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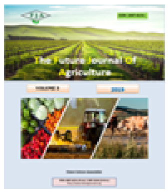

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AGRO-PHYSIOLOGICAL AND SEED VIABILITY RESPONSE OF FIVE EGYPTIAN CLOVER GENOTYPES TO DIFFERENT LEVELS OF SALINE IRRIGATION WATER

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ABSTRACT: Salinity is a major barrier to Egyptian clover production because it disrupts its growth, physiological, and biochemical processes, hence economically reducing yield. Laboratory and lysimeter experiments were conducted to evaluate five Egyptian clover genotypes (Sakha 4, Sakha 2000, Sakha 2014, Gemmiza 1 and Serw1) under three levels of saline irrigation water (control, 4.5 and 9.0 dS m-1) during 2018 / 2019 and 2019 / 2020 seasons. Results demonstrated that irrigation with 9.0 dS m-1 recorded the lowest values of all seed viability parameters, fresh, dry forage yields, plant height, number of stem, fresh leaf / stem and dry leaf/ stem percent, chlorophyll content, relative water content, K/ Na ratio, on contrary the contents of both proline and malondialdehyde (MDA) increased under irrigation with 9.0 dS m-1 as a result of salt stress. Among the studied five genotypes, Sakha 2014 was superior to other genotypes and ranked first in all studied traits except K/Na ratio, followed by Serw1 and Sakha 4 with insignificant differences between them, while Gemiza 1 and Sakha 2000 recorded the lowest values of physiological and agronomic traits also with insignificant differences between them. It could be concluded that the cultivation of Sakha 2014, Serw1, and Sakha4 is suitable to obtain high yield of Egyptian clover under saline conditions.

Key words: Egyptian clover genotypes, Salinity levels, physiological traits, seed viability.

INTRODUCTION

Forage crops play a crucial role in Egypt's sustainable agricultural system. (**Badawy, 2013**). Egyptian clover (*Trifolium alexandrinum* L.) is an annual legume crop well adapted to Mediterranean, center Europe, India, Pakistan, Southern USA, the Near East, West and South Asia regions to improve the soil fertility, hay production, and grazing **(Badawy. 2013** and **Muhammed *et al.*, 2014).** Insufficient production of forage crops and wide competition between Egyptian clover and wheat in winter season, made the extension to cultivate marginal lands necessary.

About 7% of the world's total land area is affected by salts, which is similar to the percentage of its arable land **(Munns, 2002)**. Salinity has reached a level of 19.5% of all irrigated land and 2.1% of dry-land agriculture worldwide (**Rani *et al.* 2012)**.

It is known that legumes are generally more sensitive to salinity **(Ghassemi-Golezani *et al.*, 2009),** this led to research for salinity tolerant genotypes with the aim of improving crop plants **(Zhu, 2001)**. Seed germination is the first critical stage affected by salinity in crop growth cycle, where salts are foremost dominated at surface layer **(Niste *et al*., 2015)**. Seed germination and seedling characteristics are the most substantial criterion used to select salinity tolerance in plants **(Mondal and Borromeo, 2016).** It has been established that salt tolerance in the germination stage of forage seeds such as berseem clover, alfalfa, and red clover is a heritable parameter that might be exploited for population selection of salt tolerance **(Mandic *et al.*, 2014).** Water stress acts by decreasing the percentage and rate of germination and seedling growth **(Rahimi *et al.*, 2006** and **Hamidi and Safarenjad, 2010)**. Seed germinate is the first critical and the most sensitive step in the life cycle of plants. Abiotic stresses including salting and draught are the major factors which reduce seed germination and seedling growth **(Kaydan and Yagmur, 2008 & Shitole and Dhumal, 2012).** Salinity reduces length and dry mass of the stem and affects the length and conductivity of the root of clovers *(Trifolium sp.)* **(Orak and Ates, 2005).** Also, **Abd El_Galil *et al.* (2007)** estimated the stability for sixteen Egyptian clover genotypes at four locations (Sakha, Gemmiza, Serw and Sids) during two seasons. They mentioned that genotypes hatour, Sakha4, Gemmiza 1, Narmer and Giza 6 surpassed other genotypes with no differences among them regarding the fresh yield.

In addition, studying differential responses of genotypes with contrasting stress tolerance will help underlying salt stress tolerance mechanisms **(Kumar *et al.* 2008)**. Highly attention should be directed to develop new varieties tolerant to salinity to increase the productivity of unit area / land in such regions **Abo-El-Goud *et al.* 2015)**. **Badran *et al.* (2015)** indicated that plant height in alfalfa significantly reduced as salinity concentration increased. Also**, Badawy (2018**) reported that synthetic Sakha 2000 had the lowest average of plant height and No. of stem. Salinity affects crops yield throw its physiological and biochemical effects. Salt stress harms plants primarily through osmotic stress, ion toxicity, where increasing salt ion concentrations in soil prevent water uptake and an excessive accumulation of Na^+ in cells causes ion toxicity, physiological disturbance, nutritional imbalance, and oxidative damage. **(Motos *et al.*, 2017).** Chlorophyll content has been widely used to identify tolerance to environmental stress in various plant species, such as salinity stress in white clover **(Li *et al.*, 2022)**. Also the Na^+/K^+ ratio is a key factor for salt tolerance in plants under saline conditions **(Sun *et al.*, 2014).**

As a result of yield losses under saline conditions and the wide variation of clover genotypes in their ability to tolerate salinity stress, the goal of this study was to evaluate five clover genotypes to determine the most tolerant genotypes and obtain maximum yield per unit area.

MATERIAL AND METHODS

Laboratory (germination) and lysimeter experiments were conducted during the two winter seasons 2018/ 2019 and 2019/ 2020 to study the effect of different levels of saline irrigation water on seed viability, physiological traits and yield of five Egyptian clover genotypes (Sakha 4 (G1), synthetic Sakha 2000 (G2), synthetic Sakha 2014 (G3), Gemmiza 1 (G4) and Serw1 (G5)) .

Germination experiment

This investigation was set up under the laboratory conditions at the Seed Technology Research Department, Sakha Agriculture Research Station, Egypt during 2020 season to evaluate five genotypes of Egyptian clover. Each genotype was examined under three levels of salinity (0, 4.5 and 9.0 dS m^{-1}). 50-seed were placed in 9 cm Petri dish. After 10 days more than 2mm long shoot and root seed was counted as germinated seed.

