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Differences in Salinity Tolerance, Nutrient Concentrations, and Gene Expression among New Accessions of *Lupinus albus* L. under Greenhouse Conditions

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Abstract

Salinity is one of the main abiotic stresses, which has a major effect on plant productivity. Finding out genotypes with the ability for production under salinity stress could be one of the promising strategies to cope with this problem. In the present study, seven genotypes of *Lupinus albus* L. were exposed to three different levels of salinity stress (2, 4 and 8 dS m^{-1}). Physiological and biochemical responses such as nutrient concentrations, protein content, and peroxidase (POX) activities in the leaves were investigated. In addition, the genetic diversity using Sequence-related amplified polymorphism (SRAP) among the seven genotypes was determined. The current work shows that two white lupin accessions were tolerant to salt stress, namely CGN 10106 and CGN 10112. However, CGN 10102, CGN10104, and CGN10108 genotypes were moderately-tolerant. In contrast, CGN 10109 and Balady showed sensitivity to salt stress. The nutrient balance was influenced differently according to the interaction between the genotypes and salinity. The SDS-PAGE analysis of the total proteins extracted from the leaves of seawater-stressed plants and the control recorded an increase or decrease in the protein content depending on the genotype. Furthermore, salinity induces the synthesis of new proteins in both of the tolerant and sensitive genotypes. Activities of antioxidant defense appeared to be associated with the differential regulation of distinct POX. POX isoenzymes displayed an increase or decrease in the genotypes' tolerance to seawater stress. On the other hand, the SRAP technique was used to amplify coding regions of DNA of the tested seven white lupin genotypes with eleven primers targeting open-reading frames (ORFs). Eleven polymorphic SRAP primer sets generated fifty-one alleles with average 0.710 polymorphism information content (PIC). The percentage of polymorphic bands was 35.29 %. The UPGMA dendrogram, showing genetic similarity among seven genotypes, was clustered into three groups ranging from 0.80 to 0.94. The results show the possibility of using diluted seawater to grow white lupin plants taking into consideration the suitable genotype for the available dilution factor.

Keywords: White lupin, Seawater, Salt stress tolerance, Protein, Peroxidase isozymes, SRAP marker.

1. Introduction

Greenhouse farming is a widely applied cultivation system to supply a controlled environment convenient for optimal crop production **(**Yazgan *et al*., 2008**)**. Recently, the Egyptian government is planning the construction of 100,000 of novel glass-houses for increasing horticultural crops such as vegetable and fruit crops in the reclaimed lands. In the reformed soil, saline water can be applied for irrigation due to the absence or a limited supply of fresh water. Furthermore, the groundwater used for irrigating glass-houses near the coast regions is often saline. A number of researches on the subject were performed in this respect. The tolerance of horticultural crops to salinity level may alter depending on the meteorological and land conditions in the area including irrigation methods (Wu *et al*., 2001; Katerji *et al*., 2003). It is also suggested that a seawater desalination technology is applied to treat the salinity troubles of irrigation water and land in the glass-houses located in littoral regions (Zarzo *et al*., 2013). In designing the desalination technology, the target salinity level of irrigation water essentially affects the product water cost. Accordingly, it is important to study the tolerance of salt for crops cultivated in the glass-house conditions to estimate the optimal salinity of irrigation water to reduce the negative effects on crop production.

Salt stress induces two different kinds of stress. Osmotic stress produced from the high solute concentrations and low soil water potential primarily limit the growth of plants through the first stage of salt stress (Sümer *et al*., 2004). Salt-stressed plants rarely suffer from wilting (De Costa *et al*., 2007). In the second stage of salinity, ions aggregate in the plants and may reach to toxic concentrations. Generally, sodium (Na^+) and chloride (Cl^-) ions are the prevailing ions in saline lands. Both Na^+ and Cl^- ions may expose harmful impacts on the metabolism of the plant and may induce growth inhibition of salt-sensitive cultivars or species (Marschner, 1995). Salinity triggers a broad assortment of plant responses,

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ranging from changing gene expression and cellular metabolism for alterations in the rate of growth and crop yield (Amor *et al*., 2005). The plant's ability to adapt with these conditions depends on the cultivar or species such as the ability of plants to perceive the stimulus, produce and transfer signals and instigate bio-chemical alterations that adjust the metabolism (Dolatabadian and Saleh, 2009). A study on white lupin states that salinity decreased growth, the rate of transpiration, pigments content, and photosynthetic rate (Fernandes *et al*., 2004). There is still a deficiency of data on the effects of using saline irrigation water on the growth and yield of crops cultivated under glass-house conditions. Therefore, the aim of this work is to examine the response of seven white lupin genotypes and the content of the mineral nutrients to different salinity levels in irrigation water under glass-house conditions. In addition to, the detection of gene expression under seawater stress by biochemical markers to help in the selection of salinity tolerance. Moreover, DNA fingerprinting among the tested seven accessions will be determined by the SRAP marker.

2. Materials and Methods

2.1. Plant Materials and Glass-house Experiment

Six white lupin accessions were imported from The Centre for Genetic Resources, Netherlands; and one local cultivar Balady to be used in this investigation. The names, pedigree, and origin of lupin genotypes are presented in Table 1. In this study, pot experiment was conducted in a glass-house at the Virology Laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, University of Ain shams, Egypt. The experiment was set up as a completely randomized block design with three treatments and four replications. Pots were filled with loam (1:1 sand: clay) and its chemical characteristics were analyzed according to Jackson (1973) as shown in Table 2. Three white lupin seeds were sown in each pot on November 29 in season 2017/18. The seeds were left to grow inside the greenhouse under natural lighting, (22/14) ± 2°C (day/night) and 70% relative humidity. Salinity treatments were started four weeks later from the planting time. Tap water was used as a control treatment [an electrical conductivity of water (ECw) = 0.6 dS m^{-1}]. Three salinity levels of irrigation water (ECw) were prepared by diluting seawater to achieve the target levels of 2, 4, and 8 dS m^{-1}. Some of the chemical characteristics for the used seawater were determined and presented in Table (2). Recommended doses of chemical fertilizers (N:P:K) for white lupin production were applied to all treatments. The symptoms of salt stress were recorded after fourteen days of treatment with seawater. Leaves of seven white lupin genotypes were collected in paper bags after forty days of the treatment.

Table 1. Pedigree of seven *Lupinus albus* L. genotypes used in this study.

No.	Accessions	Type	Name	Country
1	*L. albus* CGN 10102	Research material	N92/50	Italy
2	*L. albus* CGN 10104	Research material	N105/50	Italy
3	*L. albus* CGN 10106	Research material	N107/50	Italy
4	*L. albus* CGN 10108	Research material	N121/50	Italy
5	*L. albus* CGN 10109	Research material	N122/50	Italy
6	*L. albus* CGN 10112	Land variety	Przehendowski Wezesnv	Poland
7	*L. albus* cv. Balady	Land variety	Balady	Egypt

Table 2. Chemical properties of the experimental soil and seawater.

