Research Journal of

Seed Science

ISSN 1819-3552

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Seed Science

ISSN 1819-3552
DOI: 10.3923/rjss.2017.17.26

Research Article
Effect of Salinity on Germination and Seeding Parameters of Forage Cowpea Seed

[1]A.A. Kandil, [1]A.E. Shareif and [2]M.A. Gad

[1]Department of Agronomy, Faculty of Agriculture, Mansoura University, Egypt
[2]Ministry of Justice, 35516 El-Mansoura, Dahkhlia District, Egypt

Abstract
Objective: To investigate the effects of osmotic stress on performance forage cowpea varieties at critical stage of germination to salinity stress. **Methodology:** A study was conducted at the Seed Technology Laboratory of Mansoura University, Egypt during April and May 2014. Two forage cowpea cultivars (Buff and Baladi) were evaluated in the salinity concentrations 0, 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl. **Results:** The results exposed that germination parameters namely final germination percentage, germination index and seedling vigor index were estimated. Seedling characters namely shoot and root length, shoot and root foliage weight, shoot and root dry weight and chlorophyll content were measured. Forage cowpea cultivars significantly diverse in means of final germination percentage, germination index, seedling vigor index, shoot and root lengths, shoot and root fresh weights, shoot and root dry weights and chlorophyll content. Increasing salinity levels from 0-16 dS m^{-1} significantly diminished germination percentage, germination index, seedling vigor index, shoot and root lengths, shoot and root fresh weights, shoot and root dry weights and chlorophyll content and the control treatment recorded the highest averages of these characters. Thus, the lowest averages of these characters were recorded with highest salinity levels i.e., 16 dS m^{-1}. **Conclusion:** Results clearly displayed that germination index, seedling vigor index, shoot and root lengths, shoot and root fresh weights, shoot and root dry weights and chlorophyll content significantly affected by the interaction between forage cowpea cultivars and salinity concentrations. It could be determined that Buff cultivar was more tolerant to salinity stress and produced highest germination and seedling parameters which must be put in breeding program of forage cowpea under salinity conditions.

Key words: Forage cowpea cultivars, germination parameters, seedling characters, salinity levels, seedling chlorophyll content

Received: September 05, 2016 Accepted: October 19, 2016 Published: December 15, 2016

Citation: A.A. Kandil, A.E. Shareif and M.A. Gad, 2017. Effect of salinity on germination and seeding parameters of forage cowpea seed. Res. J. Seed Sci., 10: 17-26.

Corresponding Author: A.E. Shareif, Department of Agronomy, Faculty of Agriculture, Mansoura University, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cowpea *Vigna sinensis* L., is one of the most important legume crops in Egypt as well as other tropic and sub-tropic countries. It is grown in Egypt as vegetable and plus corps is favorable to the Egyptian consumers especially in form of dry seeds. Its dry-seeds have high percentage of protein (20-30%) that characterized as a complete protein compared with those of other vegetables. Also, they are rich in the essential amino acid lysine[1]. Moreover, forage cowpea residues serve as a filling material for animal feed it intercropped with maize in summer. Under Egyptian conditions there was shortage in forage dry matter production, legumes forage crops are produce large amount of biomass in summer season. The cultivated land and water in Egypt is limited source. So, it can be easily grown forage cowpea in the newly reclaimed sandy soil and irrigate it by saline water instead of delta soil. There are many factors effecting productivity of cowpea such as varieties therefore, this study was carried out to investigate the varietal productivity of cowpea for seeds which irrigate by saline water if sown in newly reclaimed soils. Many varieties are grown in tropical and sub-tropical agricultural areas of the world, where soil salinity is a yield-limiting factor. Dantas *et al.*[2] concluded that seeds of cultivar pitiúba where is more tolerant to salinity, than the cultivars Canapu and Epace-10. It is also cultivated as a dryland crop under different climatic conditions ranging from semi-arid too sub-humid[3]. Similar conclusion was reported by Wilson *et al.*[4]. Kaya *et al.*[5] found that mean frequency of germination, germination time, germination index, root length, shoot length and seedling fresh weight showed responses of cultivars to salt stress. Padilla *et al.*[6] found that the largest reductions were found in the genotype Cubanita 666, while in IT 86 D 715 showed few affectations in the evaluation of inhibition index for salt stress. El-Hefny[7] revealed that Kafr El-Shekh cultivar surpassed than cream 7 cultivars in plant fresh weight. In addition, Patel *et al.*[8] showed that the germination percentage of seeds was highly affected by salinity in all cultivars in all salinity levels from 2-10 dS m^{-1}. On the other hand, height and weight of all cultivars were less affected by the salt stress. Only at 10 dS m^{-1} EC, significant reduction in plant height and root length could be found for all three cultivars. Moreover, El-Shaieny[9] concluded that the differences among genotypes were significant with all traits. Black eye Crowder has more tolerance to water salinity than other studied genotypes.