Germination percentage **(ISTA, 2018)** was calculated using the following formula:

$$\text{Germination \% (G\%)} = \frac{\text{Number of normal seedling}}{\text{Total number of seed tested}} \times 100$$

Germination index **(G I)** Mean day germination **(MDG)** was calculated as described by **AOSA (1986**) using the following equation:

$$\text{Germination index} = \frac{\text{number of germinated seeds}}{\text{days of first count}} + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

MDG = FGP/D where FGP is the final germination percentage, and D show the days up to achieve maximum germination rate.

At 10th day after seed placement, five seedlings of each Petri dish were sampled to estimate the following traits seedling length (cm), fresh and dry weight of seedling (g)

Seedling growth rate and seedling vigor index were calculated using the following formula (ISTA, 2018):

$$\text{Seedling growth rate} = \frac{\text{seedling length (cm)}}{\text{number of days}}$$

Seedling vigor index = Germination (%) × seedling length (cm)

Lysimeter experiment

The experiment was carried out at Greenhouse Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt. The site of the experiment has an elevation of 6 meter above sea level with latitude of 31º 7′ E and longitude of 30º 57′ N. The used experimental design was completely randomized design (CRD) arranged in split plot with three repetitions. The three levels of saline irrigation water S_1 (tap – water as control EC=0.65 dS m^{-1}), S_2= (EC 4.5 dS m^{-1}) and S_3= (EC 9.0 dS m^{-1}) were allocated in the main plot, while the sub plots were assigned to the five forage Egyptian clover genotypes. The lysimeter plot area was $2m^2$ (2 m length and 1 m width)(photo1).

Photo 1. The shape of lysimeter

Seed were broadcasted at the rate of 20 kg fed^{-1} and the rate of 150 kg calcium super phosphate 15.5% P_2O_5 fed^{-1} was applied at land preparation and all recommended agriculture practices were applied. Sowing dates were 28th and 30th October in the first and second seasons respectively.

Table 1. Soil structure and chemical analysis of lysimeter plots in the two studied seasons

Determination	Season	
	2018- 2019	2019- 2020
Physical Analysis		
Sand %	14.23	17.73
Silt %	33.79	29.55
Clay %	51.98	52.72
Texture	Clay	Clay
Chemical Analysis		
pH	8.9	8.27
EC (dS/m)	3.99	4.49
Anion and cation (meq/L)		
K^+	0.4	0.53
Na^+	28.5	32.8
Ca^{++}	7.97	9.23
Mg^{++}	5.2	7.3
$HCO3^-$	2.0	3.0
CL^-	19.83	23.9
$SO4^{--}$ (meq/L)	20.20	21.63

Analyzed at soil improvement and conservation Res., Depart, Soil, Water and Environment Res., Isn't., ARC.

Saline solutions for germination test and lyzimeter experiment were prepared by dissolving commercials salt in tap water to obtain 4.5 dS m^{-1} and 9 dsm^{-1} and chemical analysis of the used solutions were detailed in Table 2.

Table 2. Chemical analysis of the used irrigation water during 2018/ 2019 and 2019/ 2020 seasons

Irrigation water treatment	pH	EC dS m^{-1}	* SAR	Soluble cations (meq/L)				Soluble anions (meq/L)		
2018-2019 season				Na^+	Ca^{2+}	Mg^{2+}	K^+	Hco^{3-}	cl^-	$S04^{--}$
Control (tap water)	7.68	0.6	3.53	4.1	1.2	1.5	0.3	1.05	2.9	2.7
4.5 dS m^{-1}	8.11	4.5	10.46	30.6	7.2	9.9	0.8	4.5	21.4	23.3
9 dS m^{-1}	8.36	9	14.79	61.2	14.4	19.8	1.5	6.0	42.8	48.10
2019- 2020 season										
Irrigation water treatment	**pH**	**EC dS m^{-1}**	***SAR**	**Soluble cations (meq/L)**				**Soluble anions (meq/L)**		
				Na^+	Ca^{2+}	Mg^{2+}	K^+	Hco^{3-}	Cl^-	$S04^{--}$
Control (tap water)	7.83	0.70	3.99	4.8	1.3	1.6	0.3	2.00	3.3	2.7
4.5 dS m^{-1}	8.15	4.50	12.59	34.6	6.2	8.9	0.9	5.00	25.4	20.2
9 dS m^{-1}	8.37	9.00	17.89	67.2	12.4	15.8	1.2	7.50	46.8	42.3

Analyzed at soil improvement and conservation Res., Depart, Soil, Water and Environment Res., Inst., ARC. *Sodium absorption ratio

Four cuts were taken through the growing period of both seasons. The 1st cut was taken at 55 days after sowing, the 2nd cut was taken at 45 days after the 1st cut. The 3rd cut was taken at 30 days after the 2nd cut and the 4th cut obtained after 28th days from the 3rd cut in the two seasons. Before cutting samples of ten plants from each plot were randomly taken to determine

Physiological determinations and K / Na ratio

Total chlorophyll content (chl.a + chl.b, µg ml^{-1}) using N-N Dimethylformamide according to the method of **Moran 1982**.

Relative water content (RWC %) was determined as described by **Gonzalez and Var-Gonzalez (2001)** using leaves of five plants. It was determined using the formula:

RWC % = (FW-DW)/(SW-DW) *100

Where FW is leaf sample fresh weight, DW the weight of the sample after drying in oven at 70°c, SW is the saturated weight of the leaf sample after placed it for four hours in distilled water.

Proline content (mg g^{-1} FW) was determined as shown by **Bates *et al.*, 1973** using Spectrophotometer apparatus at wave length 520 nm.

Lipid peroxidation content (malondialdehyde content µmols g^{-1} FW) were determined using the method described by **Heath and Packer (1968**).

K / Na ratio

K and Na were determined using Flame Photometer according to **Chapman and Pratt (1978).**

Agronomic traits and reduction percent

1-Fresh yield, kg /plot.

2- Dry yield, kg /plot: where dry matter percentage was multiplied by fresh yield.

3- Plant height (cm) from the crown to the plant top on randomly 5 plants for every plot.

4-Number of stems within ($0.25m^2$).

5- Fresh leaf/stem ratio: samples 200 g/plot.

6- Dry leaf/stem percent: samples dried in oven on 105ºc until constant weight.

7-Reduction percent (%) for plant characters

Statistical analysis

The date was recorded statistically by analysis of variance (ANOVA) using **MSTAT-C version 4 (1986)** according to **Gomez and Gomes (1984)** combined analysis over two years was performed, when the assumption of homogeneity of error was no rejected according to **Bartlett's (1937).** The differences between the means were tested by the least significant differences (L.S.D.) values at 0.05 and 0.01 levels of Probability determined for various genotypes as descried by **Steel *et al*. (1997)**.

