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Evaluation of Genotypic Diversity of some Varieties of Egyptian Clover under Salinity Levels of Sea Water

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ABSTRACT

This investigation was conducted at the laboratory of Seed Technology Research Department at Sakha Agric. Res. Station ARC, Egypt, during the year of 2016-2017, to study the effect of salinity of four sea water levels i.e. 0, 4, 6 and 8ML^{-1} mixed with distillated water which was used as the evaluation included a control seed germination, shoot length, root length, seedling fresh and dry weight of all 16 genotype varieties. The experimental design was factorial completely randomize arrangement Excluded in a design with three replicates. Seeds were sowed in patri-dishes. The effect of seawater concentrations varied significantly where the germination percentage, shoot length, root length, fresh weight and dry weight decreased with the increasing of the seawater concentrations from 0 to 8ML^{-1}. The maximum germination percentage was found with fahl genotype (mono cut type) and for Sakha 4 (mult- cut type). Among the 64 treatments combinations the lowest germination percentage was found in population 46 and differed, in fresh weight for the seedling which ranged between 185.83 and 50.83 (mg) for Fahl and Sakha4, respectively while genotype Helaly gave 237.50(mg). The highest germination percentage was obtained for Serw 1, Fahl, Sakha4 and Sakha 96 genotypes, respectively. On the other hand, the lowest germination percentage under the highest level was Sakha comp 2000. ISSR (Inter Simple Sequence Repeat) and Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis were utilized in this study to evaluate at molecular level the differences between levels of water salinity stress of Egyptian clover. Five primers of ISSR generate highly genetic variation for resistance, moderate and sensitive. Clover genotypes resulting in salinity stress. 72, 68, 62, 31, and 25% of genetic similarity were reflected for third, fifth, fourth, second and first ISSR primers. Common important protein fraction with 54 KDa was expressed in all resistance, moderate and sensitive as well Egyptian clover which refers to its importance role in salinity metabolism cycle. Varied protein interactions were distinguished clearly and showed different responses or reaction for salinity level.

Keywords: Sea water, Salinity, Egyptian clover SDS – PAGE, ISSR markers.

INTRODUCTION

Egyptian clover (Trifolium alexandrinum L.) is considered to be the main winter forge leguminous crop in Egypt. Significant improvement has been made in many crops, such as cereals but few investigations have been made for improving yield potential of Egyptian clover, because of narrow genetic base for Egyptian clover.

There is a high degree of self-sterility and incompatibility limits. Which directed Egyptian clover breeders to relay on selection and developing synthetic varieties. However salinity is a main abiotic stress in semiarid and arid areas and it affects about 7 % of world's land area of about 93O million hectares *(Mendham and Salisbuiy , 1995)*. *(Bakheit, 2013)*. Moreover, high devoted attention should be given to develop new tolerant genotypes for salinity stress to increase the productivity and quality under, more than one third the cultivated area is salt affected soil.

Various abiotic stresses including high salinity, which affects crop metabolism and plant growth. Soil salinity is a major factor limiting the crop production globally (Kumar et al., 2010). Soil salinity affects enormous areas cultivated land causing significant reduction in crop yield.

Soil salinity may be happen because of natural progresses or initiated thru irrigation with saline water under bad circumstances. Over than 5O% of all watered lands are salt affected soils *(El swaify et al., 1983)*.

Salinity major abiotic stress adversely affects plant processes at physiological, biochemical and molecular level and reduces plant productivity *(Tester and Davenport 2003)*

Salt is a key environmental stress factor that badly impacts seed germination. Salinity tolerance of germination of seeds of forage such as rape berseem clover, alfalfa, and red clover which has been shown to be a heritable trait which could be used as a good ceriteion for selection of salt tolerance populations *(Mandic et al., 2014)*.

Forages play an important role in achieving sustainable agriculture systems.

It is well known that there is a significant variation among genotypes different with respect to salt tolerance. *(Rana, 1986)*. Legumes are generally more sensitive to salinity especially in germination stage *(Ghassewi – Golezani et al., 2009)*. Germination is a vital phase in the life-cycle of crop plant, however because has at toxic effect on germination.

Molecular marker techniques would identify such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), Amplified Fragment length Polymorphism (AFLP), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), microsatellites (SSR) … etc, (Lin *et aL,* 1996). Also, there are several different DNA analytical procedures that have been used to identify, characterize and determine genetic diversity among genotypes. They are a reliable method of genetic fingerprinting and have been successfully used for characterization and evaluation of genetic relationships in several species (Vos *et al,* 1995;Neqief tf/., 2000).

ISSR markers were used as useful tools to assess the genetic variations in Capparis spp. (caper) and Solenostemma arghel (arghel) species which is considered as an important prerequisite for the improvement of these species and for the conservation of their germplasm *(Said, 2005)*. However, the ISSR-PCR method using primers based on di-tri-tetra-penta-nucleotide repeats without the requirement for prior knowledge of the genome sequence seems particularly suitable for germplasm comparison. Advantage of the use of ISSR as a dominant marker compared with RAPD has been the repeatability of ISSR methodology reported for several species *(Jain et aL, 1999; Fernandez et al, 2002; SicaeJ et al, 2005)*. Molecular

markers prepared through utilizing recent potentials of genome genotyping, offer a valuable molecular evidence for assessing genetic diversity in plants *(Badr et aL, 2012).* Studies reported that Inter simple sequence repeat (ISSR) is considered a molecular marker method that was established by *Zietkiewicz et al (1994)*. ISSR markers are highly polymorphic and are valuable in investigations performed on genetic diversity, phylogeny, genome mapping ,and gene tagging *(Reedy et aL, 2002)*.