Cowpea can cultivate in newly reclaimed soils as summer crop tolerant to drought condition to reduce the globe of protein shortage because it seed had high nutritive value. Seed germination of cowpea is an important parameter which is affected by various factors such as cultivars, temperature levels and water salinity stresses. Optimization of these factors can lead to enhance seed germination and crop productivity. Salinity stress is a major environmental factor that drastically affects the crop productivity throughout the world, it is a menace to both agriculture and the soil body. Moreover, salinity is known to exercise depressive effects on germination percentage, length of shoot and root, fresh and dry weight of shoot and root. Salinity, as a biotic hazard, induces numerous disorders in seeds and propagules during germination, it either completely inhibits germination at higher levels or induces a state of dormancy at lower levels[10]. Dantas *et al.*[2] reported that presence of salt at concentrations higher than 50 mol m^{-3} NaCl affect the germination, seedlings growth and cotyledons total protein synthesis of all cowpea cultivars. Zhang *et al.*[11] reported that salinity damage is mainly due to altered water relations caused by high salt accumulation in the intercellular spaces. Van den Berg and Zeng[12] stated that drought, like salinity, plays an important role not only in determining germination rates, but also influences seedling development. Taffouo *et al.*[13] showed that K^+ concentration K/Na ratio, seedling height and total chlorophyll were significantly decreased by salt solutions. Abusuwar and Abbaker[14] found that plant seed germination significantly ($p \leq 0.01$) decreased with increasing sea water in the irrigation water. Fateme *et al.*[15] showed that salinity stress caused reduction of speed and percentage germination of seed, shoot length, fresh and dry weights of shoots and roots of plants. Moreover, Hadi *et al.*[16] showed that increasing salinity levels from 10-30 mM. decreased the growth parameters of various varieties of cowpea. Similar conclusions were reported by working on rice[17], wheat[18], chickpea[19], sorghum[20], canola[21] and on sugar beet[22-24]. Moreover, it is indicated that salinity levels had a significant effect on all of studied traits[9]. Therefore, the present investigation was carried out to study performance of cowpea cultivars on seed germination and seedlings properties, the effect of salinity stress on seed germination and seedlings characters as well as the effect of the interaction between studied factors on seed germination seedlings properties of cowpea plants.

MATERIALS AND METHODS

This study was conducted at Agronomy Department Laboratory Faculty of Agriculture, Mansoura University, Egypt during April and May, 2014 the objectives of this investigation aimed to study the effect of two cowpeas *Vigna sinensis* L., cultivars to germination and seedling under salinity stress and their interaction. The experiments were arranged in factorial experiment in Randomized Complete Block Design (RCBD). The first factor included two cowpeas (Buff and Blade) cultivars which were obtained from Agriculture Research Center (ARC), Legume Research Institute Ministry of Agriculture, Egypt. The second factor included eight different salinity concentrations 0, 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl.

Seeds of cowpea cultivars were surface sterilized by immersion for 5 min in sodium hypochlorite solution, then repeatedly washed with deionized water. Fifty seeds of uniform size in each treatment for each cultivar were allowed to germinate on a filter paper in 9 cm diameter petri dishes. Each filter paper was moistened with a water solution at eight different NaCl concentrations. Thus, the whole experiment comprised 64 petri dishes. After pre-treatments of cowpea, seeds were germinated for 14 days, incubating in a growth chamber adjusted to 20 ± 1°C in a dark. For each treatment, 50 seeds were counted at random and placed on a moisture germination paper in a petri dishes. There were four replicates for each treatment. Dishes were inspected daily and sterilized water added as required. The number of normal seedlings, abnormal seedlings, dead seeds and hard seed in each replicate was recorded. Every 24 h, the number of germinated seeds was counted. Seeds were categorized as germinated (radical 2 mm), hard (no imbibition or swelling) or nonviable (abnormal, dead or infected seeds) as described by ISTA[25].

Hard or (dormant) seeds can be distinguished from dead seeds by pushing down on each no germinated seed with the flat part of a pencil eraser. If the seed does not flatten with gentle pressure, it is considered hard. All seeds which had taken up no water in 6 days were considered as hard seed. Seeds which at the end of test period are neither hard nor have produced any part of a seedling were considered dead. Normal seedlings show the potential for continued development into satisfactory plants when grown in good quality soil and under favorable conditions of moisture, temperature and light, normal and abnormal seedling can have recognized according to ISTA rules.