Correlation

Simple correlation was calculated according to **Steel and Torries (1980)**.

RESULTS AND DISCUSSION

Laboratory experiment

The obtained results cleared that different levels of saline treatments were significantly different from each other in germination (%), germination index (G I), mean day germination (M D G), seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), seedling growth rate (S G R) and seedling vigor index (SV I) Table (3).

Increasing salt concentration to 9 dSm^{-1} led to significant decrease in all studied traits when compared with the control treatment. The highest values of all studied traits were obtained from control treatment and the lowest values were observed with 9.0 d S m^{-1} concentration.

The genotype Serw 1 (G5) followed by Synthetic Sakha 2014(G3) gave the maximum values in all studied traits except for fresh and dry weight which were higher in genotype Gemmiza 1 (G4). To genotypes Synthetic Sakha 2000 (G2) we observed the minimum values in all studied traits except for fresh and dry weight which were lower in genotype Sakha 4 (G1). Variability in salinity tolerance among rice genotypes at germination have also been reported by **Hakim *et al*. (2010).**

Decreasing germination because of higher salinity may be due to high osmotic stress or because of specific ion toxicity **(Huang and Redmann, 1995).** In addition to toxic effects of certain ions, higher concentration of salts reduces water potential in the medium which in turn hinders water absorption by germinating seeds and resulting in reduction in germination **(Hakim *et al*., 2010).** Similar result was also found in sunflower **(Shila *et al*., 2016).**

Table 3. Mean values of germination (%), germination index (G I), mean day germination (M D G), seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), seedling growth rate (S G R) and seedling vigor index (S V I). of Egyptian clover genotypes as affected by saline irrigation levels over two seasons

Treatment	G %	G I	M D G	Seedling length (cm)	Fresh weight (mg)	Dry weight (mg)	S G R	S V I
Saline solution (s)								
S_1 Control	94.40	79.83	9.44	6.74	281.70	35.33	0.67	635.60
S_2 4.5 dS m^{-1}	90.13	76.22	9.01	5.82	209.10	21.40	0.58	530.70
S_3 9.0 dS m^{-1}	83.20	70.36	8.32	4.79	223.70	24.27	0.49	415.40
F test	**	**	**	**	**	**	**	**
LSD0.05	2.14	1.81	0.12	0.33	15.68	2.97	0.033	27.02
Genotype (G)								
Sakha 4(G1)	84.44	71.41	8.44	5.52	219.10	21.78	0.55	474.40
Sakha 2000(G2)	80.44	68.03	8.04	4.58	236.80	23.33	0.46	383.60
Sakha 2014(G3)	93.56	79.11	9.36	5.94	240.30	27.22	0.59	557.50
Gemmiza 1(G4)	90.67	76.67	9.07	4.98	263.10	35.56	0.49	454.20
Serw1(G5)	97.11	82.12	9.71	7.90	244.80	27.11	0.79	766.50
F test	**	**	**	**	**	**	**	**
LSD0.05	2.44	2.07	0.24	0.25	26.83	3.29	0.02	23.38

** highly significant at 5% levels probability

Seedling length is the most important parameter in sugar beet and cabbage for selection under salt stress **(Jamil and Rha, 2004)**. The reduction in seedling length may be due to toxic effects of saline water used along with unbalanced nutrient uptake by the seedlings **(Niste *et al*., 2015 and Hakim *et al*., 2010).**

Reduction in seedling growth as a result of salt stress has been reported by **Akram *et al*. (2010)** in several other crops. High salinity level may slow root and shoot growth because of decreasing essential mineral nutrients absorption from soil by the plant which could adversely affect seedling growth and vigor **(Berhanu and Berhane, 2014).**

It was found that seedling dry weight was decreased at all salinity levels **(Berhanu and Berhane, 2014). Akram *et al*. (2007)** found that seedling dry weight of all corn hybrids showed a decline towards increase in salinity level.

Highly significant differences were observed between all genotypes for germination (%), germination index (G I), mean day germination (M D G), seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), seedling growth rate (S. G. R) and seedling vigor index (S V I) as can be seen in Table 3.

The interaction between salinity levels and genotypes varied significantly in all traits under study (Fig.1). The highest values in germination percentage and germination index were found in Serw1 (G5), Synthetic Sakha 2014 (G3) and Gemmiza 1 (G4) at control treatment. On contrast, Synthetic Sakha 2000 at 4.5 dS m^{-1} concentration gave the lowest values in all studied traits.

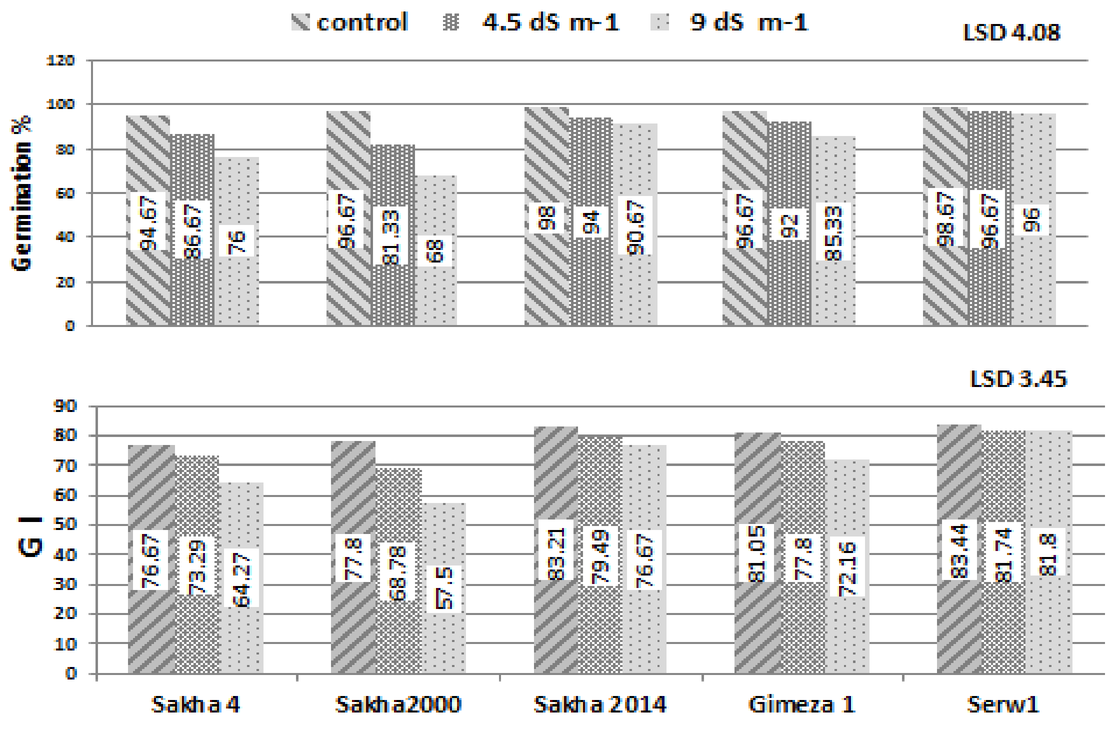

Fig. 1. interaction effect between saline treatments and genotypes on germination % and GI.

