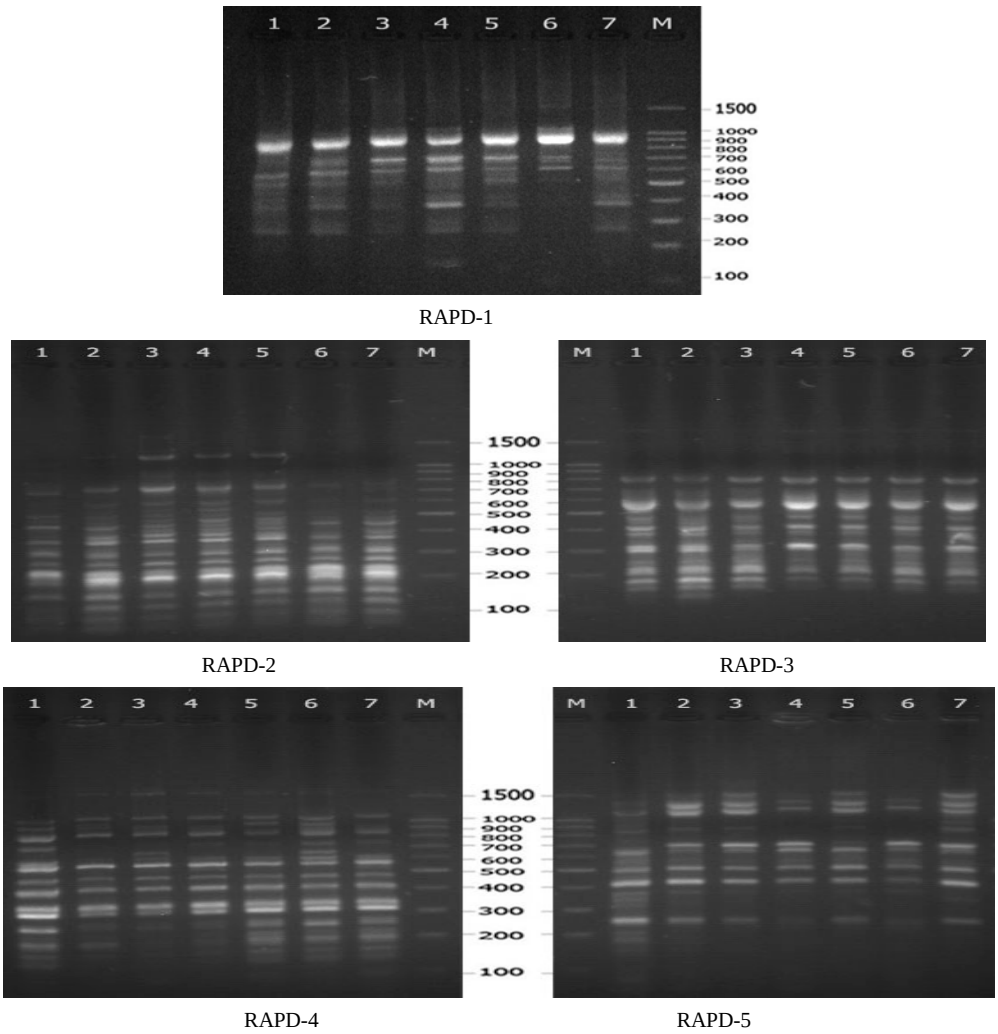

Figure 3. Amplified products of RAPD-PCR using of five primers for analyzed seven genotypes *Lupinus albus*. Lane M= DNA ladder 100 bp. Lane 1: CGN 10105; lane 2: CGN 10106, lane 3: CGN 10108; lane 4: CGN 10109; lane 5: CGN 10112; lane 6: CGN 10113 and lane 7: Balady cultivar.

3.6. ISSR Profiles

The four primers were used in ISSR loci, produced a total of 57 amplified fragments, ranging from 185 (ISSR-4) to 1500 (ISSR-1) bp. Forty amplicons were monomorphic (70.18%), 17 were polymorphic (29.82%) of the total number of bands (Figure 4 and Table 9). The number of amplicons using single primers ranged from 11 (ISSR-2) to 21 (ISSR-1) with a mean of 14.25 bands per primer. The highest degree of polymorphic among accessions for each primer was 33.33% for primers (ISSR-1 and ISSR-4), followed by ISSR-3 (30.77%). However, the lowest degree of polymorphism was scored in primer ISSR-2 (18.18%). The polymorphism of all amplification fragments was 29.82% for the genotypes investigated. Furthermore, seven out of the 57 bands were unique markers (12.28%). On other hands, CGN 10105 record the maximum number of unique loci (four) with molecular sizes (+433; +491; +619 and +1200 bp). Followed, CGN 10109 scored three specific bands of (+530; +595 and +715 bp) (Table 9). On the contrary, the other accessions have not showed any markers.