Studied characters

Germination parameters

Final germination percentage: It was calculated according to the germination count was taken after 14 days and expressed as percentage according to the following equation described by Ellis and Roberts[26] and Ruan *et al.*[27] equation:

$$FGP = \frac{\text{No. of germinated seeds after 14 days}}{\text{Total No. of germinated seeds}} \times 100$$

Germination Index (GI): It was calculated according to the following equation of Karim *et al.*[28]:

$$GI = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in the control}} \times 100$$

Seedling Vigor Index (SVI): Seedling vigor index was calculated according the formula as suggested by Abdul-Baki and Anderson[29] and expressed in whole number:

$$(SVI) = (\text{Average shoot length (cm)} + \text{Average root length (cm)} \times \text{Germination percentage (\%)}$$

Seedling parameters

Shoot length: The length of the 10 seedlings from the seed to the tip of the leaf blade were recorded and expressed in centimeters as the shoot length.

Root length: The root length of 10 seedlings from the seed to the tip of the root and recorded and expressed in centimeters (cm) as the root length.

Shoot fresh weight: The weight of 10 seedling shoots was measure and expressed in gram as the shoot fresh weight.

Root fresh weight: The weight of 10 seedling roots was measured and expressed in gram (mg) as the root fresh weight.

Root dry weight: The weight of 10 seedling roots was recorded and expressed in gram after oven drying at 70°C for 72 h.

Shoot dry weight: The weight of 10 seedling roots was recorded and expressed in gram after oven drying at 70°C for 72 h.

Total chlorophyll (SPAD): Total chlorophyll (SPAD): Chlorophyll content in leaves samples was assessed by SPAD-502 (Minolta Co. Ltd., Osaka, Japan).

All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for factorial experiment in Randomized Complete Block Design (RCBD) as published by Gomez and Gomez[30] by using means of "MSTAT-C" computer software package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5 and 1% level of probability as described by Snedecor and Cochran[31].

RESULTS AND DISCUSSION

Cultivars performance: Mean of final germination percentages, germination index, seedling vigor index, root and shoot length, shoot and root fresh weight and shoot and root dry weight and chlorophyll content as affected by studied cultivars under different salinity concentrations are showed in Table 1 and 2. The results clearly indicated that studied cultivars significantly differed in final germination percentages, germination index, seedling vigor index, root and shoot length, shoot and root fresh weight and shoot and root dry weight and chlorophyll content. Highest final germination percentage, seedling vigor index, root and shoot length, shoot and root fresh weight and shoot and root dry weight and chlorophyll content were produced from germinated Buff cultivar, which was 75.35, 81.85, 1031.8, 5.42, 7.30, 2.29, 1.03, 0.503, 0.141 and 6.77, respectively while, the lowest final germination percentage was recorded from germinated Baladi cultivar, which was 59.65, 77.01, 624.7, 3.93, 5.68, 1.85, 0.51, 0.328, 0.103 and 5.51, respectively. The results clearly revealed that Buff cultivar exceeded Baladi cultivar in final germination percentage, germination index, seedling vigor index, root and shoot length, shoot and root fresh weight and shoot and root dry weight and chlorophyll content by 26.32, 6.28, 65.15, 37.91, 28.52, 26.78, 101.0, 53.35, 36.89 and 22.90%, respectively. The differences between forage cowpea cultivars in final germination percentage might be due to genetically factors and heredity variation among the two forage cowpea cultivars under study. Rao *et al*.[32] found that some cultivars may have a degree of salt adaptation due

Table 1: Means of final germination percentage, germination rate, seedling vigor index, root and shoot length as affected by studied cultivars and salinity levels

Treatments	Final germination (%)	Germination rate	Seedling vigor index	Root length (cm)	Shoot length (cm)
Cultivars					
Buff	75.43	81.85	1031.8	5.42	7.30
Blade	59.63	77.01	624.7	3.93	5.68
F-test	**	**	**	**	**
Salinity levels (dS m^{-1} NaCl)					
0	84.87	100.00	1668.5	8.31	10.97
2	81.87	96.63	1469.9	7.63	10.03
4	77.43	91.35	1218.0	6.17	9.36
6	72.68	85.64	874.0	4.94	6.97
8	67.43	79.12	661.7	4.03	5.80
10	64.81	76.08	608.7	4.01	5.38
12	61.81	72.73	512.8	3.71	4.51
14	53.12	62.46	283.8	2.12	3.14
16	43.43	50.89	156.9	1.21	2.36
F-test	**	**	**	**	**
LSD at 5%	6.75	4.76	121.0	0.46	0.80
Interaction					
F-test A×B	NS	**	**	**	**