Physiological traits

Presented data in Table 4 demonstrated remarkable decrease in total chlorophyll content with increasing the electrical conductivity (EC) of the irrigation water in the four cuts and in the mean of all cuts, where the lowest concentration of total chlorophyll (chl.a + chl b) were obtained in plants irrigated by S3 (9.0 dSm^{-1}) as compared with control treatment. These results are in agreement with **Rahman *et al*., (2015) and Daneshnia and Chaichi (2018),** they reported that under salinity the oldest leaf starts to show chlorosis and leaf abscission occurs. Relative water content (RWC%), which indicates the balance between water supply to the leaf and transpiration rate was assessed to give an indicator of the plant's water dehydration condition during exposed to salinity stress (**Seiam *et al.*, 2020).** In this study it's clear that RWC% decreased as a result of increase the salinity level of the irrigation water in the four cuts and in the mean of the all cuts **(Bhattarai *et al.*, 2020**). This decrease in the water content of the leaves is a normal result of the first effect of salinity on plants through the osmotic stress which occurs as a result of the high osmotic pressure of irrigation water, causing physiological drought stress, and inability of plants to take sufficient water **(Motos *et al.*, 2017).**

Among the five studied genotypes, (Sakha2014) G3 gave the highest concentrations of total chlorophyll content and relative water content in all cuts and mean of cuts, followed by (Serw1) G5 and (Sakha 4) G1 with insignificant differences between them. While (Sakha 2000) G2 and (Gemmiza 1) G4 recorded the lowest values of the two traits and ranked last (Table 4).

Rahman *et al.*, (2015) and Lei *et al.*, (2018) mentioned that chlorophyll content and RWC decreased significantly with increasing salinity and they were higher in the tolerant genotypes than the sensitive ones. The results revealed that G3(Sakha 2014) had the highest RWC and total chl. Water content of the leaf and chlorophyll pigments are the main factors in the photosynthesis process, and that means G3 had the highest efficiency of photosynthesis process, and that would reflect on both fresh and dry yields as can be seen in Table 7.

Data in Fig. 2, illustrated that there were insignificant differences in total chlorophyll content between the two irrigation saline level S_2 and S_3 (4.5 and 9.0 dS m^{-1}) for G3, G5 and G1. While irrigating G4 and G2 with 9.0 dSm^{-1} gave the lowest chlorophyll content. On the other hand the highest percent of RWC were obtained from G3 and G1 either with control or 4.5 dS m^{-1}.

Table 4. Mean values of total chlorophyll and RWC % of Egyptian clover genotypes as affected by saline irrigation levels over two seasons

Treatment	Total chlorophyll (µg ml^{-1})					RWC (%)				
Saline solution (S)	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**
S1 Control	12.33	13.99	14.44	12.12	13.22	84.34	83.30	80.69	78.78	81.78
S2 4.5 dSm^{-1}	12.57	14.25	13.66	10.44	12.73	83.81	82.24	79.94	71.42	79.35
S3 9.0 dSm^{-1}	11.04	11.46	11.35	8.56	10.60	80.78	77.82	75.89	66.61	75.27
F test	**	**	**	**	**	**	**	**	**	**
LDS	0.240	0.421	0.297	0.373	0.194	0.740	0.431	0.735	0.444	0.430
Genotype (G)										
Sakha 4(G1)	12.00	13.33	13.36	10.50	12.30	83.73	81.39	79.62	73.08	79.45
Sakha 2000(G2)	10.87	12.40	12.31	9.33	11.23	80.91	79.12	76.77	69.61	76.60
Sakha 2014(G3)	13.06	14.08	13.98	11.27	13.10	85.91	83.77	81.82	75.77	81.82
Gemmiza 1(G4)	11.75	12.91	12.52	10.12	11.82	81.13	79.57	76.69	70.19	76.89
Serw1(G5)	12.21	13.44	13.59	10.63	12.47	83.20	81.74	79.32	72.71	79.24
F test	**	**	**	**	**	**	**	**	**	**
LSD	0.165	0.171	0.349	0.263	0.122	0.573	0.520	0.544	0.295	0.294

