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Seed Germination Percentage and Early Seedling Establishment of Five (*Vigna unguiculata* L. (Walp) Genotypes Under Salt Stress.

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ABSTRACT

Abiotic stress such as salinity of soil or water is a general problem and of special concern in countries with low rainfall and hot temperatures such as Egypt. The main objective of these experiments is to study some physiological and morphological characters of five cowpea genotypes viz. Sudany, Chinese red, Kaha 1, TVU 21 and Black eye Crowder. As influenced by salinity levels viz. Normal water 153 as control, 2500, 3500 and 4800 ppm. Germination percentage over control decreased insignificantly with increasing salinity levels. On contrary, salinity significantly reduced shoot length, root length, seedling fresh weight and seedling dry weight, relative water content, ion leakage and chlorophyll content. There were genotypic differences among the test genotypes in response to salt stress exposure. The results indicated that salinity levels had a significant effect on all of studied traits. On the other hand, the differences among genotypes were significant with all traits except, germination percentage. While, the interaction between genotypes and water salinity levels were significant with root length, seedling fresh weight, seedling dry weight, relative water content, ion leakage and chlorophyll content. While, germination percentage and shoot length, exhibited insignificant effect. All studied traits for cowpea seedlings decreased with increasing water salinity. In general, Black eye Crowder has more tolerance to water salinity than other studied genotypes.

Keywords: Ion leakage, Chlorophyll, seedling growth, salt stress.

INTRODUCTION

Cowpea is one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, southern Europe and Central and South America., [1]. In Egypt, cowpea is a popular vegetable crop. The total cultivated area of the crop was estimated at 9155 feddans for dry seed in 2008 with a mean production of 980kg/fed. Also, the area that produced green pods was 10064 feddans with a mean of 5.19 ton/fed. (Dep., Agric., Statistics. Ministry of Agriculture, Giza, Egypt). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes [2], [3], and [4]. Furthermore, it decreases plant growth and yield, depending on the plant species, salinity levels, and ionic composition of the salts [5] and [4]. Saline stress is one of the main factors limiting legume productivity in arid and semi-arid regions [6]. Salinity adversely affects the plant growth and development, hindering seed germination [7]. Seed germination is usually the most critical stage in seedling establishment, determining successful crop production [8] and [9]. The germination of seed is a complex process depending on the genetic and environmental factors, such as temperature, light, and salinity [10] and [11].

Salinity is one of the most important factors limiting plant growth and delaying seed germination as well as final germination percentage [12]. The effect of salinity on plant growth is related to the stage of plant development at which salinity is imposed [13]. Salinity stress affects seed germination either through osmotic effects, by preventing or delaying germination [14], or through ion toxicity, which can render the seeds unviable [15]. Salt stress leads to suppression of plant growth and development at all growth stages, however, depending upon plant species, certain stages such as germination, seedling or lowering stage could be the most critical stages for salts stress [16]. Salinity has reached a level of 19.5% of all irrigated land and 2.1% of dry-land agriculture worldwide [17]. Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options [18]. Some plants can germinate under high concentrations of NaCl. However, other species are more sensitive during germination. Consequently, the study of salt tolerance during germination, early and late growth of plants is important for determining saline limits at each developmental phase [19]. [20] Observed that the lengths of the main root and shoot of cowpea decreased to 23 and 44 % respectively when treated with 100 mM NaCl. Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (25 mM annual rainfall) and irrigation with saline water [21], [22]. Different plants have different abilities saline conditions. Difference between salt tolerance not only form genus and species, also even within a species can be observed. Reduced germination rate and plant growth under salinity conditions depends on combine salt, salt concentration and stage of plant growth. [23]. Crop establishment depend on an interaction between seedbed environment varieties at early seedling stages for successful crop production in a saline environment [24]. A decrease in plant growth in salinity soils is caused by the osmotic and water potential of soil, specific toxicity, and nutritional deficit. After these primaries effects, secondary stresses happen as the oxidative damage [25]. Plants exposure to salt stress results in degradation of photosynthetic pigments, destruction of chloroplast, diminution of chlorophyll fluorescence and reduction in net photosynthetic rate [6], [27]. [28]. have found that salinity induces the structural changes in bean plant roots by increasing the cell membranes permeability and thus ions leakage.