Parameters	Experimental soil	Seawater
$CaCO_3$ %	3.22	-
Organic matter (OM) %	1.97	-
pH	7.5*	8
EC dSm^{-1}	0.54**	53
Soluble cations and anions	**meq $100g^{-1}$**	**meq l^{-1}**
Ca^{++}	1.67	30.3
Mg^{++}	1.73	114
K^{+}	0.37	13.7
Na^{+}	1.67	642
$CO_3^{=}$	-	-
HCO_3^{-}	1.70	2.5
Cl^{-}	1.74	671
$SO_4^{=}$	2.0	126.5

EC is electrical conductivity. * determined in 1:2.5 soil suspension, ** measured in 1:5 soil extraction.

2.2. Nutrients Determination in White Lupin Genotypes

The leaf samples were collected from three plants for each genotype. They were rinsed with deionized water and oven-dried at 65ºC for constant weight. Then, the samples were ground and kept in a plastic bag for nutrient analysis. The portion of the dried samples was dissolute in acid mixtures (sulfuric and perchloric acids) to be digested as described by Cottenie, (1980). Nutrients were determined in the digested aliquots. Sodium, potassium, and calcium were measured by flame emission (Cottenie, 1980). The total nitrogen was estimated by Kjeldahl method and phosphorus was determined by the ammonium-vanadate and molybdate method according to Motsara and Roy, (2008). The results were expressed as an ionic percentage of the dry matter (g $100g^{-1}$ DM).

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

SDS-PAGE was done on 15% polyacrylamide gels, according to (Laemmli, 1970) as modified by (Studier, 1973).

2.4. Peroxidase (POX) Isoforms

The POX isozymes of the antioxidant enzymes were extracted based on the method described by (Stagemann *et al*., 1985). POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX were determined according to (Baaziz *et al*., 1994).

2.5. Extraction of Genomic DNA

Young 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; Mahfouze *et al*., 2018).

2.6. Sequence-Related Amplified Polymorphism (SRAP)

A set of eleven SRAP primers (Table 3) was designed following Li and Quiros, (2001) and used to search for polymorphism among the seven white lupin genotypes. The total reaction mixture was 25 µL containing 10X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 *p*mol primers, 1.25 U *Taq* polymerase, and about 150 ng genomic DNA. The amplification regime followed the recommendation of Li and Quiros, (2001) as follows: An initial denaturing step was performed at 94°C for five minutes followed by five cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, subsequently followed by thirty-five cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 7 min.

The 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. The gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

Table 3. List of the tested SRAP primers. The selective nucleotide sequences for each primer are underlined.

Primers	Sequence	
name	Forward	Reverse
SRAP1	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGTC
SRAP2	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCGA
SRAP3	TGAGTCCAAACCGGTCC	GACTGCGTACGAATTCAG
SRAP4	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTCTG
SRAP5	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTAAT
SRAP6	TGAGTCCAAACCGGTTG	GACTGCGTACGAATTTGA
SRAP7	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCTG
SRAP8	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTGTC
SRAP9	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCGA
SRAP10	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAG
SRAP11	TGAGTCCAAACCGGAAT	GACTGCGTACGAATTTGC

2.7. Data analysis

A matrix for SRAP was generated by scoring reproducible bands as one for their presence and as zero 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 the SRAP analysis.

2.7.1. Principal Coordinated Analysis (PCA)

PCA was also carried out to show multiple dimensions of the distribution of the genotypes in a scatter-pot by PAST software version 1.62 (Hammer *et al*., 2001) The Bootstrap analysis PAUP, version 4.0b10 software (Swofford, 2003), was performed to evaluate the tree topology reliability for 1,000 simulations. To investigate the discriminatory power of each SRAP primers, the polymorphic information content (PIC) was calculated according to (Smith *et al*., 2000) as follows: $PIC = 1-\Sigma n\ fi^2$ where fi is the frequency of the allele in the set of seven white lupin genotypes. The discrimination power (Dp) for each primer was calculated by dividing the number of polymorphic alleles amplified by primer by the total number of polymorphic alleles obtained (Khierallah *et al*., 2011).

2.8. Statistical Analysis

The data were analyzed by the two-way analysis of variance procedure using SigmaStat software, version 3.5 and the differences were declared significant at $P \leq 0.05$ probability level according to Fisher LSD method

3. Results

3.1. Effect of Salt Stress on White Lupin Genotypes

Seven genotypes of white lupin were irrigated with three different levels of diluted seawater (2, 4 and 8 dS m^{-1}). In addition to, tap water as control at the early seedling and these developed seedling stages. These genotypes demonstrated different responses of salinity tolerance depending on individual genotypes and the growth stage (Figures 1 and 2 and Table 4). Both white lupin accessions CGN 10106 and CGN 10112 were salinity-tolerant; they have not recorded any symptoms at the three levels of the salinity. However, two accessions CGN 10102 and CGN 10108 were moderately-tolerant to seawater stress until 4 dS m^{-1}; they have not shown any symptoms at 2 and 4 dS m^{-1}, except CGN 10108 whose shoot growth decreased slightly compared to the control. However, at 8 dS m^{-1} level, different symptoms appeared, and these include yellowing, wilting, leaf roll and reduction of shoot growth (Figures 1 and 2 and Table 4). In addition, CGN 10104 was also moderately-tolerant, for no visual symptoms were recorded within all the studied salinity levels except that the shoot growth reduced slightly compared with the control (Figure 2). On the other hand, the two genotypes CGN 10109 and Balady were sensitive to salt stress, and displayed typical symptoms of salinity including leaf roll, yellowing, tips of leaves turning white, darker green, decreasing of shoot growth and death, compared with the control (Figure 1). However, genotype CGN 10108 recorded different symptoms at the seedling stage reaching to the flowering stage (Figure 2 and Table 4). In contrast, CGN 10109 and Balady have not given any flowers.

Table 4. Symptoms of seawater salt stress on seven *Lupinus albus* L. genotypes under the glass-house conditions.

No.	Genotypes	Treatment (dS m^{-1})	Symptoms of salinity	Period of symptoms appearance (days)	Tolerant/Sensitive
1	*L. albus* CGN 10102	Control	Ns	-	Moderately Tolerant
		2	NS	-	
		4	NS	-	
		8	Y, W, R, D	8-29	
2	*L. albus* CGN 10104	Control	Ns		Moderately Tolerant
		2	R	29	
		4	R	29	
		8	R	29	
3	*L. albus* CGN 10106	Control	Ns	-	Tolerant
		2	NS	-	
		4	NS	-	
		8	NS	-	
4	*L. albus* CGN 10108	Control	Ns	-	Moderately Tolerant
		2	NS	-	
		4	R	29	
		8	Y, LR, R	8-29	
5	*L. albus* CGN 10109	Control	Ns	-	Sensitive
		2	NF	29	
		4	LR, Y, R, NF	25-29	
		8	LR, TW, R NF	8-29	
6	*L. albus* CGN 10112	Control	Ns	-	Tolerant
		2	Ns	-	
		4	Ns	-	
		8	Ns	-	
7	*L. albus* cv. Balady	Control	Ns	-	Sensitive
		2	DG, NF	25-29	
		4	Y, DG, NF	25-29	
		8	D, NF	25-29	

D= Dead, DG=Darker green, LR= Leaf roll, NF=Non- flowering, NS= No symptoms, R= reduction of shoot growth, TW= tips of leaves turn white, Y= Yellowing, W= Wilting.