Table 9. ISSR-PCR analysis, a total number of loci, monomorphic, polymorphic, unique loci of seven *L. albus* genotypes.

Primer Code No.	Primer sequences	Size range of the scorable loci (bp)	Total loci	No. of monomorphic loci	No. of polymorphic loci	% Polymorphism	Unique loci	Molecular size of markers (bp)
ISSR-1	$(CA)_6AC$	191-1500	21	14	7	33.33	2	+433, +1200
ISSR-2	$(CT)_8GC$	216-740	11	9	2	18.18	1	+619
ISSR-3	$(GA)_6CC$	202-1441	13	9	4	30.77	1	+491
ISSR-4	$(CAC)_3GC$	185-850	12	8	4	33.33	3	+530, +595, +715
Total		**185-1500**	**57**	**40(70.18%)**	**17**	**29.82%**	**7**	**12.28%**

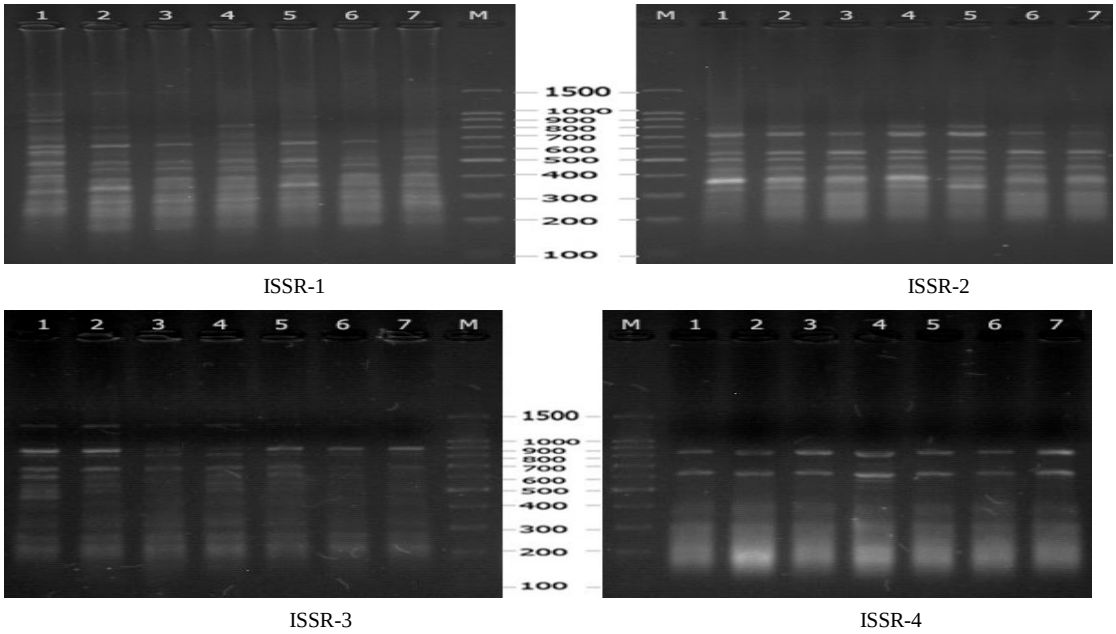

Figure 4. Amplified products of ISSR-PCR using of four primers for analyzed seven genotypes *Lupinus albus*. Lane M= 100 bp DNA ladder. Lane 1: CGN 10105; lane 2: CGN 10106, lane 3: CGN 10108; lane 4: CGN 10109; lane 5: CGN 10112; lane 6: CGN 10113 and lane 7: Balady cultivar.

3.7. Cluster Analysis

The Nei genetic similarity index ranged from 0.74 between (CGN 10105 and CGN 10108); (CGN 10105 and CGN 10109); (CGN 10105 and CGN 10113) and (CGN 10109 and CGN 10113) to 0.88 between (CGN 10108 and CGN 10109) (Table 10). The genetic identity between *L. albus* genotypes felled into the range of 0.74 to 0.88 as shown in the UPGMA tree (Figure 5)

Three major groups were observed. The lupin genotypes CGN 10106; CGN 10113 and Balady cultivar were put in the first group (I): (similarity range 0.74 to 0.83), whereas the accessions CGN 10108, CGN 10109 and CGN 10112 were placed within the second group (II): (similarity range 0.74 to 0.88). However, CGN 10105, which were put in the third group: (similarity range 0.74 to 0.81), referred to be the most distinct but joined with groups I and II (Figure 5).