Electrophoetic data analysis of native or denatured proteins of diverse species have been widely utilized to offer information concerning genetic plants/examples involve *Trifolium* L. *(Badr, 1995)* and soybean *(El-Kholy, 2013)*.

The use of protein data for *Trifolium* genotype identification and differentiation is suggested by *Sheidai et al., (1999)*. They revealed differences among species / populations of Trifolic repens, T. italic, T. alexandrinum, T. fragiferum, T. subterraneum, T. resupinatum and T. hybridum using the morphometric analysis and SDS-PAGE seed-protein, The present study aimed to study the effect of water salinity stress towards germination, plant growth traits, total protein and determine the most suitable concentration and combination of growth regulator for improvement and to evaluate the salt tolerance through SDS-PAGE analysis in Egyptian clover as a way to solve water quality problem.

MATERIALS AND METHODS

The current study was performed at the laboratory of Seed Technology Research Department at Sakha Agric. Res. Station, ARC, Egypt, during 2016-2017 season for examining the effect of four salinity concentrations (0, 4, 6 and 8 ML-1) from sea water mixed with distilled water while, zero concentration used as a control (normal water). The 16 Egyptian clover genotypes studied were Gemmeiza1, Sakha Comp.2014, Giza6, Company variety (008-001-2013), Sakha4, Sids Comp., Sakha96, Sakha Comp., Sakha Comp.2000, Fahl, Population10, Population6, Sakha Comp.2011, Serw1, Population46 and Helaly The traits study were : germination percentage, shoot length (cm), root length (cm), seedling fresh weight (mg) and seedling dry weight (mg).

Table 1. Sea water composition (meq/L)*.

EC	Hco3	Cl	So4	Ca	Mg	Na	K
50.50	3.00	502.08	2.22	45.00	142.92	308.23	11.15

*** melgram per liter.**

Studied laboratory traits were as follow:

Standard germination test: Percentage of germination was expressed by percentage of normal seedling at the end of experiments period as stated by International Seed Testing Association (ISTA, 1993). Seeds were nursed in a growth chamber at 2OoC and were considered to be germinated after the emergence of radical. Germination was scored when a 2mm radical had readout from the seed coat. Seeds were germinated for 1O days at 2OoC. Germination count was carried out after four days. Normal seedling was counted expressed as germination % at final count.

$$\text{Germination \%} = \frac{\textit{Number of normal seedling}}{\textit{Number of tested seed}} x\,100$$

Seedling vigor: 1O normal seedlings from each replicate were obtained to determine shoot and root length (cm), seedling fresh weight (mg) and seedling dry weight (mg) according to ISTA 1993.

At the end of screeming peried,

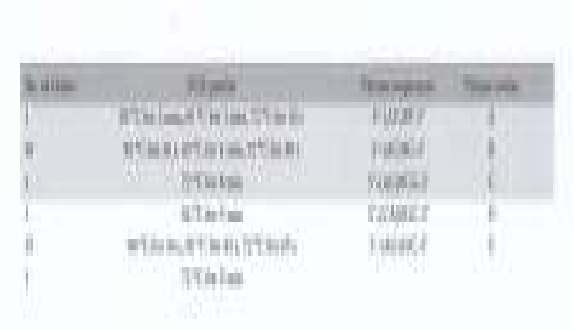
Five genotypes were selected according to germination percentage, they were one sensitive (pop 46), two moderate tolerance (Helaly and Serw1) and two tolerance (Fahl and Sakha4) for the evaluate of molecular behavior.

Molecular and Biochemical analysis:

Tolerance, moderate and sensitive genotypes of clover (Trifolium alexandrinum) were selected depending on the germination percentage under study. Total soluble proteins were examined through SDS Polyacrylamide gel electrophoresis (SDS-PAGE) with 12% of Polyacrylamide T % according to the methods of Lammli (1970). Total soluble protein was extracted and fractionated.

ISSR (Inter Simple Sequence Repeat) – PCR amplification: Total genomic DNA was amplified through Gene Amp Polymerase Chain Reaction (PCR) system cycler. ISSR (Inter Simple Sequence Repeat) PCR for amplified genomic DNA was carried out. Table (1) showed five ISSR (Inter Simple Sequence Repeat).

Table 2. ISSR (Inter simple sequence Repeat) DNA Pimers under study



Data were statistically analyzed using the analysis of variance for three replicates according to vicar arraignment factorial in a completely randomized design. Analysis of variance was computed according to Snedecor and Cochran (1981) and treatment means was compared by Duncan,s multiple range test, (Duncan 1955). All statistical analyses were performed using analysies of variance technique by "MSTAT-C" computer software package (1990).

RESULTS AND DISCUSSION

1- Effect of sea water concentrations :

The results indicated that levels effect of seawater concentration were significantly varied from each other in terms of germination percentage, shoot length, root length, seedling fresh weight and seedling dry weight Table 3.