**, highly significant at 5% levels probability

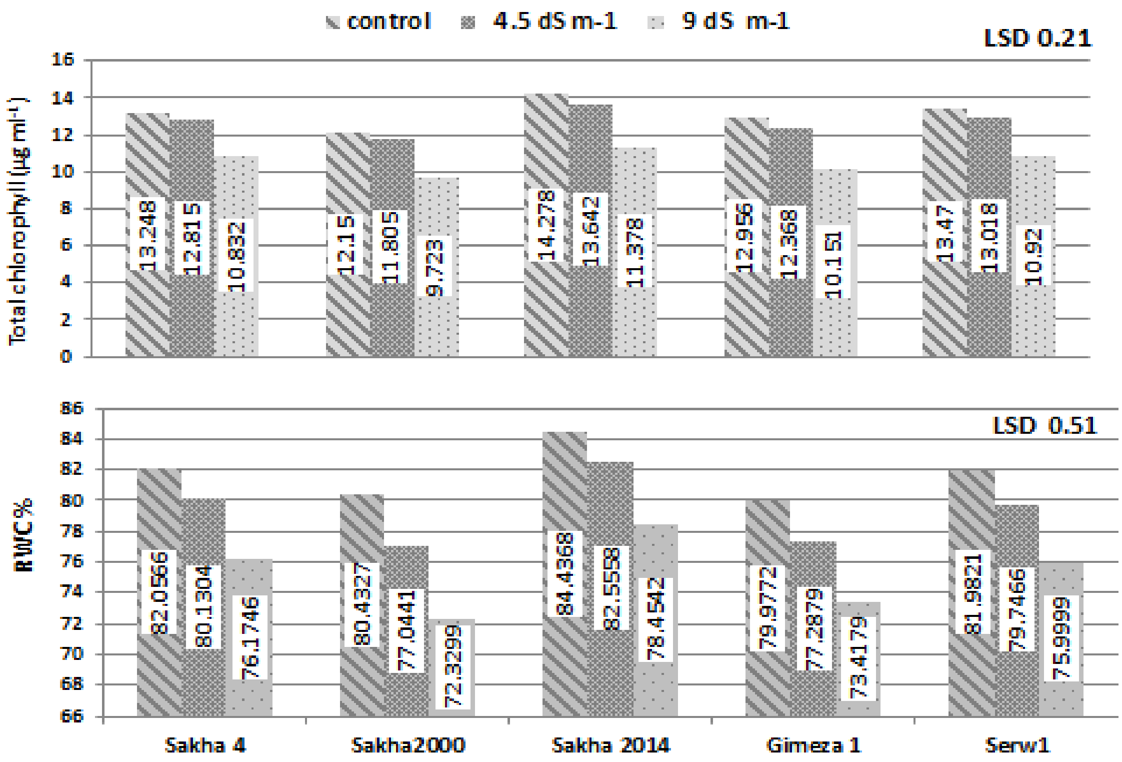

Fig. 2. Interaction effects between saline water and genotypes on total chlorophyll and RWC % over the two seasons.

Data in Table 5 revealed that both proline and MDA (malondialdehyde) increased significantly with increasing the level of salinity of irrigation water. Where the lowest values were obtained from control treatment while 9.0 dSm^{-1} recorded the highest ones in the four cuts and the mean of all cuts. These results reported previously by **Melo *et al*. (2022)**, where they revealed that proline is water soluble and it has been observed to accumulate in the cytosol of plant cell in response to salinity. Proline acts as non-Enzymatic antioxidant, and it has a role in keeping cells from dehydration under osmotic stress conditions. Reactive oxygen species (ROS) increased under salinity stress conditions and its accumulation harms all cell components and causes lipids peroxidation in the cell membrane, the final product of the peroxidation process is malondialdehyde (MDA) as reported by **Bhattarai *et al.*, 2020.** On contrary of the increase of proline, the increase in MDA is unfavorable and it is evidence of the plant's inability to tolerate salinity stress. Estimating MDA is considered an important indicator of determination ability of the plant in tolerating salinity stress**.**

G3 recorded the lowest values of MDA followed by G1 and G5, while the highest content was obtained from G2. **Lie *et al.*, (2018)** reported that under salinity stress, the alfalfa genotype which had the lowest content of MDA is most tolerant than other genotypes.

Table 6 show the ratio between K and Na in shoot of berseem plants under saline irrigation water. It's obvious that K content decreased and Na increased with increasing salinity of irrigation water, where the highest ratio were obtained from control treatment, and decreased with using 4.5 and 9.0 dsm/1.

Concerning the studied five genotypes, G5 (Serw1) was superior other studied genotypes and ranked first followed by G3 **(Sakha, 2014)**.

Sun *et al.*, (2014) demonstrated that K^+/Na^+ ratio is a key factor for salt tolerance in plants under saline conditions, where genotypes which had the highest ratio are more tolerant to salinity stress. The K^+/Na^+ ratio could be considered as an essential indicator of salt tolerance **(Rasel *et al.*, 2021).**

Table 5. Mean values of proline and malondialdehyde (MDA) contents of Egyptian clover genotypes as affected by saline irrigation levels over the two seasons

Treatment	proline (mg g^{-1} Fw)					MDA (μ mol g^{-1} Fw)				
Saline solution(S)	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**
S1 Control	0.218	0.208	0.2358	0.176	0.210	289.88	277.045	305.08	327.00	299.75
S2 4.5 dSm^{-1}	0.306	0.244	0.3301	0.218	0.274	298.39	286.151	324.39	359.78	317.18
S3 9.0 dSm^{-1}	0.376	0.326	0.3801	0.264	0.337	396.77	385.435	451.38	506.93	435.13
F Test	**	**	**	**	**	**	**	**	**	**
LSD	0.006	0.019	0.059	0.005	0.007	7.46	31.96	39.56	38.07	28.28
Genotype (G)										
Sakha 4(G1)	0.297	0.261	0.313	0.230	0.275	314.74	303.55	346.24	388.97	338.38
Sakha 2000(G2)	0.272	0.226	0.284	0.190	0.243	362.76	353.42	396.47	430.31	385.74
Sakha 2014(G3)	0.343	0.302	0.35	0.238	0.308	298.37	288.66	329.85	369.25	321.54
Gemmiza 1(G4)	0.283	0.242	0.303	0.209	0.259	341.33	325.72	373.21	409.27	362.38
Serw1(G5)	0.307	0.266	0.326	0.230	0.282	324.54	309.68	355.64	391.71	345.40
F Test	**	**	**	**	**	**	**	**	**	**
LSD	0.007	0.021	0.022	0.020	0.021	5.024	8.59	5.132	6.00	5.46

** highly significant at 5% levels probability

Table 6. Mean values of K / Na ratio in leaves of Egyptian clover genotypes as affected by saline irrigation levels over two seasons

Treatment	K / Na ratio				
Saline solution (s)	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**
S1 Control	1.284	1.315	1.359	1.402	1.340
S2 4.5 dS m^{-1}	1.077	1.116	1.146	1.194	1.133
S3 9.0 dS m^{-1}	0.938	0.962	0.976	1.004	0.970
F Test	**	**	**	**	**
LSD	0.06	0.019	0.018	0.027	0.019
Genotype (G)					
Sakha 4 (G1)	1.102	1.143	1.174	1.216	1.159
Sakha 2000(G2)	1.046	1.071	1.113	1.148	1.094
Sakha 2014(G3)	1.123	1.155	1.176	1.232	1.171
Gemmiza 1(G4)	1.058	1.088	1.113	1.139	1.099
Serw1(G5)	1.169	1.200	1.224	1.264	1.214
F Test	**	**	**	**	**
LSD	0.03	0.04	0.03	0.04	0.03

** highly significant at 5% levels probability

Agronomic traits

Fresh and dry forage yields

The data in Table 7 revealed that fresh and dry weight yields decreased significantly with increasing the level of salinity irrigation water in all cuts and total fresh and dry forage yields, where S1(control treatment) gave the greatest fresh and dry forage yields (20.9 and 2.480 kg/plot) respectively, while S3 recorded the lowest one (16.9 and 2.099 kg/plot) for both fresh and dry forage yields respectively over the two studied seasons. Also, data showed that synthetic Sakha 2014 (G3) genotype had the highest values of total fresh and dry forage yields (20.1 and 2.429 kg/plot) respectively and exceeded the rest of the genotypes over the two seasons, followed by genotypes Sakha 4 (G1)(19.3 and 2.336 kg/plot) and Serw 1 (G5) (19.3 and 2.342 kg/plot) over the two seasons for total fresh forage yields, respectively. While the genotype Synthetic Sakha 2000 (G2) was the lowest with values 17.0 and 2.079 kg/plot for total fresh and dry forage yields, respectively.