Table 2: Means of shoot and root fresh weight and shoot and root dry weight and chlorophyll content as affected by studied cultivars and salinity levels

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (mg)	Root dry weight (mg)	Chlorophyll content
Cultivars					
Buff	2.29	1.03	0.503	0.141	6.77
Blade	1.85	0.51	0.328	0.103	5.51
F-test	**	**	**	**	**
Salinity levels (dS m^{-1} NaCl)					
0	3.33	1.26	0.771	0.265	10.89
2	3.01	1.14	0.673	0.215	9.60
4	2.77	1.03	0.584	0.165	8.43
6	2.28	0.94	0.517	0.143	6.79
8	2.24	0.82	0.411	0.104	6.11
10	1.82	0.66	0.335	0.083	5.26
12	1.49	0.61	0.256	0.071	4.34
14	1.02	0.25	0.116	0.031	2.36
16	0.66	0.19	0.073	0.020	1.50
F-test	**	**	**	**	**
LSD at 5%	0.21	0.08	0.041	0.012	6.14
Interaction					
F-test A×B	**	**	**	**	**

to water retention capacity, membrane permeability and osmo-protection and or genetical and morphological factors. These results in good agreement with those reported by Kaya *et al.*[5], Padilla *et al.*[6], El-Hefny[7], Patel *et al.*[8], El-Shaieny[9], Ashebir *et al.*[23], Thiam *et al.*[33] and Wada and Abubakar[34].

Salinity concentrations: The results in Table 1 and 2 indicated a significant differences due to salinity concentrations on final germination percentage. Increasing salinity concentrations significantly reduced final germination percentage. Highest final germination percentage produced from the control treatment, which was 84.88%. Increasing salinity levels up to 16 dS m^{-1} NaCl significantly recorded the lowest final germination percentage, which was 43.44%. It could be noticed that increasing salinity concentrations from 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl reduced final germination percentages by 3.53, 8.77, 13.84, 20.55, 23.65, 27.18, 37.42 and 48.82%, respectively compared with the control treatment. Germination index as affected by salinity levels are presented in Table 2. The results clearly point out a significant difference due to salinity levels on germination index. Highest germination index was recorded the control treatment, which was 100.00%. Increasing salinity levels up to 16 dS m^{-1} NaCl significantly recorded the lowest germination index, which was 50.89%. It could be stated that increasing salinity levels from 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl reduced germination index by 9.63, 15.83, 22.03, 27.67, 33.24, 39.64 and 45.63%, respectively compared with the control treatment.

Salinity decreased germination index of seeds which is directly related to the amount of absorbed water by the seeds. A significant difference due to salinity concentrations on seedling vigor index. Highest seedling vigor index was produced from the control treatment, which was 1668.5 Increasing salinity concentrations up to 16 dS m^{-1} NaCl significantly recorded the lowest seedling vigor index, which was 156.9. It could be concluded that increasing salinity levels from 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl reduced seedling vigor indexes compared with the control treatment. The undesirable effect of high concentration of salinity on seedling vigor index might be due to the toxic effect of salinity, which decreases seedling vigor index. Regarding to root and shoot length, root fresh weight and shoot and root dry weight and chlorophyll content a significant difference due to salinity concentrations on root and shoot length, root fresh weight and shoot and root dry weight and chlorophyll content. Tallest roots and shoots, highest root fresh weight and as well as shoot and root dry weight and chlorophyll content were produced from the control treatment, which were 8.31, 10.97, 1.26, 3.33, 0.771, 0.265 and 10.89, respectively. Increasing salinity concentrations up to 16 dS m^{-1} NaCl significantly recorded the shortest roots and shoots, highest root fresh weight and as well as shoot and root dry weight and chlorophyll content, which were 1.21, 2.36, 0.19, 0.66, 0.073, 0.021 and 1.50, respectively. It could be concluded that increasing salinity levels from 2, 4, 6, 8, 10, 12, 14 and 16 dS m^{-1} NaCl reduced root and shoot length compared with the control treatment.