Increasing seawater concentration from 0 to 8 ML-1 led to a significant decreasing in the mean germination percentage, shoot length, root length, seedling fresh weight and seedling dry weight when compared with the control as (81.27 to 56.88%), (2.93 to 1.74cm), (2.70 to 1.42cm), (247.92 to 187.08mg) and (21.13 to 12.58mg), respectively. Declining germination because of higher salinity may be correlated to the salinity nature, which diminishes imbibitions of water because of depressed osmotic potential of medium and alterations in metabolic activities *(Yupsanis et al., 1994)*. Salinity prompted seed

germination inhibition could be indorsed to high osmotic stress or because of specific ion toxicity *(Huang and Reddman, 1995)*. It seems that declining in germination rate is correlated to salinity-induced disturbance of the metabolic process that causing elevation in phenolic compounds levels *(Ayaz et al., 2000)*. The reduction in root and shoot development may be because of toxic effects of saline used along with unbalanced nutrient uptake by the seedlings. It moreover, may be due to the ability of a root system to control movement of ions to the shoot, which is of crucial importance to plant survival in the presence of NaCl *(Hajibagheri et al., 1989)*. Similar results were reported by *Amira (2011), Asci (2011), Almas et al (2013), Begum et al (2013) and Niste et al (2015)*.

Table 3. Effect of different concentrations of sea water on germination (%) shoot length, root length, seedling fresh weight and seedling dry weight of Egyptian clover.

Characters / Treatments	Germination %	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
Sea water concentration					
0 ML^{-1}	81.27a	2.93a	1.84b	247.92a	21.13a
4ML^{-1}	74.71b	2.57b	2.70a	232.08a	17.96ab
6ML^{-1}	73.17b	1.83c	2.35a	212.71b	14.92bc
8ML^{-1}	56.88c	1.74d	1.42b	187.08c	12.58c
F-test	**	**	**	**	**
Genotypes					
Gemmeiza	83.75c	2.27bc	2.26a-c	237.50ab	12.83b
Sakha Cm.	59.75h	2.38b	2.17a-c	185.83d	12.25b
Giza6	84.33c	2.22cd	2.18a-c	233.33ab	17.75b
Comp. Variety	82.92c	2.06ef	1.79c	193.33d	22.50ab
Sakha4	90.50ab	2.24cd	1.77c	214.173b-d	16.42b
Sids Comp.	68.42f	2.17c-e	1.73c	195.83cd	31.33a
Sakha96	82.92c	2.18c-e	2.33a-c	228.33a-c	15.08b
Sakha Comp.	73.58e	2.30bc	1.92bc	235.00ab	14.67b
Sakha Comp.2000	46.92i	2.03f	1.89bc	214.17b-d	15.25b
Fahl	93.67a	3.75a	2.69ab	250.83a	16.92b
Population10	48.58i	2.06ef	1.89bc	194.17d	13.42b
Population6	65.92fg	2.10d-f	2.05bc	235.00ab	15.08b
Sakha Comp.2011	63.42g	2.11d-f	1.91bc	212.50b-d	13.75b
Serw1	88.92b	2.26bc	2.14bc	235.00ab	15.83b
Population46	31.58j	1.87g	1.51c	216.67b-d	12.83b
Helaly	78.92d	2.30bc	3.03a	237.50ab	20.42b
F-test	**	**	**	**	*

***, ** indicated highly significant at 0.01%. Means followed by the same letter are not significantly different at the 5% level, using Duncan's multiple range tests.**

2- Effect of genotypes:-

The effect of genotypes on examined viability factors of Egyptian clover seed are displayed in Table 3. Highly significant differences were detected between all genotypes for all the chartistices traits under the study. It was noticed that the Fahl genotype had the highest percentage of germination (93.67%). While, the lowest average of germination percentage was observed in population46 genotype (31.58%).

Root length also significantly varied among control genotype. Root length of Helaly genotype recorded the highest value (3.03cm). Concerning the shoot length, the situation is different, highest value was recorded for Fahl (3.75cm) and the lowest value was observed in population46 (1.87cm).

The data showed that mean seedling fresh weight had the highest for Fahl genotype (250.83mg) and the genotypes which gave the lowest were; Sakha Comp. 2014, company variety and Population 10 with average of 185.83, 193.33 and 194.17mg respectively. Seedling dry weight was observed in Sids comp. genotype (31.33mg) was the highest value.

3- Effect of interaction:-

The interaction of salinity and genotypes in terms of percentage of germination was significant ($p \leq$ O.O1). The optimum germination was observed in Fahl genotype at zero concentration, while, the minimum germination was found in population6 at 6 mM^{-1} sea water concentration Figure 1.

Although some genotypes had significant effect on shoot length of Egyptian clover under saline conditions. Fahl genotype had the highest shoot length (4.43 and 4.48 cm) at zero and other 4 concentrations, while, population6 had the lowest shoot length (1.10 cm) at 8 ML^{-1} concentration Figure 2.

The effect of the interaction between salinity levels and genotypes on length of root was significant ($p \leq 0.01$). The longest root was determined in Helaly genotype at zero concentration, whereas minimum length of root was measured in Sids Comp. at 8 ML^{-1} saline conditions (Figure 3). Lengths of shoot and root were the most important parameter for selection under salt stress circumstances, as roots are indirect contact with soil and absorb water and nutrient from soil *(Jamil and Rha, 2004)*, and shoot grows until the r oot can fully support it. Toxic effects of saline and unbalanced nutrient up taking by seedlings may be responsible for decreasing in root and shoot growth under salinity concentrations. High salinity level may slow root and shoot growth because of decreasing in water and essential mineral nutrients absorption from soil by the plant (Neumann, 1995). Furthermore, the initial decreasing in shoot elongation is possibly because of hormonal signals generated by roots (Munns, 2002).