MATERIALS AND METHODS

Experimental site:

In this study, the effects of salt stress on germination rate, seedlings development and pigment contents were investigated in different genotypes of Cowpea (*Vigna unguiculata* (L.)Walp.). The seedlings of *Vigna unguiculata* viz. Sudany, Chinese red, Kaha 1, TVU 21 and Black eye Crowder were treated with four saline water (153 as a (control), 2500, 3500 and 4800 ppm, and tap water as a control). This work was carried out at the Agricultural Experimental Farm of the Faculty of Agriculture, South Valley University, Qena, Egypt.

Plant materials and salt treatments:
Germination:

Twenty seeds were germinated in 25-cm plastic pot filled with peat moss. Five replicates (Pots) per treatment were used. The seeds were irrigated with saline water of (control) 153, 2500, 3500 and 4800 ppm. The germinated seeds were counted after two weeks to calculate the germination percentage (GP) as the following:

$$(GP\ \%) = \frac{g}{20} \times 100$$

As *g* is the number of germinated seeds and 20 is the total number of seeds

Seedling growth:
Seedlings of cowpea (*Vigna unguiculata* (L) Walp.) were grown in plastic pots (25 cm diameter, 22 cm height) filled with peat moss. After 3 weeks, the seedlings were subjected to salt treatment. Salt concentrations in water were tap water (control) 153 ppm, 2500, 3500 and 4800 ppm. All measurements on the youngest and expanded leaves were made after 7 weeks, when plants had achieved a steady state.

Measurements:

Several parameters will be measured to understand the mechanism of stress tolerance trait. These parameters include.

1. Estimates germination percentage
2. Shoot length (cm), root length (cm)
3. Seedling fresh weight (g) and seedling dry weight (g)
4. Chlorophyll fluorescence as indicator of stress tolerance
5. Ion leakage
6. Relative water content.

The seedling length was measured using a ruler. Water status of the seedlings was determined by measuring relative water content (RWC) according to [29]. (%) $\frac{FW - DW}{TW - DW}$ X 100

Where FW is the fresh weight, DW is the dried weight and TW is the turgid weight of tissue after being soaked in water for 12 h at room temperature. Cell membrane stability was measured by ion leakage (IL) from seedlings using the method described by [30]. Chlorophyll content: A hand-help SPAD-502 (Minolta Camera Co., Osaka, Japan) was used to estimate chlorophyll content, as described by [31].

Statistical analysis:

Statistical analysis of the results was evaluated by analysis of variance using the Statistical Analysis System software v. 9.1 [32]. The experimental design comprised complete randomized blocks (CRD) with a split plot arrangement of treatment in five replicates in which cowpea genotypes were allocated to the main plots while irrigation water salinity levels were allocated to the sub plots. Treatments means were considered significantly different at $p<0.05$. Using Duncan's multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Water salinity is tremendous causes of lose of crops and other food plants. In view of importance of salinity, considerable efforts have been invested in this field. Using water with low quality may be the first choice. Scientists are working on means to compensate deficiencies. Moderately saline water could be used for growing salt tolerant plants for foods, fuel and fodder. Selection of proper crop species of economic importance that can tolerate prevailing salt stress, adaptation of proper cultivation practices and undertaking great precautions to avoid salt accumulation in soil profile due to saline water irrigation could provide solution.

The present investigation work was conducted to evaluate and compare the performance of five cowpea genotypes, Sudany, Chinese red, Kaha 1, TVU 21 and Blackeye Crowder under different water salinity levels, 153, 2500, 3500 and 4800 ppm.
Analysis of variance results showed that over salinity treatments, varietal differences were significant for most studied traits, in Table (1). Expect GP%. On the other hand, as for water salinity, these treatments exhibited significant or high significant response for all studied traits. In addition, the interaction between genotypes and salinity was insignificant with germination percentage (GP %) and shoot length (SL). On contrary, was significant with root length (RL), seedling fresh weight (SFW), seedling dry weight (SDW), relative water content (RWC), chlorophyll content (CC) and ion leakage (IL).