Figure 1. Effect of the seawater salt stress on *Lupinus albus* L. plants growth of CGN 10109, Balady, CGN 10108 and CGN 10112 genotypes at the seedling stage, compared to the control. 1- *L. albus* CGN 10109 (control), 2- *L. albus* CGN 10109 (2 dS m^{-1}), 3- *L. albus* CGN 10109 (4 dS m^{-1}) , 4- *L. albus* CGN 10109 (8 dS m^{-1}), 5- Balady (control), 6- Balady (2 dS m^{-1}),7- Balady (4 dS m^{-1}), 8- Balady (8 dS m^{-1}), 9- *L. albus* CGN 10108 (control), 10- *L. albus* CGN 10108 (2 dS m^{-1}), 11- *L. albus* CGN 10108 (4 dS m^{-1}) , 12- *L. albus* CGN 10108 (8 dS m^{-1}), 13- *L. albus* CGN 10112 (control), 14- *L. albus* CGN 10112 (2 dS m^{-1}), 15- *L. albus* CGN 10112 (4 dS m^{-1}) and 16- *L. albus* CGN 10112 (8 dS m^{-1}).

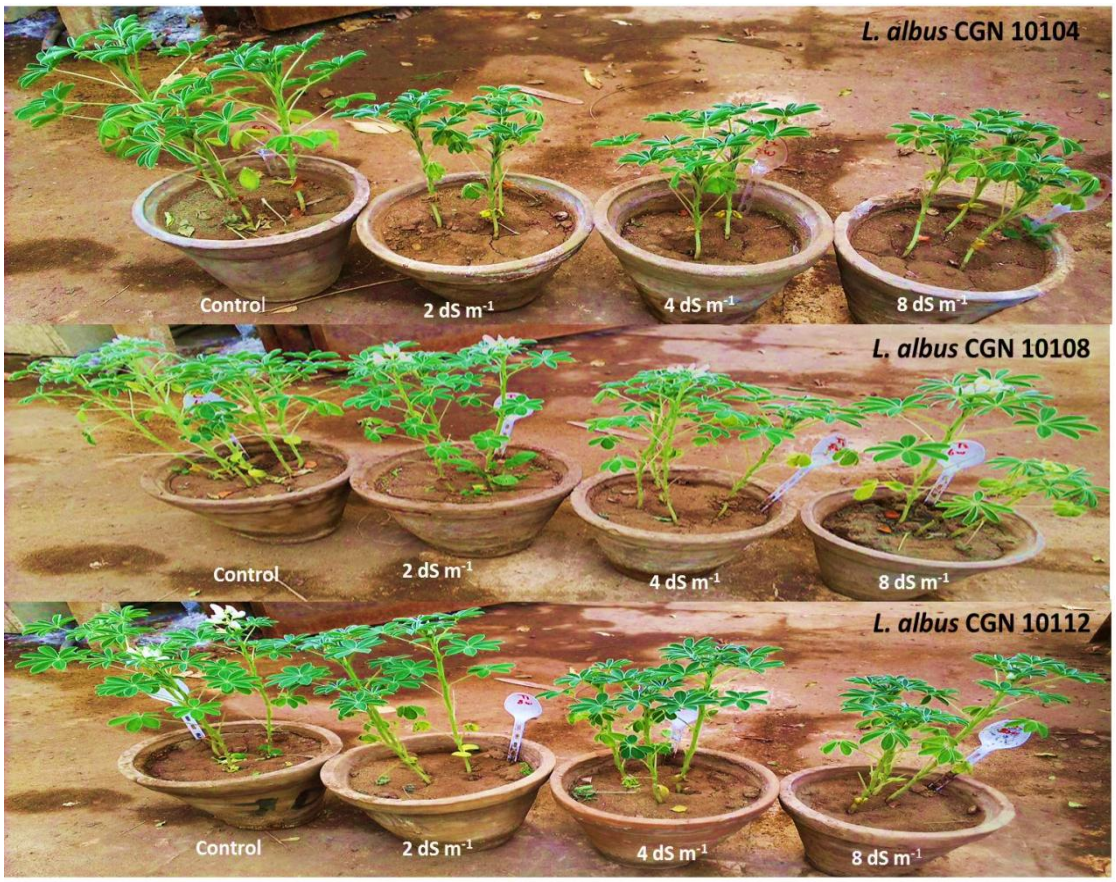

Figure 2. Effect of salt stress on *Lupinus albus* L. genotypes at the developed stage of CGN 10104, CGN 10108 and CGN 10112 genotypes, compared to the control.

3.2. Influence of Salinity in Irrigation Water on Nutrient Contents in White Lupin Genotypes

The results of variance analysis on the effect of genotype and salinity treatments on nutrient concentrations in the leaves are presented in Table 5. Seven genotypes of white lupin as a sole factor and the interaction between them and the salinity treatments recorded significant effects on all of the studied nutrients. However, the salinity as the sole factor was only significant to the content of Ca, Na, and P ions. The impact of salinity on nutrient concentrations (%) and Na ratio in respect to each genotype are shown in Figures 3 and 4, respectively. Among all of the tested genotypes, Balady cultivar has the highest N, P, Ca, and Na content compared to the control. The absorption of nutrients behaves differently according to the salinity of the irrigation water within the studied genotypes. The absorption of nitrogen was enhanced by increasing the salinity within the genotypes, compared to the control such as CGN 10102, CGN 10109, and CGN 10112. However, it was reduced or stable for Balady, CGN 10104, CGN 10106, and CGN 10108 (Figure 3). In all white lupin genotypes, the content of P was low, ranging between 0.11 % recorded for CGN 10106 and CGN 10109 and 0.26 % for Balady cultivar (data not shown). Accession CGN 10106 had the highest concentration of K among all the accessions compared to the control. Increasing the salinity of irrigation water decreased the K content by 5, 20, 13, and 15% for the accessions CGN 10102, CGN 10106, CGN 10109, and CGN 10112, respectively compared to the control. For the other genotypes, the K content in the leaves of plants either slightly increased or had no changes. The content of Ca was enhanced significantly by 22, 14, 16, and 11% for CGN 10102, CGN 10104, CGN 10108, and Balady, respectively. These behaviors were reversed for the genotypes CGN 10109 and CGN 10112, where the content of Ca was reduced by 41 and 7%, respectively, compared to the control. There was a positive relationship between the salinity of the irrigation water and the Na content in the white lupin leaves. The different Na ratios [K/Na, Ca/Na and Ca/(Na+K)] were influenced significantly by the salinity of irrigation water (Table 5). Accession CGN 10106 had the highest K/Na ratio compared with the control, while CGN 10104 and CGN 10112 showed the highest ratio value at the level 8 dSm^{-1} of ECw, compared to the other genotypes (Figure 4).

Table 5. Variance analysis results of the effect of accession and salinity on N, P, K, Ca and Na concentrations of the white lupin leaves.