The interaction of genotypes and salinity of seedling fresh weight was significant ($p \leq 0.01$). The highest seedling fresh weight was found in Sakha96 at zero concentration (310.00 mg), while, the lowest

seedling fresh weight was found in Sids Comp. 2014 at zero saline conditions Figure 4.

Although genotypes effects showed significant differences ($p \leq 0.01$) under saline conditions on seedling dry weight. Sids Comp. had the highest seedling dry weight (42.00 mg) at zero concentration Figure 5.

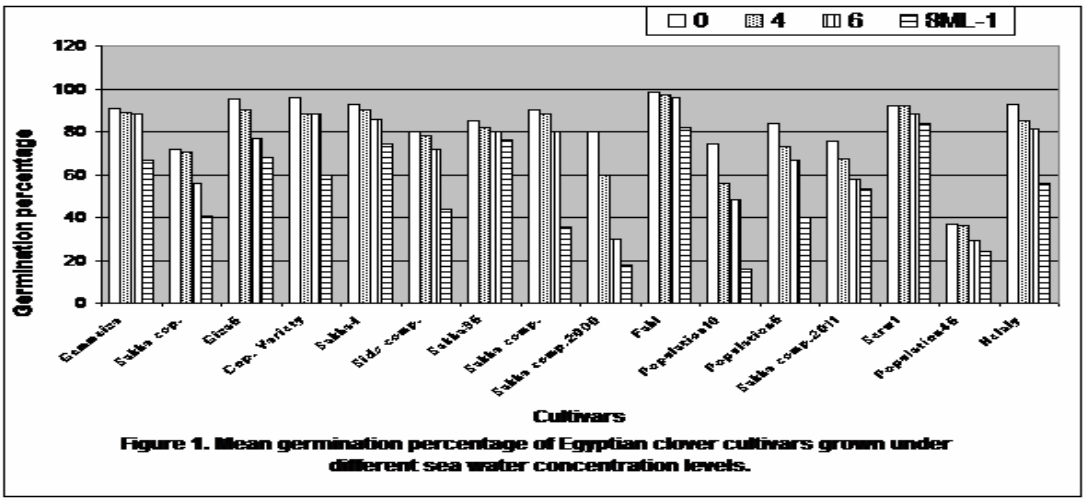


Figure 1. Mean germination percentage of Egyptian clover cultivars grown under different sea water concentration levels.

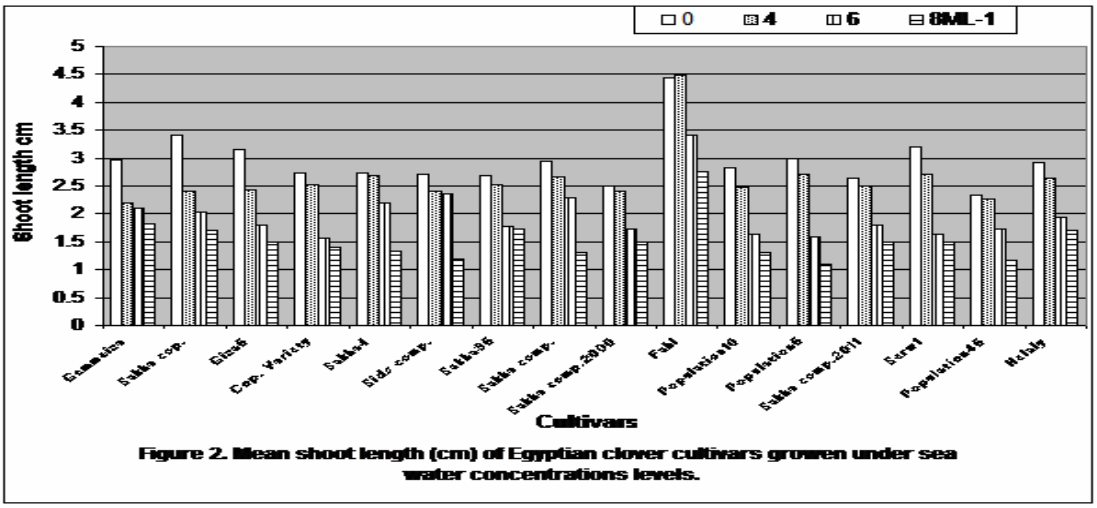


Figure 2. Mean shoot length (cm) of Egyptian clover cultivars grown under sea water concentrations levels.