The undesirable effect of high concentration of salinity root and shoot length might be due to the toxic effect of salinity, which decreases root and shoot length. Munns[35] concluded that soluble salts lead to osmotic stress, which results in specific ion toxicity and ionic imbalance and the consequences of these can be plantdemise. Similar conclusion was reported by El-Shaieny[9], Taffouo *et al.*[13], Fatme *et al.*[15], Hadi *et al.*[16], Kandil *et al.*[17-21], Wada and Abubakar[34] and Lobato *et al.*[36].

Interaction effects: The interaction between studied cultivars and salinity levels significantly affected germination index as graphically illustrated in Fig. 1. Results revealed that highest

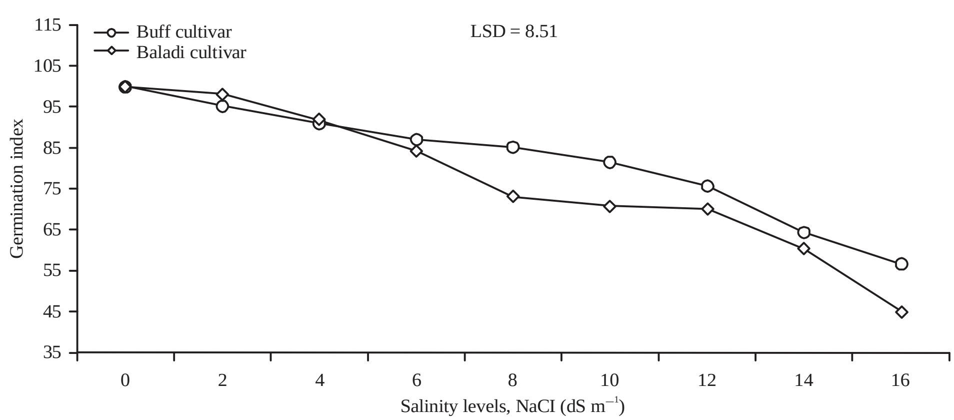

Fig. 1: Means of germination index as affected by studied cultivars and salinity levels

germination index was produced from the control treatment (100%) in both studied cultivars. It could be noticed that under of all salinity levels Buff cultivar exceeded Baladi cultivars in germination index. The lowest germination index percentages were produce from sown Baladi cultivar under highest salinity level of 16 dS m^{-1} NaCl (45.09%). Similar conclusion was reported by Taffouo *et al.*[13], Ashebir *et al.*[23], Singh *et al.*[24], Thiam *et al.*[33] and Wada and Abubakar[34].

The resulted demonstrated in Fig. 2 clearly indicated that the interaction between studied cultivars and salinity levels significantly affected seedling vigor index. It could be stated that highest values of seedling vigor index (2138.8) was obtained from sown Buff cultivar under the control treatment. Whilst the lowest values of seedling vigor index (91.3) was obtained from sown Baladi cultivar under highest salinity level of 16 dS m^{-1} NaCl. Similar conclusion was reported by El-Shaieny[9], Ashebir *et al.*[23], Rao *et al.*[32], Thiam *et al.*[33] and Lobato *et al.*[36].

With respect to the effect of interaction between studied cultivars and salinity levels, the results illustrated in Fig. 3 clearly showed a significant difference on root length. Tallest roots (10.91 and 9.96 cm) were produced from sown Buff cultivar under without salinity or 2 dS m^{-1} NaCl, respectively without significant differences between. However, the shortest roots (1.56 and 0.85 cm) were obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level, respectively without significant differences between. Similar conclusion was reported by El-Shaieny[9], Ashebir *et al.*[23], Rao *et al.*[32], Thiam *et al.*[33] and Lobato *et al.*[36].

Regarding to the effect of the interaction between studied cultivars and salinity levels significantly affected on shoot length, the results graphically illustrated in Fig. 4 clearly revealed that tallest roots (12.3 and 12.0 cm) were produced from sown Buff cultivar under without salinity or 2 dS m^{-1} NaCl, respectively without significant differences between. Whilst, the shortest roots (2.68 and 1.80 cm) were obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level, respectively without significant differences between.