Table 7. Mean values of fresh and dry yields (kg/plot) of Egyptian clover genotypes as affected by saline irrigation levels over the two seasons

Treatment	Fresh yield kg/plot					Dry yield kg/plot				
Saline solution (S)	Cut1	Cut2	Cut3	Cut4	Total	Cut1	Cut2	Cut3	Cut4	Total
S1 Control	4.3	5.1	6.5	5.0	20.9	0.408	0.523	0.814	0.735	2.480
S2 4.5 dSm-1	4.0	4.6	5.7	4.2	18.5	0.397	0.483	0.739	0.637	2.256
S3 9.0 dSm-1	3.8	4.2	5.1	3.8	16.9	0.375	0.456	0.680	0.588	2.099
F test	**	**	**	**	**	**	**	**	**	**
L.S.D	0.10	0.10	0.11	0.06	0.16	0.007	0.007	0.007	0.012	0.019
Genotype (G)										
Sakha 4(G1)	4.2	4.7	5.9	4.5	19.3	0.413	0.490	0.762	0.671	2.336
Sakha 2000(G2)	3.6	4.3	5.4	3.7	17.0	0.349	0.460	0.696	0.574	2.079
Sakha 2014(G3)	4.3	4.9	6.1	4.8	20.1	0.415	0.514	0.786	0.714	2.429
Gemmiza 1(G4)	3.9	4.4	5.6	4.1	18.0	0.383	0.473	0.722	0.625	2.203
Serw1(G5)	4.2	4.7	5.9	4.5	19.3	0.406	0.499	0.754	0.683	2.342
F test	**	**	**	**	**	**	**	**	**	**
L.S.D	0.62	0.13	0.10	0.11	0.22	0.004	0.008	0.008	0.008	0.021

**, highly significant at 1% level probability

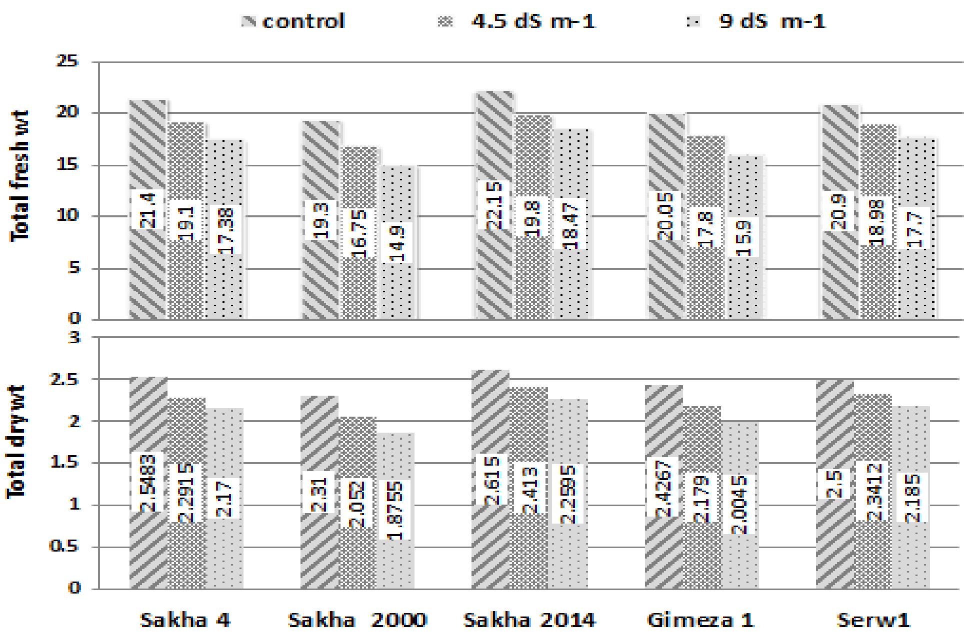

Fig. 3. Interaction effect between saline water and genotypes on fresh and dry yields over the two seasons.

Data in Fig. 3, showed that there were insignificant differences for total fresh forage yield while it had highly significant difference in total dry forage yield. Under the three saline irrigation water treatment (control, 4.5 dS m^{-1} and 9.0 dS m^{-1}) the best genotype was Synthetic Sakha 2014 (G3) for fresh and dry forage yields. While, the lowest one was Synthetic Sakha 2000 (G2) under all saline treatments.

These results are in harmony with those obtained by **Omar (1991),** who reported that Egyptian clover cultivars are sensitive to salinity stress and it could be use saline water in irrigation up to 1000 ppm with a small reduction in fresh and dry forage yields.

Similary, **Abo El-Goud *et al.* (2015)** who studied nine Egyptian clover to develop highly productive populations tolerant to high level of salinity and they found the genotypes (108 from Faraskour district) was the highest for total fresh and dry forage yields and a new promising to produce as a variety tolerant for high level of salinity. Also**, Badawy *et al.* (2018)** evaluated thirteen Egyptian clover genotypes for salinity tolerance under serw area conditions and they reported that the genotypes Helaly, population 46 and serw 1 are tolerant to saline soil under EC 6 dS/m. **Kazemeini *et al.* (2018)** who found that fresh and dry forage yields decreased with increasing saline irrigation levels on berseem clover. **Badawy *et al.* (2022)** who observed significant reduction in fresh and dry forage yields with increasing salinity water irrigation in cowpea genotypes.

Plant height and number of stem (0.25m^2)

Regarding in Table 8 showed that plant height and No. of stem were highly significantly effects due to salinity levels of irrigation water and these characters had decreased by increasing salinity of irrigation water.