Table 10. Genetic similarity and genetic distance statistics for seven genotypes of *L. albus.*

Accessions	*L. albus* CGN 10105	*L. albus* CGN 10106	*L. albus* CGN 10108	*L. albus* CGN 10109	*L. albus* CGN 10112	*L. albus* CGN 10113	*L. albus* cv. Balady
L. albus CGN 10105	1.00						
L. albus CGN 10106	0.81	1.00					
L. albus CGN 10108	0.74	0.81	1.00				
L. albus CGN 10109	0.74	0.80	0.88	1.00			
L. albus CGN 10112	0.77	0.81	0.86	0.86	1.00		
L. albus CGN 10113	0.74	0.83	0.79	0.74	0.78	1.00	
L. albus cv. Balady	0.79	0.83	0.80	0.78	0.82	0.83	1.00

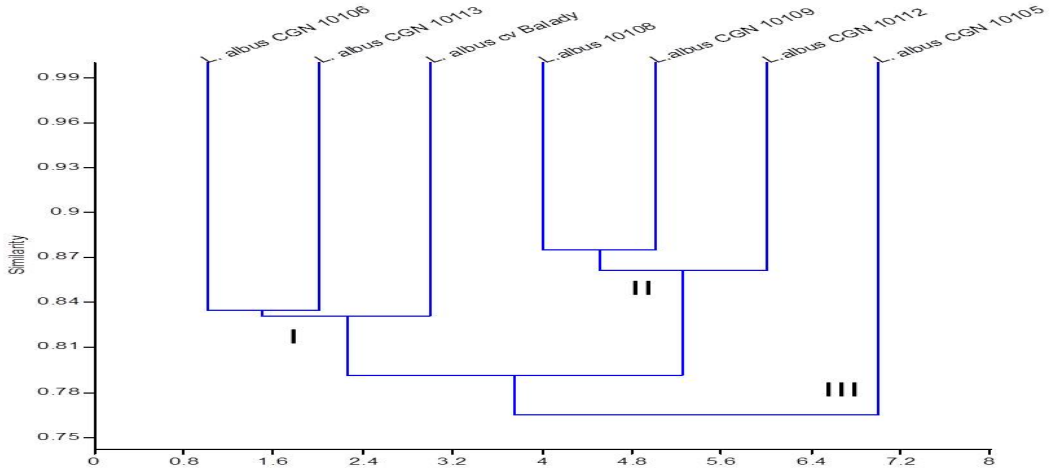

Figure 5. UPGMA dendrogram of seven genotypes *Lupinus albus* depend on Jaccard"s similarity coefficient

4. Discussion

White lupin (*L. albus* L.), is a member of the family *Fabaceae*. Lupin seeds are used as source of a protein for the human and animal nutrition due to their nutritional value (high in lipids, protein and dietary fiber). The present study is conducted to characterize the genetic relationships among six white lupin imported from Centre for Genetic Resources, and one local Egyptian cultivar Balady. Seven genotypes of white lupin seeds were exposed to water deficit. Our results showed highly significant differences in the field performance and drought tolerant among tested seven genotypes. Two accessions CGN 10106 and CGN 10108 were tolerant of drought compared with other genotypes. Thus, these genotypes recorded the highest seed yield when exposed to water stress. These results are in agreement with Annicchiarico *et al*., (2010), Mut *et al.,* (2012) and EL-Harty *et al*., (2016) who found significant differences among the Egyptian landraces of white lupin in crop components in various environments but seasonal variance was non-significant for the number of branches, plant height, seeds pod^{-1} and pods $plant^{-1}$.

In the present investigation, the content of the macronutrients in the studied genotypes was compared with those of other studies on different legume seeds, including *L. albus*, carried out by Özcan *et al*., (2013). From the previous results, it is observed that the highest concentration of N recorded in (CGN 10106); (K, P and Na (CGN 10113 and Balady); Mg and Ca (CGN 10105 and CGN 10112) genotypes. However, the maximum contents of micro-nutrients were scored in genotypes CGN 10112, CGN 10113 and Balady. Concentration of micronutrients in the studied white lupin seeds was of less values than those in the *L. albus* reported by Özcan *et al*., (2013) and of similar or higher values than those reported in other varieties (Bartkiene *et al*., 2016). In general, essential elements are necessary for physiological and metabolic processes in the human body (Alsafwah *et al*., 2007; Bartkiene *et al*., 2016).