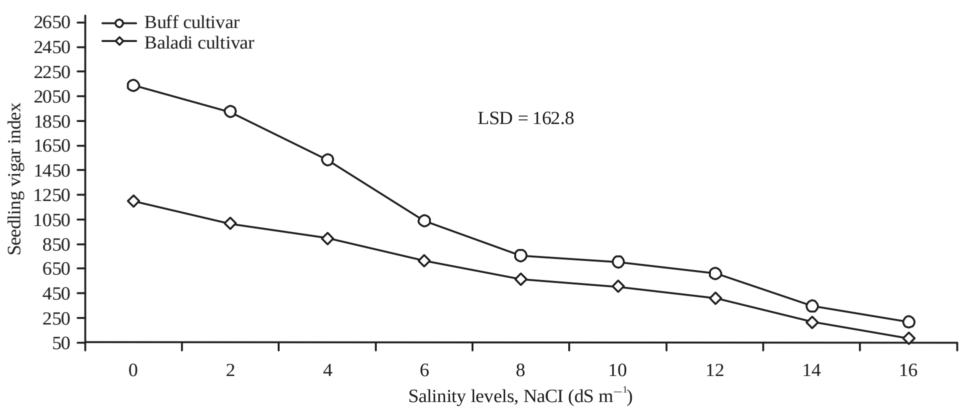

Fig. 2: Means of seedling vigor index as affected by studied cultivars and salinity levels

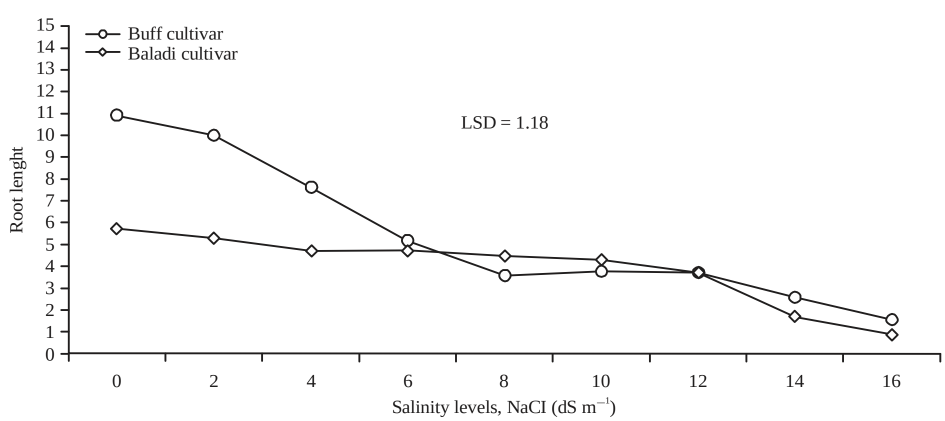

Fig. 3: Means of root length (cm) as affected by studied cultivars and salinity levels

Similar conclusion was reported by El-Shaieny[9], Ashebir *et al*.[23], Rao *et al*.[32], Thiam *et al*.[33] and Lobato *et al*.[36].

The interaction between studied cultivars and salinity levels significantly affected shoot fresh weight as graphically illustrated in Fig. 5. Results revealed that maximum shoot fresh weight (3.73 and 3.42 g) was produced from sown Buff cultivar under the control treatment or 2 dS m^{-1} NaCl, respectively. Whilst, the lowest shoot fresh weight (0.66 g) was obtained from sown Baladi cultivar under highest salinity level of 16 dS m^{-1} NaCl. Similar conclusion was reported by El-Shaieny[9], Rao *et al*.[32] and Lobato *et al*.[36].

With respect to the effect of interaction between studied cultivars and salinity levels, the results illustrated in Fig. 6

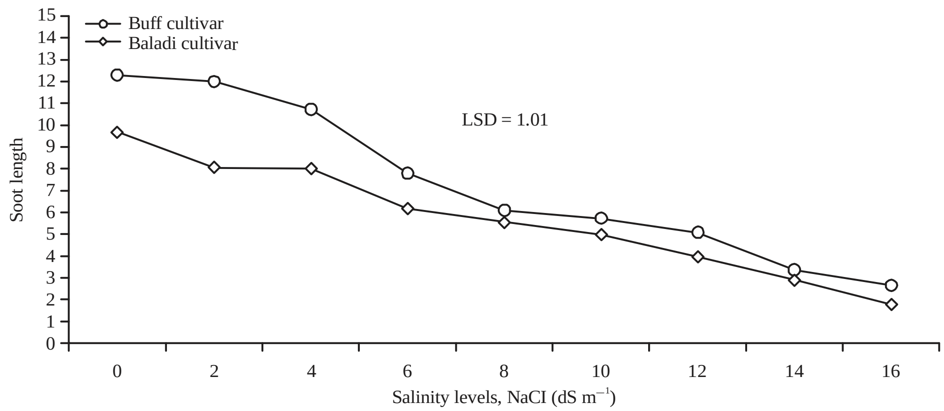

Fig. 4: Means of shoot length (cm) as affected by studied cultivars and salinity levels