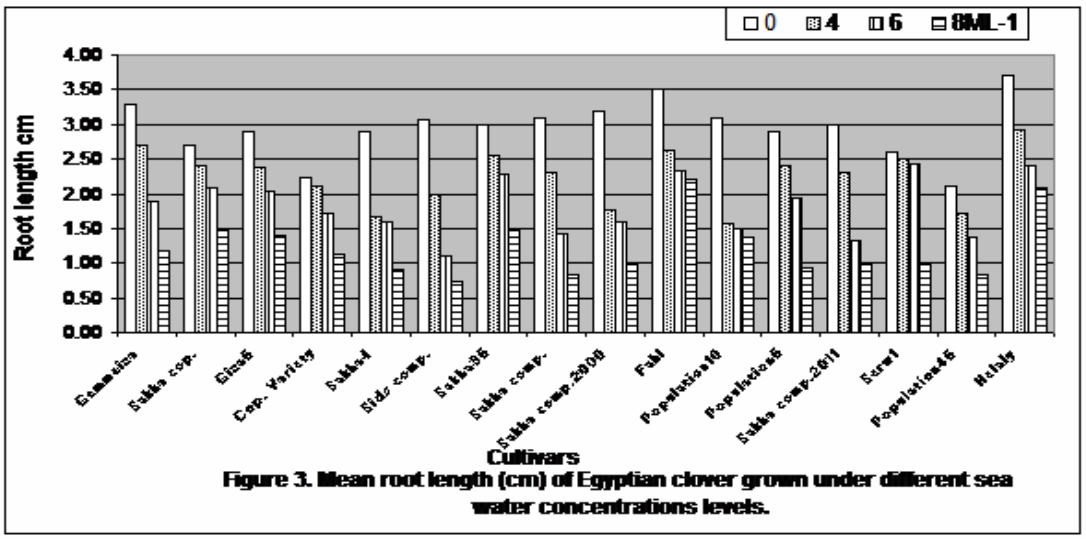


Figure 3. Mean root length (cm) of Egyptian clover grown under different sea water concentrations levels.

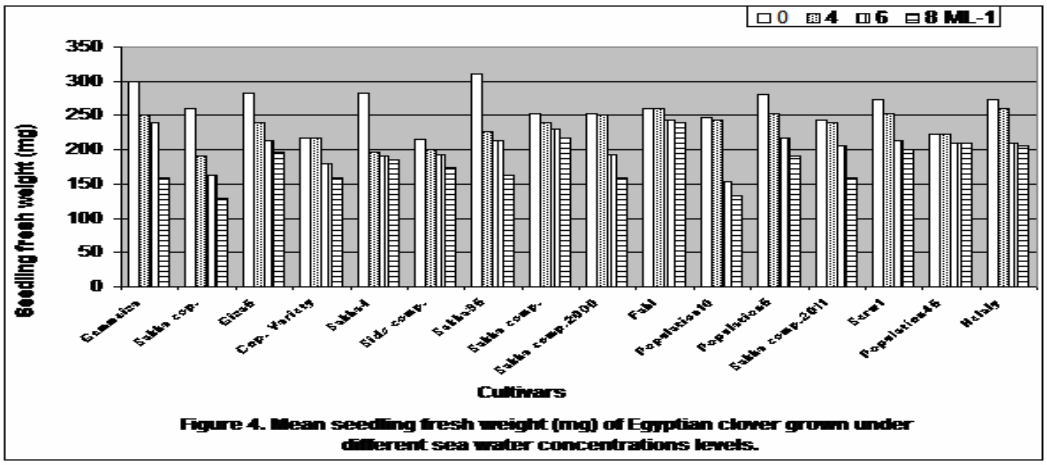


Figure 4. Mean seedling fresh weight (mg) of Egyptian clover grown under different sea water concentrations levels.

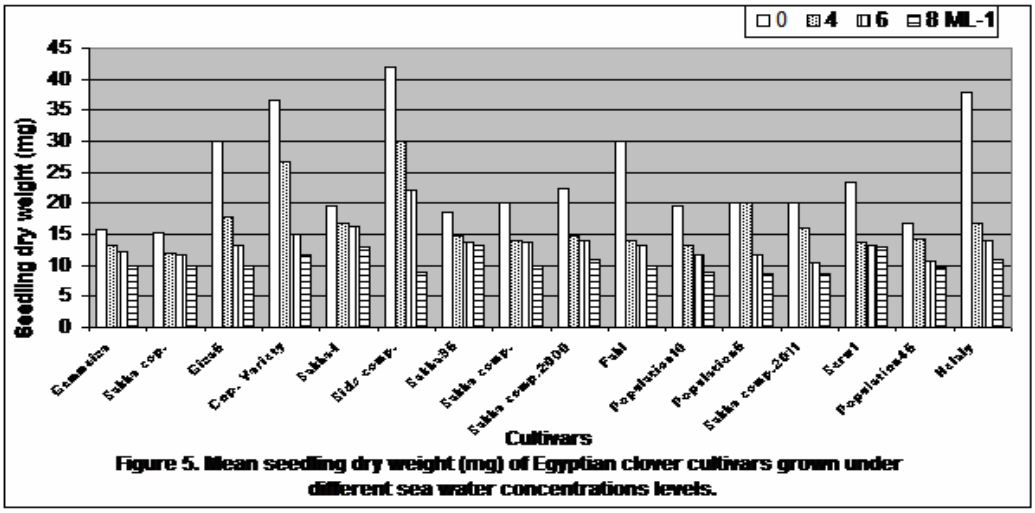


Figure 5. Mean seedling dry weight (mg) of Egyptian clover cultivars grown under different sea water concentrations levels.

Biochemical analysis:-

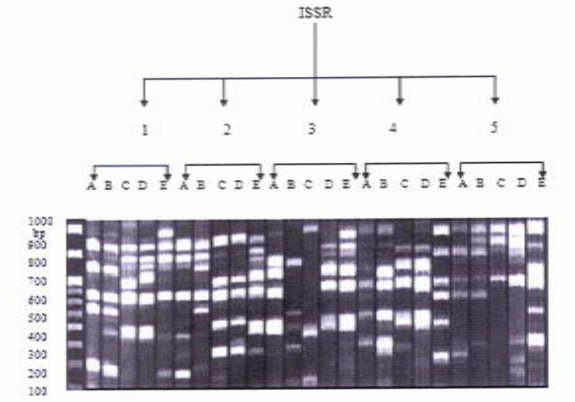


Figure 1. ISSR -PCR amplification technique for five clover with different salinity response.