Factors	Nutrients					Na ratios		
	N	P	K	Ca	Na	K/Na	Ca/Na	Ca/(K+Na)
Accession	***	***	***	***	***	***	***	***
Salinity	ns	***	ns	***	***	***	***	***
Interaction	***	***	*	***	***	***	***	***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ ns: non-significant

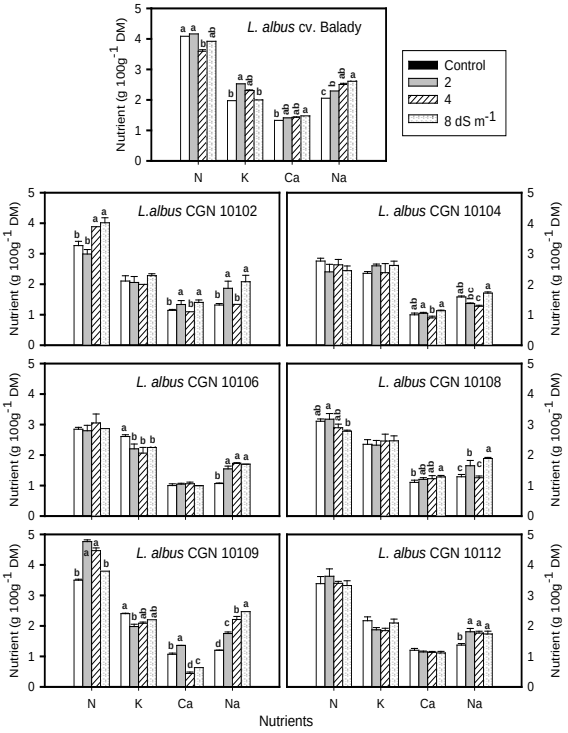

Figure 3. Means of nutrient concentrations for the studied seven *Lupinus albus* L. genotypes as a function of ECw. Different letters within each nutrient are significantly different at $P \leq 0.05$ according to Fisher LSD method. Nutrient column with no letter indicates no significant difference among the salinity levels. Vertical bars represent standard errors. DM: dry matter. N (0.388), K (0.349), Ca (0.143) and Na (0.237) are nitrogen, potassium, calcium and sodium, respectively, with their LSD values in brackets.

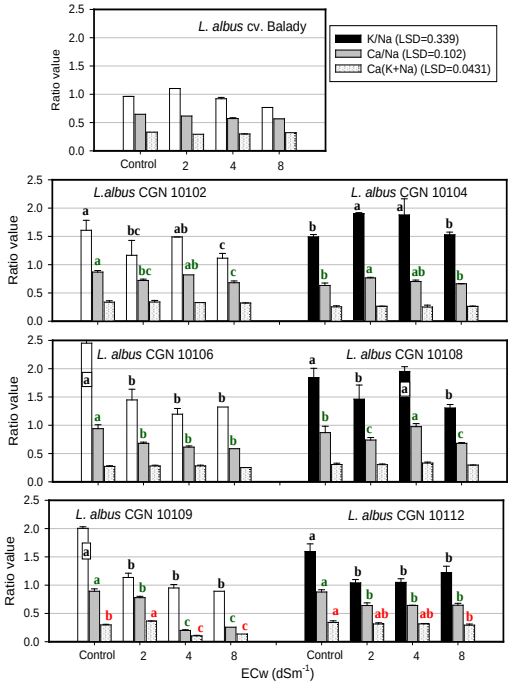

Figure 4. Means values of Na ratios in the studied seven *Lupinus albus* L. genotypes as a function of ECw. Vertical bars represent standard errors. Different letters have same color are significantly different at $P \leq 0.05$ within each nutrient according to Fisher LSD method. Column with no letter indicates no significant difference among the salinity levels.

3.3. Effect of Salinity on Gene Expression by SDS-PAGE

SDS-PAGE demonstrated the differences in protein-banding patterns of seven genotypes from *L. albus* L., treated with three different levels of salt stress 2, 4, and 8 dS m^{-1} as illustrated in Figure 5.

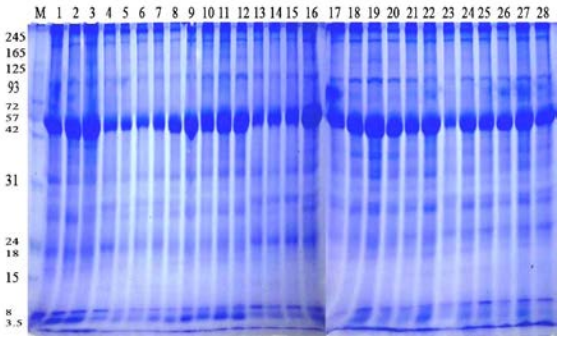

Figure 5. SDS-PAGE banding patterns of leaf proteins extracted from seven *Lupinus albus* L. genotypes under seawater salt stress at levels 2, 4, and 8 dS m^{-1}, compared with the control. Lane M: protein marker. Lane 1: *L. albus* CGN 10102 (control), lane 2: *L. albus* CGN 10102 (2 dS m^{-1}), lane 3: *L. albus* CGN 10102 (4 dS m^{-1}) , lane 4: *L. albus* CGN 10102 (8 dS m^{-1}), lane 5: *L. albus* CGN 10104 (control), lane 6: *L. albus* CGN 10104 (2 dS m^{-1}), lane 7: *L. albus* CGN 10104 (4 dS m^{-1}), lane 8: *L. albus* CGN 10104 (8 dS m^{-1}), lane 9: *L. albus* CGN 10106 (control), lane 10: *L. albus* CGN 10106 (2 dS m^{-1}), lane 11: *L. albus* CGN 10106 (4 dS m^{-1}) , lane 12: *L. albus* CGN 10106 (8 dS m^{-1}), lane 13: *L. albus* CGN 10108 (control), lane 14: *L. albus* CGN 10108 (2 dS m^{-1}), lane 15: *L. albus* CGN 10108 (4 dS m^{-1}) , lane 16: *L. albus* CGN 10108 (8 dS m^{-1}), lane 17: *L. albus* CGN 10109 (control), lane 18: *L. albus* CGN 10109 (2 dS m^{-1}), lane 19: *L. albus* CGN 10109 (4 dS m^{-1}), lane 20: *L. albus* CGN 10109 (8 dS m^{-1}), lane 21: *L. albus* CGN 10112 (control), lane 22: *L. albus* CGN 10112 (2 dS m^{-1}), lane 23: *L. albus* CGN 10112 (4 dS m^{-1}), lane 24: *L. albus* CGN 10112 (8 dS m^{-1}), lane 25: *L. albus* cv Balady (control), lane 26: *L. albus* cv Balady (2 dS m^{-1}), lane 27: *L. albus* cv Balady (4 dS m^{-1}) and lane 28: *L. albus* cv Balady (8 dS m^{-1}).

The electrophoregrams were determined depending on the molecular weight (MW) (kDa). A total number of twenty-six bands were scored ranging from 3.5 to 300 kDa; seventeen of these were monomorphic (65.38%), while nine were polymorphic (34.62% polymorphism). The highest number of protein subunits (twenty-five bands) was recorded in the seawater-sensitive Balady cultivar (4 and 8 dS m^{-1}), followed by Balady cultivar (the control and 2 dS m^{-1}), the moderately-tolerant genotypes such as CGN 10102 (2 and 4 dS m^{-1}) and CGN 10108 (8 dS m^{-1}) and the tolerant CGN 10112 (2 dS m^{-1}) (24 subunits). Besides, the control plants of CGN 10102, CGN 10108, and CGN 10112 scored 23, 22 and 22 polypeptides, respectively. In addition, the tolerant genotype CGN 10106 (4 and 8 dS m^{-1}) and the sensitive CGN 10109 (the control, 2, 4, and 8 dS m^{-1}) recorded twenty-two bands. However, the lowest number of polypeptides (18 subunits) was found in the moderately-tolerant genotype CGN 10104 (the control and 2 dS m^{-1}). On the other hand, one band with MW 66 kDa was shown in the sensitive Balady cultivar treated with 4 and 8 dS m^{-1} of salt stress (Figure 5). One polypeptide chain of 91 kDa was revealed in the tolerant genotypes e.g., CGN10102 (the control, 2, 4 and 8 dS m^{-1}), CGN10106 (4 and 8 dS m^{-1}), CGN10108 (the control and 8 dS m^{-1}) and CGN10112 (2 dS m^{-1}), and the susceptible Balady cultivar (the control, 2, 4 and 8 dS m^{-1}).