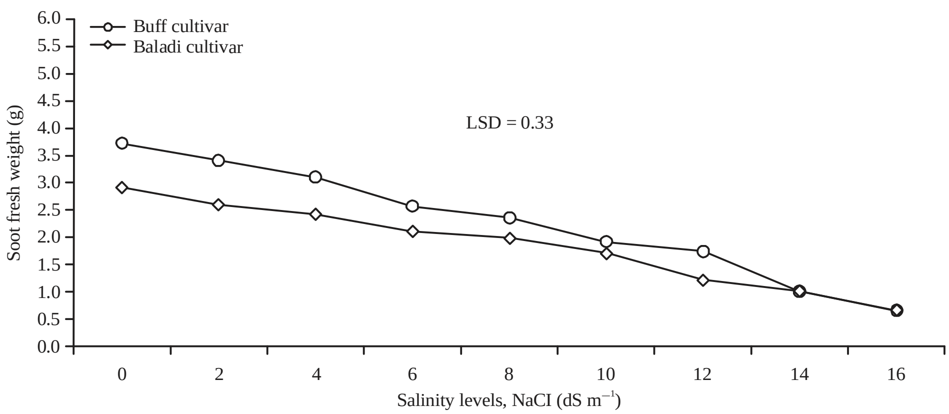

Fig. 5: Means of shoot fresh weight (g) as affected by studied cultivars and salinity levels

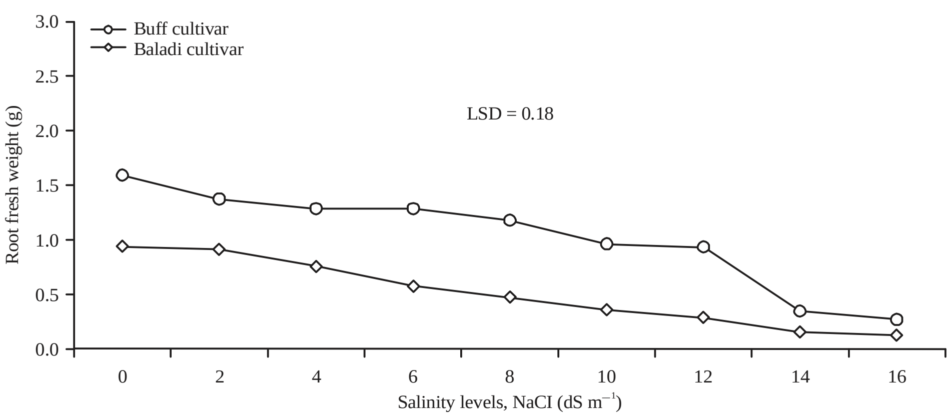

Fig. 6: Means of root fresh weight (g) as affected by studied cultivars and salinity levels

clearly showed a significant difference on fresh root weight. Highest fresh root weight (1.59 g) was produced from sown Buff cultivar without salinity levels. However, the lowest fresh root weight. (0.27 and 0.13 g) were obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level of salinity, respectively without significant differences. Similar conclusion was reported by El-Shaieny[9], Rao *et al.*[32] and Lobato *et al.*[36].

The interaction between studied cultivars and salinity levels significantly affected shoot dry weight as graphically illustrated in Fig. 7. Results revealed that maximum shoot dry weight (0.908 mg) was produced from sown Buff cultivar without salinity levels. Whilst, the lowest shoot dry weight (0.090 and 0.055 mg) was obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level of salinity, respectively without significant differences. Similar conclusion was reported by El-Shaieny[9], Ashebir *et al.*[23], Rao *et al.*[32], Thiam *et al.*[33] and Lobato *et al.*[36].

Regarding to the effect of the interaction between studied cultivars and salinity levels significantly affected on root dry weight, the results graphically illustrated in Fig. 8 clearly revealed that maximum root dry weight (0.343 mg) was produced from sown Buff cultivar under without salinity levels. Whilst, the lowest root dry weight (0.028 and 0.013 mg) were obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level, respectively without significant differences between. Similar conclusion was reported by El-Shaieny[9], Ashebir *et al.*[23], Rao *et al.*[32], Thiam *et al.*[33] and Lobato *et al.*[36].

The interaction between studied cultivars and salinity levels significantly affected chlorophyll content as graphically illustrated in Fig. 9, results revealed that highest values of chlorophyll content (12.9) was produced from sown Buff cultivar under the control treatment. Whilst, the lowest values of chlorophyll content (1.72 and 1.27) were obtained from sown Buff cultivar under highest salinity level of 16 dS m^{-1} NaCl or sown Baladi cultivar under the same level of salinity, respectively without significant differences.