Table 8. Mean values of plant height and No. of stem of Egyptian clover genotypes as affected by saline irrigation levels over two seasons

Treatment	Plant height (cm)					No. of stem (0.25m^2)				
Saline solution (s)	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**	**Cut1**	**Cut2**	**CCut3**	**Cut4**	**mean**
S1 Control	51.1	78.7	90.8	83.2	75.95	158.4	178.8	184.9	183.2	176.3
S2 4.5 dSm^{-1}	48.5	71.6	84.8	69.4	68.58	156.4	176.7	181.7	176.9	172.9
S3 9.0 dSm^{-1}	45.0	65.7	79.4	62.8	63.23	154.1	172.6	177.5	172.4	169.2
F test		**	**	**	**		**	**	**	**
L.S.D		0.49	0.34	1.54	0.62		1.10	1.01	0.74	1.08
Genotype (G)										
Sakha 4(G1)	49.9	73.0	86.1	72.5	70.4	160.0	182.7	184.3	180.7	176.9
Sakha 2000(G2)	43.4	67.1	81.2	66.1	64.5	150.6	166.0	173.8	173.5	166.0
Sakha 2014(G3)	53..6	75.8	89.0	76.7	73.8	161.9	184.6	189.9	182.2	179.7
Gemmiza 1(G4)	45.4	69.9	82.7	69.1	66.8	153.6	172.5	178.6	174.7	169.9
Serw1(G5)	48.7	74.1	86.2	74.5	70.9	156.0	174.4	180.3	176.3	171.8
F test	**	**	**	**		**	**	**	**	
L.S.D	0.19	0.58	1.16	0.57		1.14	0.97	1.06	1.25	

**, highly significant at 1% level probability

Data in Fig. 4, illustrated that plant height and No. of stem (0.25) affected significantly by saline treatments, where Synthetic Sakha 2014(G3) was the highest genotype but Synthetic Sakha 2000 (G2) was the lowest one under saline water irrigation, respectively.

The interaction S1 G3 gave the maximum average for plant height and No. of stem which were 80.0 cm 181.9 while S1 G2 recorded the minimum average (71.8cm and 169.8) for plant height and No. of stem over two years, respectively. Also S2 G3 had the highest averages (73.1cm 180.1) while S2 G2 had the lowest average value were 63.8 cm and 166.1 for plant height and No. of stem over two years, respectively. However, S3G3 recorded the highest average were 68.2cm and 177.0, while S3G2 gave the lowest average value were 57.7cm and 162.0 for plant height and No. of stem over two years, respectively.

These results are in agreement with **Omar (1991).** Similarly, these results are harmony with**, Abd El-Galil (2007)** who found that significant differences were recorded among the varieties as syn 2-ranked 1st followed by Ismailia_94 in alfalfa. The same trend, **El-Hefny (2010), Patel *et al.* (2010), Salih and Kia (2013)** who reached that increasing salinity decreasing plant height of cowpea**.** The same trend, **Kazemeini *et al.* (2018)** who revealed that plant height decreased by increasing salinity levels of irrigation water. **Badawy *et al.* (2022)** who found that highly significant differences among the three saline water treatment. Also, they decided that addition of saline irrigation water significantly decreased plant height of cowpea when compared to natural irrigation water with saline water. Where control treatment (fresh water) recorded the highest average of plant height 80.51cm, while S3 (3.5 dS m^{-1}) recorded the lowest average of plant height 56.34 cm.

It is obvious that growth reduction for plant height was noticed with the highest level of irrigation water salinity (S3) may be attributed to a reduction in cell division and elongation.

Reported synthetic Sakha 2000 had the lowest of plant height as average as was 67.3cm and No. of stem was 157.6, respectively.

Fresh and dry leaf / stem percent

The data for mean performance of fresh and dry leaf /stem percent are presented in Table 9 showed highly significant differences in both traits under salinity levels of irrigation water.

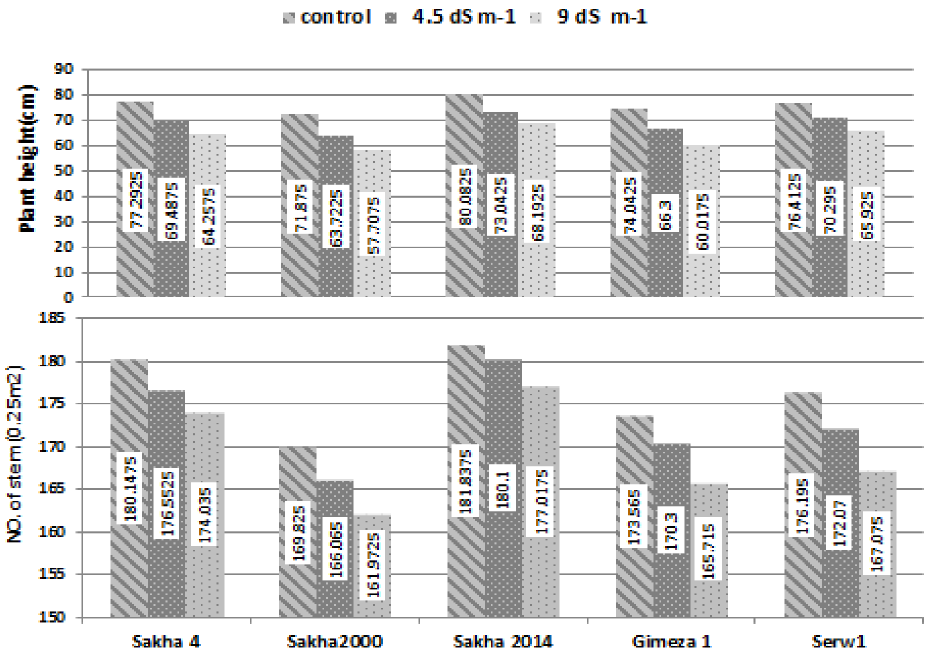

Fig. 4. Interaction effect between saline water and genotypes on plant height and number of stem over the two seasons.

Regarding in Table 9 showed that fresh and dry leaf /stem percent were decreased by increasing due to salinity levels of irrigation water. While S1(control treatment) recorded the maximum average for fresh and dry leaf/ stem percent (66.04 and 54.38) respectively, while S3 had the lowest average (57.66 and 48.99) for both fresh and dry leaf/stem percent over the two studied seasons, respectively. Also, data revealed that synthetic Sakha 2014 (G3) genotype had the highest average of fresh and dry leaf/ stem percent (60.80 and 53.30) respectively and exceeded the rest of the genotypes over the two seasons, followed by genotypes Sakha 4 (G1) 59.38 and Serw 1 (G5) (52.94 over the two seasons, respectively. While the genotype Synthetic Sakha 2000 (G2) was the lowest with average 53.86 and 49.96 for fresh and dry leaf/ stem percent, respectively.