Where:
1- First ISSR primer. 2- Second ISSR primer.
3- Third ISSR primer. 4- Fourth ISSR primer.
5- Fifth ISSR primer.
A-Salinity Resistance Egyptian clover.
B-Salinity Resistance Egyptian clover.
C-Salinity moderate Egyptian clover.
D-Salinity moderate Egyptian clover.
E-Salinity sensitive Egyptian clover.

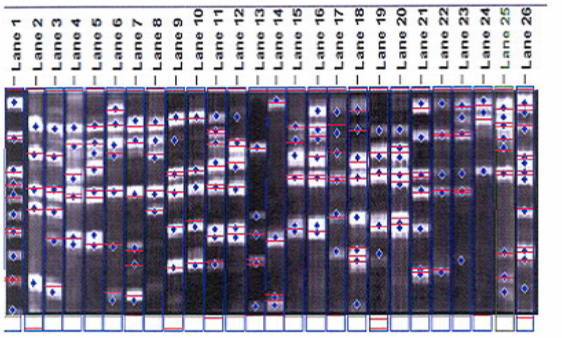


Figure 2. Genomic band detection for ISSR (Inter Simple Sequence Repeat) -PCR amplification technique for five Egyptian clover with different salinity response.

1- First ISSR primer. 2- Second ISSR primer.
3- Third ISSR primer. 4- Fourth ISSR primer.
5- Fifth ISSR primer.
A- Salinity Resistance Egyptian clover.
B- Salinity Resistance Egyptian clover.
D- Salinity moderate Egyptian clover.
C- Salinity moderate Egyptian clover.
E- Salinity sensitive Egyptian clover.

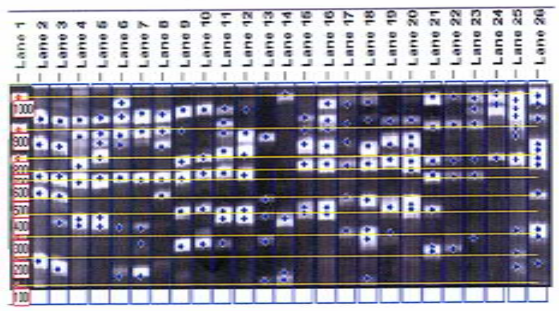
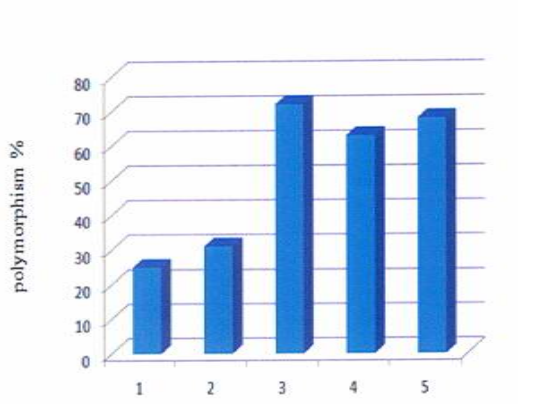


Figure 3. loci detection for ISSR (Inter Simple Sequence Repeat) -PCR amplification technique for five *Trifolium alexandrinum* with different salinity response.

1- First ISSR primer. 2- Second ISSR primer.
3- Third ISSR primer. 4- Fourth ISSR primer.
5- Fifth ISSR primer.
A- Salinity Resistance Trifolium alexandrinum.
B- Salinity Resistance Egyptian clover .
C- Salinity moderate Egyptian clover.
D- Salinity moderate Egyptian clover.
E- Salinity sensitive Egyptian clover .



ISSR (Inter Simple Sequence Repeat) Primers

Figure 4. Polymorphism % for five ISSR (Inter Simple Sequence Repeat) primers for five *Egyptian clover* with different salinity response.

Table 4. Illustrate ISSR (Inter Simple Sequence Repeat) -PCR amplification technique results for five *Trifolium alexandrinum* with different salinity response

Primer Codes	Primer sequences	Total amplified bands	Polymorphic bands	Monomorphic bands	Polymorphism%
A	5'-(AC)8T-3'	31	7	24	25
B	5'-(AC)8G-3'	30	9	21	31
C	5'-(AC)8CG-3'	27	19	8	72
D	5'-(CA)8GC-3'	33	20	13	63
E	5'-(AG)10C-3'	34	23	11	68

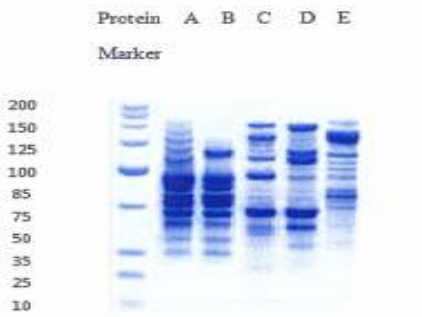


Photo 1. Protein fingerprinting patterns for five Egyptian clover with different salinity response.

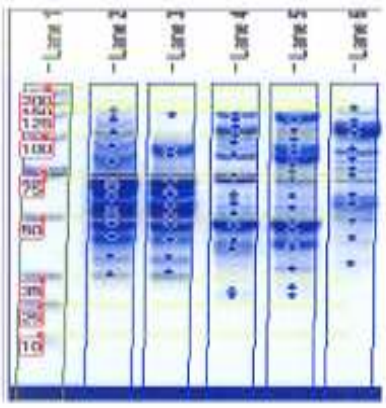


Fig 3. Computerized detection of molecular weight for five Trifolium alexandrinum with different salinity response.