Moreover, one subunit of 72 kDa was found in the tolerant genotypes i.e., CGN10102 (the control, 2 and 4 dS m^{-1}), CGN10108 (8 dS m^{-1}) and CGN10112 (2 dS m^{-1}) and the sensitive genotype such as Balady (the control, 2, 4 and 8 dS m^{-1}). On the contrary, one polypeptide of 235 kDa was scored in the moderately-tolerant accessions viz., CGN 10102 (8 dS m^{-1}) and CGN 10104 (the control, 2 and 4 dS m^{-1}), but was absent in all other genotypes.

3.4. Isozyme Patterns of the POX under Salinity Stress

Changes in the POX activities under salt stress were assessed by Native-PAGE in the leaves of seven white lupin genotypes as presented in Figure 6.

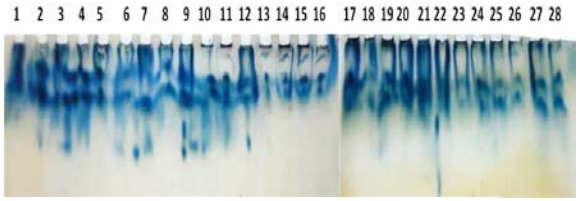

Figure 6. POX profiles of the seawater stressed seven *Lupinus albus* L. genotypes at levels 2, 4, and 8 dS m^{-1}, compared with the control. Lane 1: *L. albus* CGN 10102 (control), lane 2: *L. albus* CGN 10102 (2 dS m^{-1}), lane 3: *L. albus* CGN 10102 (4 dS m^{-1}) , lane 4: *L. albus* CGN 10102 (8 dS m^{-1}), lane 5: *L. albus* CGN 10104 (control), lane 6: *L. albus* CGN 10104 (2 dS m^{-1}), lane 7: *L. albus* CGN 10104 (4 dS m^{-1}), lane 8: *L. albus* CGN 10104 (8 dS m^{-1}), lane 9: *L. albus* CGN 10106 (control), lane 10: *L. albus* CGN 10106 (2 dS m^{-1}), lane 11: *L. albus* CGN 10106 (4 dS m^{-1}), lane 12: *L. albus* CGN 10106 (8 dS m^{-1}), lane 13: *L. albus* CGN 10108 (control), lane 14: *L. albus* CGN 10108 (2 dS m^{-1}), lane 15: *L. albus* CGN 10108 (4 dS m^{-1}) , lane 16: *L. albus* CGN 10108 (8 dS m^{-1}), lane 17: *L. albus* CGN 10109 (control), lane 18: *L. albus* CGN 10109 (2 dS m^{-1}), lane 19: *L. albus* CGN 10109 (4 dS m^{-1}), lane 20: *L. albus* CGN 10109 (8 dS m^{-1}), lane 21: *L. albus* CGN 10112 (control), lane 22: *L. albus* CGN 10112 (2 dS m^{-1}), lane 23: *L. albus* CGN 10112 (4 dS m^{-1}), lane 24: *L. albus* CGN 10112 (8 dS m^{-1}), lane 25: *L. albus* cv Balady (control), lane 26: *L. albus* cv Balady (2 dS m^{-1}), lane 27: *L. albus* cv Balady (4 dS m^{-1}) and lane 28: *L. albus* cv Balady (8 dS m^{-1}).

POX isoenzymes scored five isoforms with R_f value ranging from 0.128 to 0.723. The results regarding salt stress had different effects on the POX activities of the tested seven genotypes. An increase in the POX activities at salt level 2 dS m^{-1} has been observed in some of the moderately-tolerant genotypes CGN 10102 and CGN 10104, and the tolerant CGN 10112 compared with the control. However, there were no marked differences in POX activities at level 2 dS m^{-1} of seawater in the other genotypes. At 4 dS m^{-1} level of salinity, some genotypes displayed increase in POX activities including the moderately-tolerant accessions CGN 10102 and CGN 10104 and the susceptible CGN 10109. On the other hand, the remaining genotypes have not recorded any change in POX activities. At 8 dS m^{-1} level of seawater, it has been found that all genotypes have not scored any changes in POX activities except for the tolerant accession CGN 10106. Accordingly, POX activities declined in the tolerant genotype CGN 10106 at the three levels of salt stress, compared with the control (Figure 6).

3.5. SRAP Profiling

A total of eleven different SRAP primer sets scored fifty-one scorable fragments ranging from 100 to 1000 bp. Thirty-three out of fifty-one were monomorphic (64.71%), and eighteen bands were polymorphic (35.29%) (Figures 7 and 8 and Table 6). The number of amplified fragments generated per primer ranged from two (SRAP-3, SRAP-5 and SRAP-8) to eight (SRAP-2) with an average of 4.64 bands per primer. Primer SRAP-3 scored the highest number of polymorphism (100%), followed by primers SRAP-1, SRAP-5, SRAP-9 and SRAP-11 (50%). Moreover, Primer SRAP-7 showed the lowest number of polymorphism (16.67%), while Primer SRAP-8 has not scored any polymorphism (0%). On the other hand, eleven out of the fifty-one were unique markers (21.57%) (Table 6). The tolerant genotype CGN 10112 recorded the maximum number of positive and negative markers (three) of -100, -550 and +600 bp (Table 6). However, the moderately-tolerant genotypes CGN 10104 and CGN 10108 and the sensitive Balady cultivar displayed two negative markers with molecular sizes (-190 and -1000 bp), (-600 and -900 bp) and (-230 and -750 bp), respectively (Table 6). In contrast, CGN 10102 and CGN 10109 exhibited the minimum number of specific bands (one) of -170 and -300 bp, respectively (Table 6). The genetic identity matrix among seven *L. albus* genotypes was observed from amplicons shown by eleven SRAP markers using correlation coefficients. The genetic identity among the tested seven genotypes ranged from 0.80 to 0.94. The highest similarity was scored between two accessions (the tolerant CGN 10106 and the susceptible CGN 10109) and the tolerant genotypes (CGN 10106 and CGN 10112) (94%). However, the lowest identity was recorded between (the tolerant CGN 10112 and the sensitive Balady) (80%) as shown in Table 7. The UPGMA dendrogram showing the genetic relationship among all of the studied genotypes which fell in one main cluster (I) resolved to three sub-clusters. Sub-cluster (A) (similarity ranges from 0.85 to 0.94) contains the moderately-tolerant accessions CGN 10102, CGN 10104, CGN 10108, and the sensitive CGN 10109. Sub-cluster (B) (similarity ranges from 0.80 to 0.88) has the susceptible Balady cultivar. However, sub-cluster (C) (similarity ranges from 0.82 to 0.94) consists of CGN 10106 and CGN 10112 genotypes which are both tolerant to seawater stress (Figure 9).