Several biochemical (protein and isozyme) and molecular markers (RAPD and ISSR) have been used to assess the genetic diversity of the seven *L. albus* genotypes. In the present study, slight differences were observed in the total protein bands among seven *L. albus* genotypes, thus polymorphism was low (28.57%). These results are in agreement with Muzquiz *et al*., (2011) who mentioned that lupins contained the major storage proteins, such as albumins and three globulin kinds: α, β, γ-conglutin (Melo *et al*., 1994). β- conglutin is the main constituent; it acts the largest heterogeneity among white lupin species, showing several polypeptide chains with molecular weights ranging from (15 to 72 kDa). The α-conglutin fraction consists of heavy polypeptide subunits with molecular masses from (31 to 63 kDa) and a lighter polypeptide subunit of 20 kDa, and γ-conglutin, usually the minor constituent, including two polypeptide chains (the first chain 17 kDa and the second chain 27-30 kDa) (Melo *et al*., 1994).

Isozyme spectra of PPO and POX isoforms were determined by native-PAGE in leaves of seven *L. albus* genotypes. Our results revealed that seven white lupin genotypes varied in expression from strong to low both PPO and POX isozymes. The highest expression of antioxidant enzymes was recorded in two genotypes tolerant of drought CGN 10106 (POX) and CGN 10108 (POX and PPO). Therefore, antioxidant isozymes play a main role in the tolerance of the plant to water stress. These findings agree with those obtained by Horáček *et al*., (2009) who found that the variations in the isozyme have a great importance in the plants' breeding programs, especially in defense reactions to biotic and abiotic stresses.

All the assays used in the present study were able to uniquely fingerprint each of the seven white lupin genotypes. RAPD recorded the highest percentage of polymorphism 47.96%. On the contrary, for ISSR gave 29.82% polymorphism. Comparing between RAPD and ISSR loci, the ISSR has the capability of scoring more polymorphism to the primers barely amplify the non-coding regions of the white lupin genome, which are highly polymorphic. The RAPD loci amplifies both coding and non-coding DNA sequence of the white lupin genome, but when it amplifies in one region it does not amplify in another, decreasing the possibility of amplifying the most polymorphic sequences. According to reproducibility, the ISSR profile was indicated to be more specific in that it employs greater primers and needs higher annealing temperatures lessens the non-reproducibility that is so highly linked with RAPD (McGregor *et al*., 2000). Yorgancilar *et al*., (2009) used ISSR and RAPD loci to estimate the genetic variability among 20 old world lupin accessions and obtained that there are relationships between Egyptian and some American accessions and found that American genotype was screened from Egyptian origin materials. Talhinhas *et al*., (2003) as well as Al-Rawashdeh and Al-Rawashdeh (2015) reported that the low genetic similarity among *Lupinus* spp is most unlikely to be due to the differences in coding. You *et al*., (2005) and Yuan *et al*., (2005) mentioned that both RAPD and ISSR loci are dominant markers and their combination showed that *L. albus*, *L. luteus* and *L. angustifolius* were put in three different groups with minor genetically distances between the individuals of each group. Also, ISSR profiles are recognized to be more sensitive than RAPD technique which is again confirmed here.

Our results showed that biochemical (protein and isozymes) molecular (RAPD and ISSR) markers are beneficial to characterize the genetic diversity and evaluation of genetic distances among seven *L. albus* genotypes. Also, a combination among these assays could detect polymorphism in the tested seven *L. albus* genotypes to distinguish each genotype from the others by the unique band. Moreover, these results are important in the breeding programs for the selection process of parental strains that feasibility the prediction of crosses to generate hybrids with the best performance and drought tolerant.

5. Conclusion

The seven white lupine genotypes showed different responses under water deficit stress conditions. Analysis of variance (ANOVA) revealed that there are significant differences among the seven tested genotypes under water deficit stress. Two accessions, *L. albus* CGN 10106 and CGN 10108, were tolerant against drought compared with

other genotypes. The macro- and micro-element contents of seven *L. albus* genotypes were found to be different based on the genotype. The highest content of N was recorded for genotype CGN 10106. However, CGN 10113 seeds showed the best genotype in K, P and Na contents; this is followed the seeds of Balady cultivar. Nevertheless, CGN 10105 and CGN 10112 genotypes scored the highest content of Mg and Ca, respectively. On the other hand, the results revealed that the reduction of the K contents was depicted in CGN 10105 and CGN 10108 genotypes. In addition, the lowest contents of Na were found in CGN 10112. RAPD and ISSR analyses recorded the percentage of polymorphism 47.96% and 29.82%, respectively. The Nei genetic similarity index among genotypes ranged from 0.74 to 0.88, based on biochemical and molecular markers combined using UPGMA. So, these genotypes could be used in the future white lupin breeding programs in Egypt.

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