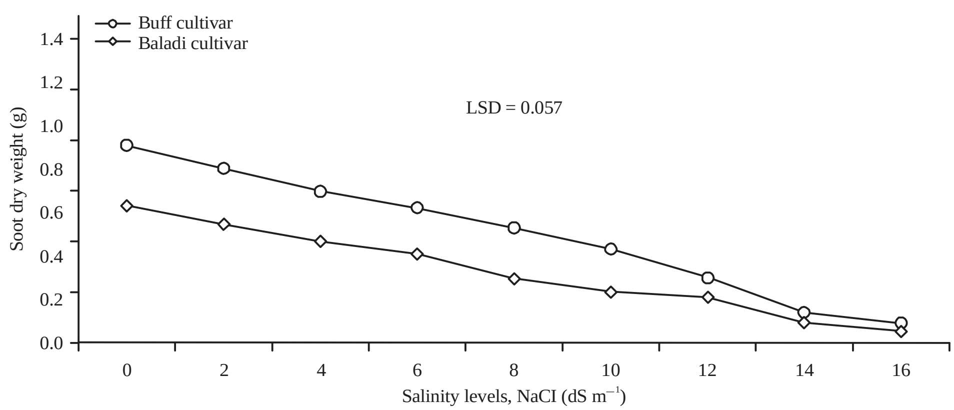

Fig. 7: Means of shoot dry weight (g) as affected by studied cultivars and salinity levels

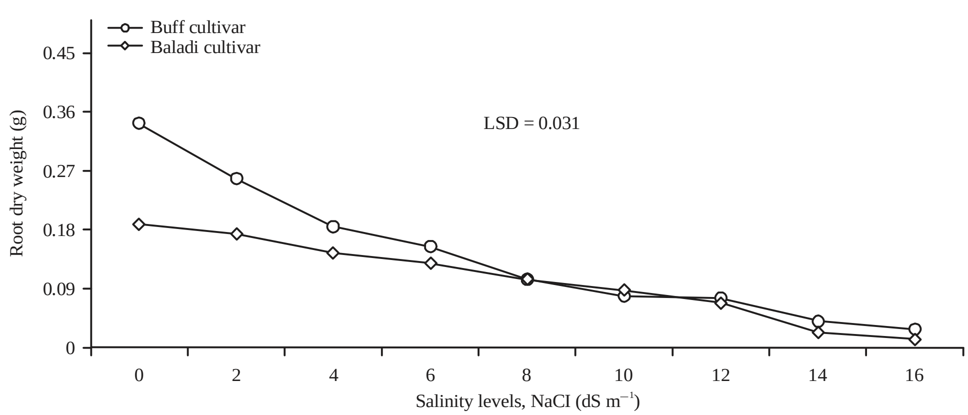

Fig. 8: Means of root dry weight (g) as affected by studied cultivars and salinity levels

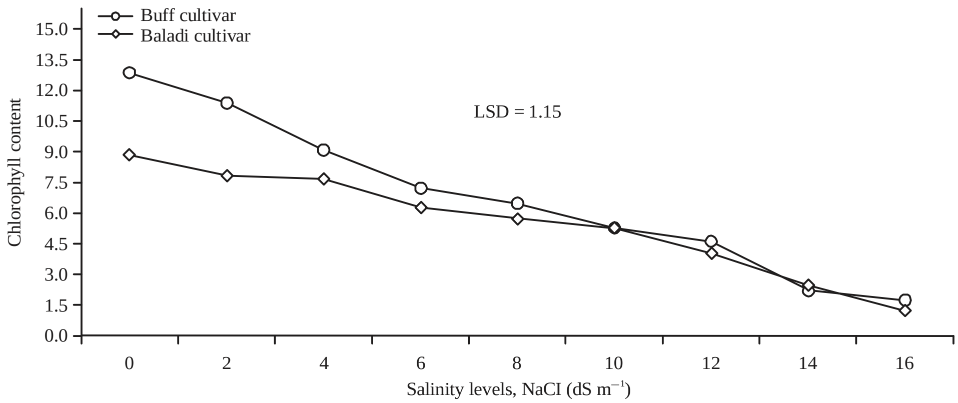

Fig. 9: Means of chlorophyll content as affected by studied cultivars and salinity levels

CONCLUSION

Accordingly, it could be recommended to cultivate Buff cultivar, it was more tolerant to salinity stress and produced highest germination and seedling parameters which must be put in breeding program of forage cowpea under salinity conditions.

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