These results are agreement with **Badawy *et al.* (2018)** who reported that thirteen Egyptian clover genotypes for salinity tolerance under Serw area conditions. They recorded population 46 were 49.2 and 63.4 for fresh and dry leafs/stem percent over two years, respectively**.** Also, **Badawy *et al.* (2022)** who mentioned that a reduction in leaf stem percent with increasing the level of salinity water irrigation on cowpea crop. While the control treatment S1 (fresh water) recorded the highest average 51.25, but the S3 (3.5 dS m^{-1}) had the lowest average was 44.36 for dry leaf stem percent. Generally, at different growth stages, salinity has been indicator to reduce the water imbibition by roots that results in reducing osmotic potentials in the root zone. This alteration may lead to alterations in metabolic activities and reduction in crop growth **Parida and Das (2005)**.

Table 9. Mean values of fresh leaf/stem percent and dry leaf/stem percent of Egyptian clover genotypes as affected by saline irrigation levels over two seasons

Treatment	Fresh leaf/stem percent					Dry leaf/stem percent				
Saline solution (s)	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**	**Cut1**	**Cut2**	**Cut3**	**Cut4**	**mean**
S1 Control	58.89	57.21	53.83	49.21	66.04	57.71	56.00	53.41	50.41	54.38
S2 4.5 dSm^{-1}	63.57	61.93	57.93	55.17	59.65	55.62	53.78	50.73	47.73	51.97
S3 9.0 dSm^{-1}	62.05	6067	55.80	52.10	57.66	52.60	50.32	48.02	45.02	48.99
F test	**	**	**	**		**	**	**	**	
L.S.D	0.38	0.84	2.26	0.50		0.70	0.47	0.53	0.21	
Genotype (G)										
Sakha 4(G1)	63.82	61.66	57.87	54.17	59.38	56.71	54.61	51.07	48.33	52.68
Sakha 2000(G2)	57.58	56.25	52.04	49.57	53.86	53.14	51.31	48.96	46.42	49.96
Sakha 2014(G3)	64.82	62.81	60.42	55.16	60.80	56.43	54.80	52.42	49.55	53.30
Gemmiza 1(G4)	58.82	57.82	52.59	49.23	54.62	53.43	51.53	49.16	45.96	50.02
Serw1(G5)	62.48	61.14	56.37	52.67	58.17	56.83	54.59	52.00	48.33	52.94
F test	**	**	**	**		**	**	**	**	
L.S.D	0.69	1.27	2.25	0.33		0.66	0.56	0.52	0.58	

**, highly significant at 1% level probability

Data in Table 10 showed that the reduction percent in total fresh and dry forage yields due to water salinity levels was the highest concentration at 9.0 dS m^{-1} salinity levels gave values (19.1 and 15.4% for fresh and dry forage yields, respectively). But, the reduction percent due to water salinity levels had the lowest concentration at 4.5 dS m^{-1} recorded (11.5 and 9.0 % for fresh and dry forage yields, respectively).

Also, the reduction in plant height and No. of stem due to salinity water levels (means of four cuts) as compared with control were 16.8 and 4.0 % over two years for salinity levels of S1 control and S3 9.0 dS m^{-1}, respectively. While, the reduction due to salinity water levels (means of four cuts) as compared with control were 9.7 and 1.9 % over two years for salinity levels of S1 control and S2 4.5 dS m^{-1} in plant height and No. of stem, respectively.

In addition, the data in Table 10 indicated the reduction in fresh and dry/ leaf stem percent due to salinity water levels (average of four cuts) as compared by control were 12.7 and 9.9 % for salinity levels of S1 (control) and S3 (9.0 dS m^{-1}) for fresh and dry leaf/stem percent over two years, respectively. But, the reduction due to salinity water levels (average of four cuts) as compared by control were 9.7 and 4.4 % for salinity levels of S1 (control) and S2 (4.5 dS m^{-1}) for fresh and dry leaf/stem percent over two years, respectively.

Table 10. Reduction percentage compared with control treatment for agronomic traits over the two seasons

Treatment	Reduction percent in 4.5 dS m^{-1}	Reduction percent in 9.0 dS m^{-1}
Fresh forage yield	11.5%	19.1%
Dry forage yield	9.0%	15.4%
Plant height	9.7%	16.8%
No. of stem	1.9%	4.0%
fresh leaf/stem percent	9.7%	12.7%
Dry leaf/stem percent	4.4%	9.9%

Correlation

Correlation among plant characters over two seasons are illustrated in Table 11. There was significant positive correlation between fresh yield and No. of stem and dry leaf/stem percent. There was significant positive correlation between both fresh yield and dry forage yield with plant height and fresh leaf / stem percent. These results are harmony with those obtained by **Bakheit *et al.* (2007) and Abo El-Goud *et al.* (2015)** who revealed that high positive correlation between fresh, dry forage yields and plant characters in Egyptian clover. Also, **Abdel-Galil *et al.* (2007) and Badawy *et al.* (2018)** showed that plant height and No. of stems are showed that plant height and No. of stems are estimated of fresh forage yield for these two characters indicated the most distinguished direct effect on such trait.

Table 11. Correlation between fresh yield and other plant characters over two seasons

Treatment	Fresh yield Kg/plot	Dry yield Kg/plot	Plant height (cm)	No. of stem ($0.25m^2$)	Fresh leaf/stem percent	Dry leaf/stem percent
Fresh yield Kg/plot	1					
Dry yield Kg/plot	0.999**	1				
Plant height (cm)	0.984**	0.985**	1			
No. of stem ($0.25m^2$)	0.931*	0.926*	0.869N.S	1		
Fresh leaf/stem %	0.970**	0.960**	0.938*	0.953*	1	
Dry leaf/stem %	0.948*	0.938*	0.944*	0.844N.S	0.965**	1

*,** and NS, significant , highly significant and not significant, at 5% levels probability respectively

Conclusion

In this study, two saline irrigation water in addition to tap water as control treatment were used to evaluate five clover genotypes to determine the most tolerant one to salinity and obtain the maximum yield from the area unit. Irrigating clover plants with 4.5 dS m^{-1} and 9 dS m^{-1} caused in significant decrease in all seed viability, agronomic and physiological characteristics over the two seasons, except for proline and malondialdehyde (MDA). The total fresh and dry forage yields were reduced under saline treatments by 11.5 and 19.1% in total fresh forage yield and 9.0%and15.6% in total dry forage yield for 4.5 and 9 dS m^{-1} respectively. G3 Sakha 2014 was superior to other studied genotypes, followed by Serw1 and Sakha 4, so it could be concluded that using these genotypes is suitable under saline conditions to obtain high productivity.

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RESEARCH ARTICLE

Agro-physiological and seed viability response of five Egyptian clover genotypes to different levels of saline irrigation water

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