Table 6. SRAP analysis of seven white lupin genotypes**.**

Primer Code No.	Size range of the scorable loci (bp)	Total loci	No. of monomorphic loci	No. of polymorphic loci	% Polymorphism	Polymorphic information content (PIC)	Discrimination power (DP)	Unique loci	Molecular size of markers (bp)
SRAP-1	100-400	6	3	3	50	0.831	0.176	2	-600, -900
SRAP-2	100-1000	8	6	2	25	0.874	0.118	1	-1000
SRAP-3	190-500	2	0	2	100	0.480	0.118	1	-190
SRAP-4	100-1000	7	5	2	28.57	0.854	0.111	0	-
SRAP-5	250-600	2	1	1	50	0.463	0.059	0	-
SRAP-6	200-800	3	2	1	33.33	0.665	0.059	1	-300
SRAP-7	150-900	6	5	1	16.67	0.833	0.059	1	-750
SRAP-8	350-470	2	2	0	0	0.500	0.000	0	0
SRAP-9	170-900	6	3	3	50	0.818	0.176	2	170, -550-
SRAP-10	230-800	5	4	1	20	0.799	0.059	1	230-
SRAP-11	100-800	4	2	2	50	0.694	0.118	2	-100, +600
Total	**100-1000**	**51**	**33**	**18**	-	-	-	**11**	-
%	-	-	**64.71**	**35.29**	-	-	-	**21.57**	-
Average	-	**4.64**	**3**	**1.64**	-	**0.710**	**0.096**	**1.0**	-

Table 7. The genetic similarity and genetic distance statistics for seven *Lupinus albus* L. genotypes.

Genotypes	*L.albus* CGN 10102	*L. albus* CGN 10104	*L. albus* CGN 10106	*L. albus* CGN 10108	*L. albus* CGN 10109	*L. albus* CGN 10112	*L. albus* cv. Balady
***L.albus* CGN 10102**	1.00						
***L. albus* CGN 10104**	0.91	1.00					
***L. albus* CGN 10106**	0.90	0.90	1.00				
***L. albus* CGN 10108**	0.89	0.85	0.88	1.00			
***L. albus* CGN 10109**	0.92	0.92	0.94	0.90	1.00		
***L. albus* CGN 10112**	0.84	0.84	0.94	0.82	0.88	1.00	
***L. albus* cv. Balady**	0.88	0.88	0.86	0.85	0.88	0.80	1.00

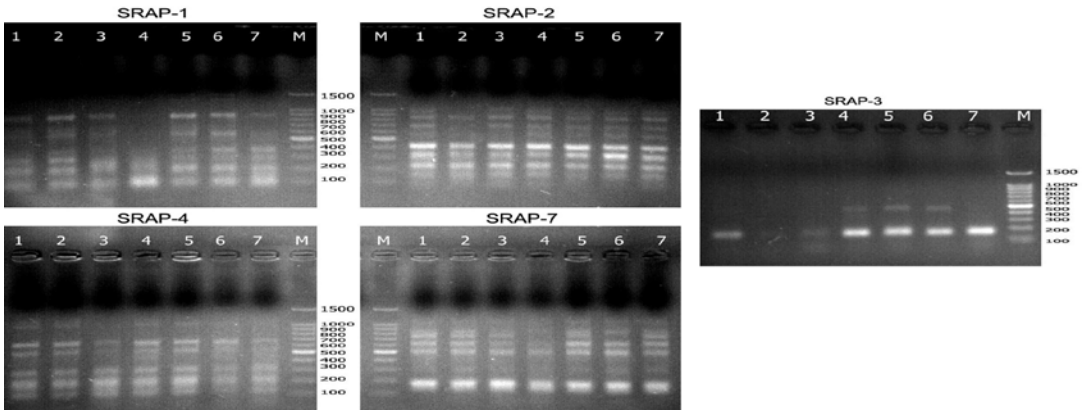

Figure 7. Amplified products of SRAP marker using of primers SRAP-1, SRAP-2, SRAP-3, SRAP-4 and SRAP-7 for analyzed seven *Lupinus albus* L. genotypes. Lane M= DNA ladder 100 bp. Lane 1: *L. albus* CGN 10102, lane 2: *L. albus* CGN 10104, lane 3: *L. albus* CGN 10106, lane 4: *L. albus* CGN 10108, lane 5: *L. albus* CGN 10109, lane 6: *L. albus* CGN 10112 and lane 7: *L. albus* cv Balady.

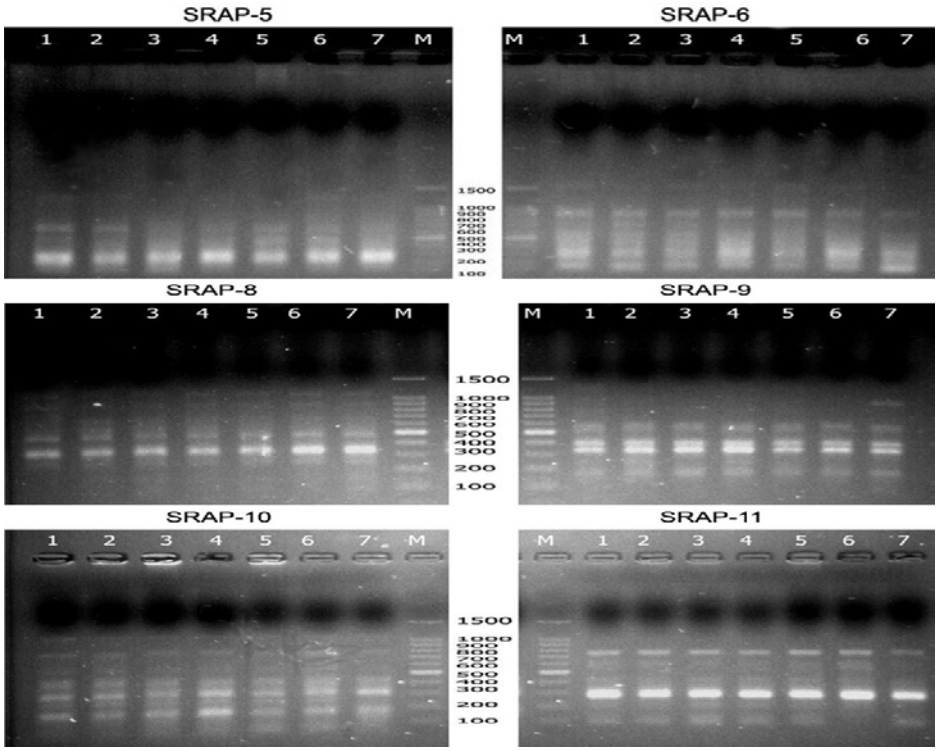

Figure 8. Amplified products of SRAP marker using of primers SRAP-5, SRAP-6, SRAP-8, SRAP-9, SRAP-10 and SRAP-11 for analyzed seven *Lupinus albus* L. genotypes. Lane M= DNA ladder 100 bp. Lane 1: *L. albus* CGN 10102, lane 2: *L. albus* CGN 10104, lane 3: *L. albus* CGN 10106, lane 4: *L. albus* CGN 10108, lane 5: *L. albus* CGN 10109, lane 6: *L. albus* CGN 10112 and lane 7: *L. albus* cv Balady.