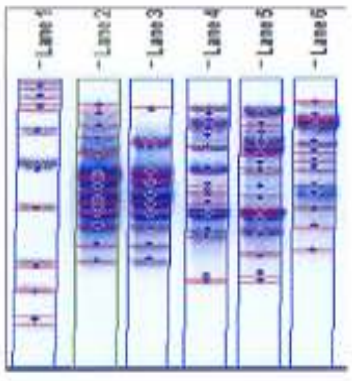


Photo 2. Computerized detection for protein patterns for five Egyptian clover with different salinity response.

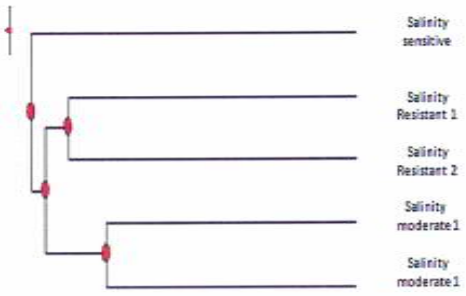


Figure 4. Phyllogenetic tree for five Egyptian clover with different salinity response based on SDS-PAGE technique.

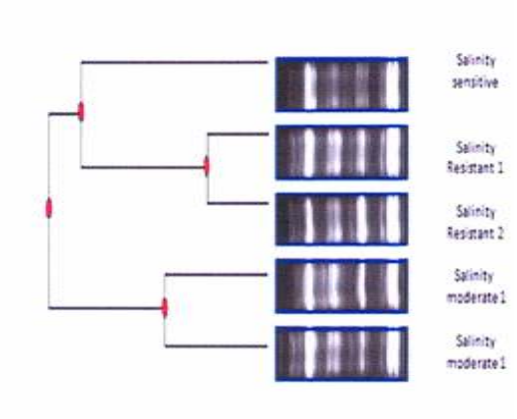


Figure 5. Phyllogenetic tree for five Egyptian clover with different levels of salinity response based on ISSR technique

Salinity stress response was evaluated for Egyptian clover via five primers of ISSR (Inter Simple Sequence Repeat) -PCR amplification technique. Generally, highly genetic variation was detected for resistance, moderate and sensitive Egyptian clover Figures 1, 2 and 3. Table 5 and Figure 4 showed that, third ISSR primer was superior for clearing genetic variation and showed 72 % of genetic variation among two resistances, two moderate and sensitive clover genotype. First ISSR primer reflected lowest genetic variation (25%) among different response levels of clover genotype. Fifth ISSR primer was superior fourth and second primers for clearing genetic variation levels among two tolera nce, two moderate tolerance and sensitive clover genotype with 68, 63 and 31 % respectively. Our obtaining results are in agreement with those of Wei (2004) who reported that DNA fingerprinting can be applied to variety identification and genetic diversity evaluation of Medicago sativa. More supported results was added to or findings by Hassan (2005) who reported that ISSR marker is the best choice for the evaluation of diversity and assessing the genetic relationships between M. oleifera and M. pregrina genotypes with high accuracy. Our findings by employed ISSR technique for clearing genetic similarity was in agreements with who applied Inter simple sequence repeat (ISSR) to evaluate salinity affect on "Sarin" subprogram using mono-nucleotide repeat based primers to detect polymorphism (41.1 %) comparable to that obtained using di-nucleotide repeat based primers (43.4 %). Selected salt tolerant genotypes showed an average similarity of 0.748 with CSR10, which was higher than the similarity (0.635) with HBC19. However, selected salt-sensitive plants showed more or less equal similarity with CSR10 (0.674) and HBC19 (0.642). According to ISSR (Inter Simple Sequence Repeat) -PCR amplification technique for five Egyptian clover with different salinity response figure 5, genetic differences were clear. As a result for significant salinity stress effect for sensitive Trifolium alexandrinum, sensitive genotype was separate in individual cluster with highly dissimilarity value. Highly genetic similarity was clearly recorded for moderate salinity Egyptian clover genotypes and superior on tolerance clover genotypes.

Table 5. SDS-PAGE analysis, showing number of peptide bands and common molecular weights.

Protein Band	Sahka 4	Fahl	Helaly	Serw I	Population 46
129	129				129
121	121	121			
114	114		114	114	114
107			107		107
103	103			103	
100			100		100
99				99	
98	98		98		
97		97		97	97
96		96	96	96	
95	95				95
89				89	89
78			78		78
70	70	70		70	
63	63				
58	58	58			
54	54	54	54	54	54
52			52		
50		50	50		50
49	49			49	
48	48	48	48		
47	47			47	47
46	46	46	46		
45				45	
44	44				44
43		43	43	43	
42			42	42	
41	41	41			
40					40
38				38	
37	37	37			
31			31	31	
			29	29	

Rl: Tolerance varityl, R2: Resiestant varity2, Ml: Moderate variety l, M2: Moderate variety2, S: Sensitive variety.