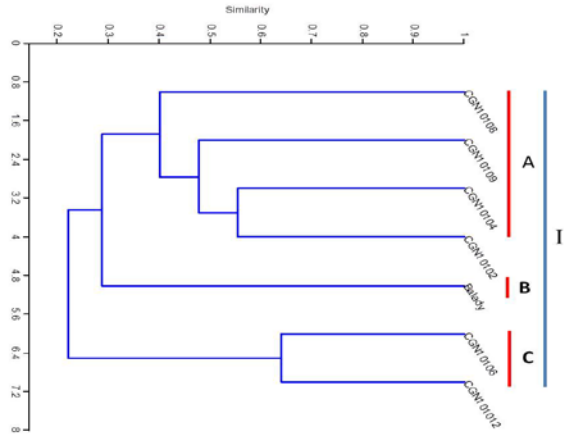

Figure 9. UPGMA dendrogram for seven *Lupinus albus* L. genotypes based on the allelic data of 11 SRAP primer combinations.

3.6. Levels of Genetic Information Generated by SRAP Primers

The level of polymorphism among the seven white lupin genotypes was estimated by calculating the polymorphic information content (PIC) values for each of the eleven SRAP markers. The PIC values varied greatly for all of the SRAP primers tested. PIC value of the eleven SRAP primers ranged from 0.463 (SRAP-5) to 0.874 (SRAP-2) with an average of 0.710 (Table 6). PIC values were positively-correlated (r = 0.948) with a number of amplified alleles per primer. The observed discrimination power (DP) was calculated for each primer and ranged from 0.059 (SRAP-5, SRAP-6, SRAP-7 and SRAP-10) to 0.176 (SRAP-1 and SRAP-9) with an average of 0.096 (Table 6). However, SRAP-8 has not recorded any DP (Table 6). The dendrogram was confirmed by principal coordinate analysis (PCA) (Figure 10). The first three principal coordinates accounted for 74.23% of the total variation. Genotypes in the PCA scatter plot, indicated by ellipses and numbered with A, B, and C, seemed to form a very close grouping in the dendrogram (Figure 10). The seawater stress-sensitive and moderately-tolerant white lupin genotypes (CGN 10102, CGN 10104, CGN 10108 and CGN 10109) were clustered into group (A). However, the seawater stress-tolerant accessions CGN 10106 and CGN 10112 were put together in group (B), while the sensitive Balady cultivar was clustered in group (C). Genotypes clustered in ellipses A, B, and C were basically from group I of the dendrogram correspondingly.

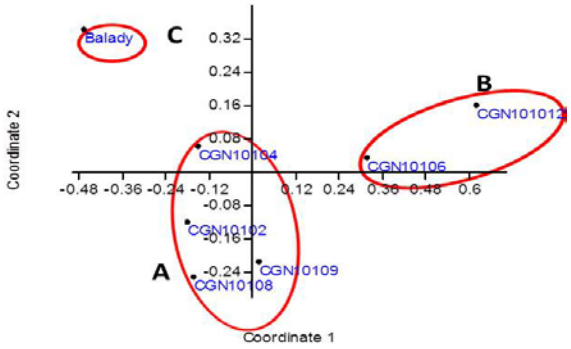

Figure 10. Principal coordinate analysis (PCA) of the seven *Lupinus albus* L. genotypes with 11 SRAP primer combinations.

4. Discussion

The present study has demonstrated significant variations in seawater stress tolerance among seven genotypes of white lupin depending on the genotypes and the growth stage. Thus, two white lupin accessions CGN 10106 and CGN 10112 were tolerant to seawater stress, compared with the control. However, the other five genotypes ranged from moderately-tolerant to sensitive controlled by the salinity level, hence showing different responses to salt stress. In the case of CGN 10104 genotype, no visual injury was pronounced within all the studied salinity levels except the reduction of the shoot growth, thus genotypes CGN 10104, CGN 10102 and CGN 10108 were considered moderately-tolerant to salinity stress. The case was different with Balady and CGN 10109 genotypes where the salinity symptoms started at levels 2 and 4 dS m^{-1}, respectively. Therefore, they were classified as sensitive to salinity. In the current study, the young white lupin seedling tissues were more sensitive to salt stress, which may be because they could not avert NaCl aggregation compared with the developed seedlings. Similar results were obtained by Krishnainurthy, 1991; Yousfi *et al*., 2007 and Ferdose *et al*., 2009, who mentioned that the response of plants to salt stress differs from species to species, plant to plant and according to the growth stage. The relative growth of seedlings decreased more severely at the early seedling stage than at the developed stage, because they could not avoid the NaCl accumulation compared to the developed plants. Ranjbar, *et al*., 2008; Tawfik *et al*., 2015; Sadak, 2016 found that plant stunting is the most popular effect of salt stress. Salinity causes several symptoms including necrosis, leaf burns and defoliation, which may appear in some woody crops; however, these visual symptoms are scarce in herbaceous crops unless plants are severely affected. Thus, it is tricky to diagnose a moderately salt-stressed crop in the field without control. There are two stages of response to salt stress (Munns, 1993). The first stage of growth decrease is due to the salt accumulating outside the roots. The growth decrease is regulated by hormonal signals of the roots. However, the second stage is due to salt accumulating in the leaves at the redundant levels. This will inhibit the younger leaves' growth by the reduction of the photosynthesis which, in turn, limits the carbohydrates supply necessary to the cells. Adverse effects of increasing seawater concentrations were clearer on leaves than on the stem and roots (Hussein *et al*., 2015). The most crucial cause of the plant's growth reduction under salt stress conditions was the suppression of cell division and cell enlargement (Allam *et al*., 2004; Munns and Tester, 2008; Radi *et al*., 2013). Krishnainurthy, (1991) observed that the impaired metabolism of nitrogen under salt stress conditions results from amino acids cumulating, which leads to a decrease of the growth of shoots in rice.

Generally, salinity induces the absorption of Na^+ in the leaves, resulting in the accumulation of Ca^{++} and K^+ ions in order to compensate for the ionic and nutritional imbalance (Chakraborty *et al*., 2013). Since the accumulation amounts of the last two ions mainly depend on the tolerance strength of plants toward salinity stress, the aforementioned observation might interpret the differentiation of the ion absorption behavior of the studied white lupin genotypes. In addition, the variation could also refer to the differences in the anatomical of the shoots part and/or the root formation among the genotypes (Loupassaki *et al*., 2002). Increasing the salinity was favorable for some genotypes which induced the accumulation of nutrients to the sufficient growth range defined according to Jones *et al*., (1991); for instance, the N content was categorized in the sufficient range for CGN 10112, CGN 10102, and Balady, while it was categorized in the range of low to sufficient for the other genotypes. The concentration of K and Ca ions was generally in the sufficient range for all of the studied genotypes. The measured Na content and Na ratio can be parameters to point out the salinity-stressed plants. The changes of Na/K ratio might be attributed to the replacement of Na to K ions on the absorption sites of the roots. The ability of plants to sustain or reduce changes in the K and Ca contents is relevant to inducing the reduction of Na accumulation. In addition, the increase of K/Na and Ca/Na ratios could be one of the physiological mechanisms to reduce the deleterious effects of salinity stress (Hussein *et al*., 2015; Patel *et al*., 2010; Aktas *et al*., 2006). Nutrient content had an effect on the biological processes of the plants under salinity stress. Nevertheless, the susceptible genotypes CGN 10109 and Balady have the highest nutrient content (particularly of N, P, and Ca), but they have not produced any flowers, which might be attributed to the highest accumulation of Na content compared with the other accessions. In these genotypes increasing the water salinity induced the content of Na in leaves of the plants and reduced the K (%). A similar trend was reported in another study carried out on common beans (Kouam *et al*., 2017).

In this study, it has been observed that salt stress causes either an increase or decrease in the protein content or absence of some proteins, compared with the control. Besides, seawater stress-tolerant, moderately-tolerant and susceptible white lupin genotypes have not differed significantly in leaf proteins. Furthermore, salinity induces the synthesis of new proteins in plants. Ashraf and Fatima, (1995) maintained that salt- stress sensitive and tolerant genotypes of safflower have not differed significantly in the leaf-soluble proteins. In addition, the increase and decrease in protein content depend on the genotype. Newly-induced polypeptides of seventy-two and ninety-one kDa were shown in both of the salt-tolerant and sensitive genotypes. In contrast, one polypeptide chain with the molecular weight 235 kDa was shown in the moderately-tolerant genotypes CGN 10102 and CGN 10104. This might interpret the enhancement of N content for some genotypes as revealed from the analysis (Demiral, 2017). These results were in an agreement with Abdel-Haleen, (2007) and Win and AZ (2017) who found that an increase in protein content might be included in mungbean tolerance. A number of induced proteins in response to salt stress were linked with the biochemical modification of the plants as a reaction to salinity stress. These proteins play the main role in salt-stress plant tolerance (Goncalo *et al*., 2003; Mahmoodzadeh, 2009). Ricard *et al*., (1996) suggested that the increase in the number of polypeptides in salt stress shows that salinity stress may induce proteins synthesis, which is probably represented as an osmoticant (Win, 2012). Bishnoi *et al*., (2006) and Zhanga *et al*., (2013) found that the treatment

of pigeon pea plants [*Cajanus cajan* (L.) Millsp.] with NaCl induced 67.5 kDa protein and 95.6 kDa protein in two genotypes, respectively due to the fact that the translation of the mRNAs is inhibited or stimulated by the increased NaCl concentrations, or perhaps because of the regulation of mRNA transcription. Others suggested that the "disappeared" proteins in response to salt stress were a result of their denaturation. These proteins may be synthesized *de novo* in response to salt stress or the increase of presently consecutive expression proteins when plants are exposed to salt stress (Qasim *et al*., 2003)

In the present study, it has been observed that the moderately-tolerant genotypes (CGN 10102 and CGN 10104), tolerant (CGN 10112), and susceptible (CGN 10109) revealed an increase in POX activities at salinity levels (2 and 4 dS m^{-1}), (2 dS m^{-1}) and (4 dS m^{-1}) respectively. These results agree with those obtained by several authors including Sekmen *et al*., (2007) who observed that the increase in the activity of antioxidant enzymes in both of the salt-stress sensitive and tolerant genotypes has been related to salt tolerance. In this study, the increase in POX activities suggests that this enzyme serve as tools to help protect white lupin plants from oxidative damage. Jakovljević *et al*., (2017) mentioned that the responses of antioxidant enzymes to salinity were different according to the growth stage and plant part. Also, it was found that the highest POX activities were observed in the control of *Ocimum basilicum* seedlings at twenty-eight days of culture, even though POX activities generally decreased with the increasing NaCl level in all treatments. In this study, it was shown that all the tested genotypes, except CGN 10106, have not recorded any changes in POX activities at the 8 dS m^{-1} level of seawater; perhaps because some enzymes need a less concentration of salinity to increase their enzyme activity. However, a high amount of saline affects the enzyme structure and subsequently its activity. Aghaei *et al*., (2009); Valderrama *et al*., (2006) and Zhanga *et al*., (2013) mentioned that POX isoenzymes play the main role in the signaling of roots to leaves, allowing young plants to activate diverse defense strategies against H_2O_2 for the generation against salinity. Consequently, increasing POX activities may improve salt tolerance in plants.

In the current investigation, eleven SRAP primer combinations generated fifty-one amplified fragments, involving eighteen polymorphic bands, with a 35.29% percentage of polymorphic bands. Mahfouze *et al*., (2018) found that RAPD and ISSR markers recorded polymorphism with 47.96% and 29.82%, respectively among seven *L. albus* genotypes (CGN 10105, CGN 10106, CGN 10108, CGN 10109, CGN 10112, CGN 10113, and Balady). However, SRAP analysis targets the coding region ORFs (open reading frames) (Liaol *et al*., 2012). Exons are usually rich with GC contents and the 'CCGG' sequence in the core of the forward SRAP primers is designed to target such coding sequences (Shao *et al*., 2010; Kaewpongumpa *et al*., 2016). Thus, SRAP profiles may be helpful in deciphering the genomic basis of complex traits which are linked to the economic value of *L. albus* and can likely reflect the genetically-determined morphological variation in a better way. SRAP primers have a high capability of recognition. These primers have the capability to record the highest number of polymorphic alleles according to the total number of differences. The polymorphisms were accounted due to the appearance and disappearance of bands. The absence of bands may be attributed to the failure of the primer to anneal at a location in some populations because of nucleotide sequence variations or by omission or insertions between primer sites (Tahir and Omer, 2017). The variation in the number of polymorphic fragments might be ascribed to the amount of GC content of the primer applied in this study.

In this study, the cluster analysis has been somehow similar to the principal coordinate analysis (PCA); both dividing the tolerant, moderately-tolerant and sensitive genotypes into three groups.

5. Conclusion

In this study, the responses of seven genotypes of white lupin, irrigated with seawater of different levels of salinity (2, 4, and 8 dS m^{-1}), were investigated under greenhouse conditions. This study confirms that the biochemical markers identification of salt-stress tolerance could be a simple and cheap tool to plant breeders for analyzing tolerance traits for the segregation of a cross-population into salinity-tolerant and sensitive genotypes. Furthermore, the obtained data show that two genotypes were tolerant (CGN 10106 and CGN 10112), three were moderately-tolerant (CGN 10102, CGN 10104, and CGN 10108), and two were sensitive to seawater salt (CGN 10102 and Balady). The absorption of nutrients was affected differently according to the genotypes and salinity levels. Salt-tolerant and sensitive genotypes displayed somewhat similar biochemical reactions at the isozyme and protein levels of exposure to seawater treatments. In this context, the expression of salt-specific proteins in the moderately-tolerant genotypes CGN 10102 and CGN 10104 under seawater stress is an important factor in studying these proteins and will help in the identification of the responsible genetic domain. Moreover, the SRAP technology is a suitable molecular marker for the genetic variability analysis among the seven white lupin genotypes. Ultimately, this study shows the possibility of using diluted seawater in the irrigation of white lupin plants under Egyptian conditions.

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