Figures 1,2 and 3 and Table 5 illustrate total soluble protein fractions among tolerancee, moderate tolerance and sensitive of clover genotypes,121, 70, 58, 54, 48, 46, 41 and 37 kda protein bands were common between salinity tolerancee genotypes. On the other hand, 114, 96, 54, 43, 42, 31 and 29 kda were recorded as common protein fragments between moderate salinity of clover genotypes. Interestingly, 129, 114, 97, 95, 50, 47, 44 were in common between tolerancee and sensitive clover genotypes. Moreover, 114, 107, 100, 97, 78, 50, 47 were in common between moderate and sensitive clover genotypes. Common protein fraction with 54 kda was expressed in all tolerancee, moderate and sensitive clover genotypes which refare to its important role in salinity metabolism cycle. 40 kda protein fraction was distinguishably expressed in sensitive clover genotypes which may be explane salinity sensitivity. Salinity resistance of clover genotypes could be explained in the light of expressed unigue protein fractions with 63, 58, 41 and 37 Kda. Distinguish low molecular weight protein fractions with 42, 31 and 29 |kda may be resulting moderate salinity resistance for clover genotypes.

More emphasize was added to the present findings by *Hamoud et al., (2005).* They applied electrophoretic detection of peotein polymorphism as markers for genetic and ecological variations have been also examined thru assessment of isozyme forms as in *Ipomea-carnea*. Moreover, *Azab et al ., (2011)* added more support to our results via SDS-PAGE analysis for the water soluble protein in the six Egyptian clover

revealed a total number of 19 bands with molecular weights (MW) ranging from about 12.24 to 121.2 kda and these six Egyptian clover genotypes cannot be uniquely identified (fingerprinted) with genotype protein markers.

As a result for salinity stress, genetic similarity was cleared for Egyptian clover based on total soluble protein fractionation via SDS-PSGE techinique. As shown in figure 4, sensitive salinity clover genotype showed highly dissimilarity and located in separate individual cluster. High genetic similarity was found within tolerant and moderate tolerant *clover* genotypes were more similar than restatant *clover* genotypes.

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تقييم التنوع الوراثي في بعض أصناف البرسيم المصري تحت مستويات الملوحة من مياه البحر

شيرين محمد النحراوى[1] و آلاء محمد المهدي أحمد شاهين[2]

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أجريت تلك الدراسة بالمعمل الخاص بقسم تكنولوجيا البذور فى محطة بحوث سخا مركز البحوث الزراعية مصر – خلال موسم 2017/2016م لدراسة أربع مستويات من مياه البحر (0، 4، 6 ، 8ML^{-1}) بعد التخفيف بماء مقطر بينما تم استخدام الماء العادي كمجموعة ضابطة والتركيز صفر استخدام مياه عادية (كنترول) بدون ملوحة علي إنبات البذرة وطول الريشة وطول الجذير والوزن الغض والوزن الجاف للبادرة وذلك ل 16 صنف من البرسيم المصري وكان تصميم التجربة في ترتيب عشوائي من ثلاث تكرارات بتصميم تام العشوائية. وكان تأثير تركيزات مياه البحر مرتفع المعنوية حيث قلت نسبة الإنبات وطول الريشة وطول الجذير والوزن الغض والوزن الجاف للبادرة بزيادة تركيز الملوحة من صفر إلي 8 ML^{-1} وكانت نسبة الإنبات الأعلى للصنف الفحل (وحيد الحشة) ويلية سخا4 صنف (متعدد الحشات) وأقل نسبة إنبات كانت لعشيره 46 واختلف الوزن الغض للبادرات مابين 185, 83 مجم لتركيبى سخا ولأعلى في الوزن الأخضر كان الفحل بوزن 250,83مجم ويلية الصنف هلالى بوزن 237,50مجم وكانت أفضل التراكيب تحت أعلى تركيز ملوحة (8) للصنف سرو 1 ، فحل ، سخا4 وسخا96 على التوالى بينما الأقل فى نسبة الإنبات تحت أعلى تركيز كان للتركيب الوراثى تركيبى سخا 2000 هذا وقد هدفت الدراسة الحالية إلى تقييم الأثر الخاص بتأثير الملوحة على *alexandrinum Trifolium* وذلك من خلال التقنية الوراثية ISSR (Inter Simple Sequence Repeat) والتقنية البيوكيميائية للتفريد الكهربي للبروتين الكلي الذائب SDS-PAGE، ولقد عكست الخمس بادئات المستخدمة في تقنية ISSR درجة عالية من التباين الوراثي لكل من التراكيب الوراثية *alexandrinum Trifolium* المقاومة والمتوسطة والحساسة للملوحة الأمر الذي يمكن عزوه إلى تأثير مستويات الملوحة المختلفة على التراكيب الوراثية، فلقد أوضح كل من البادئ الثالث، الخامس، الرابع، الثاني والأول 72، 62، 68، 31 و 25% من الاختلافات الوراثية بين التراكيب الوراثية *alexandrinum Trifolium* المقاومة والمتوسطة والحساسة للملوحة، وبدراسة البروتين الكلي الذائب لمجموعة التراكيب الوراثية المتباينة الاستجابة للملوحة فلقد تم رصد حزمه بروتينية ذات وزن جزئي يبلغ حوالي 54 كيلو دالتون في التراكيب الوراثية *alexandrinum Trifolium* المقاومة والمتوسطة والحساسة للملوحة الأمر الذي يمكن إرجاعه إلى دورها الهام في التعامل الفسيولوجي مع ضغط الملوحة على التركيب الوراثي، بالإضافة لذلك فلقد تم دراسة العديد من الحزم البروتينية في كل من التراكيب الوراثية *alexandrinum Trifolium* المقاومة والمتوسطة والحساسة للملوحة التي توضح الاستجابة المتباينة لمستويات الملوحة المستخدمة تحت الدراسة.