Means values for the traits showed that the application of 4800 ppm water salinity was significantly decreased GR, GP %, SL, RL, SFW, SDW, RWC, CC and IL, 18.960, 94.800%, 8.936, 3.808, 5.128, 5.33, 11.116, 69.936 and 11.35 respectively, as compared to control treatment, as shown in Table 2.

The results of this research reported that water salinity stress decreased germination percentage (GP) and seedling growth, SL, RL, SFW and SDW. Also, RWC and IL. Addition to plants pigment CC, of five cowpea genotypes Fig (1 to 8). Similar results were found by [33], [34], [23], [35], [36], [37].

Germination percentage (GP %):

Water salinity had a significant effect on GP %, increasing water salinity level decreased seed germination, general trend over a significant decrease in the water salinity level 4800 ppm. The highest seed germination was observed with control (153 ppm), while, the lowest percentage of seed germination with 4800 ppm, the percentage of seed germination was insignificantly decreased in all varieties, the biggest loess with genotype Kaha 1, while, Blackeye Crowder gave the best performance for that trait. Data represented in Table (2) and Fig. (1).

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Increasing salinity levels caused reduction in germination percentage of wheat and *Aeluropus lagopoides*. Theses results were found by [38], [39], [40] reported that the germination percentage decreased when the salt osmotic potential increased. . Furthermore, salt stress had a negative impact on germination percentage. These findings are in harmony with our results.

Shoot length (SL):
The maximum SL (10.644 cm) was from control treatment, salinity levels decreased root length significantly, the shortest root length was from 4800 ppm, (8.936 cm). Data presented in Table (2) and Fig. (2), indicated that shoot length (SL) of various genotypes ranged from 8.865 (cm) for Sudany genotype to 12.410 (cm) for genotype TVU 21. [41]. Founded a highly significant reduction of seeds germination of Cowpea due to salinity. The Cowpea (*Vigna unguiculata* L. Walp) showed significant a difference in it's their tolerance to salinity [42]. [35] found that the application of 8 ds m-1 salinity resulted in the loss of seedling length, seedling weight, germination percentage, germination rate and seed viability index of *Nigella sativa* L. seeds by 21, 33.3, 13.2, 32.7 and 31.3% respectively, as compared to control treatment (0 ds m-1 salinity).

Root length (RL):
As shown in Table (2) and Fig. (3), the highest (RL) was related to control treatment 5.884 (cm), and data show that increasing salinity levels from control to 4800 ppm decreased significantly (RL). The genotype Kaha 1 gave the lowest value (3.405 cm) of RL, and (5.765 cm) was observed for Blackeye Crowder genotype. [36] reported that the rate of germination, radicle, hypocotyl and plumule length, moisture content, fresh wet and dry seedling weights and seed viability index were higher in control treatment when compared to treated seeds. At -0.15MPa and more concentration, germination decreased significantly. Theses results are in line with findings by [43], [44] and [45], and theses in harmony with our results. [23], indicated that all of growth parameters usually decreased by increasing salinity levels in cowpea cultivars. Also, he showed that cowpea seed can bear on salt with density 8 ds/m. The reduction of speed of germination at high salt levels might be mainly due to osmotic stress [24].

Seedling fresh weight (SFW):
Means comparison for this trait show that the application of water salinity 4800 ppm resulted in the loss of (SFW) 6.908 (g), as compared to control treatments, data in Table (2) and Fig. (4) Show that genotype Blackeye Crowder was the heaviest (10.475 g). While, genotype Sudany gave the lowest value of (SFW), (5.171 g). [46] mention that excess salt in soil solution may adversely affect plant growth, either through osmotic inhibition of root water uptake or by specific ion effect. In many crop plants, seed germination and early seedling growth are the most sensitive stages to environmental stresses such as salinity.

Seedling dry weight (SDW):
Data in Table (2) and Fig. (5) Showed that the difference among water salinity application was decreased significantly from control treatment (7.127 g) to (5.128 g) for 4800 ppm application. The genotype Sudany gave the smallest value for SDW, (3.895). While, genotype Blackeye Crowder gave the highest value (8.024 g) for this trait. Inhibition of plant growth parameters caused by different NaCl concentrations is due to interference with plant growth processes like cell division and cell enlargement, inhibition in nutrient uptake, reduction in dry matter production due to inhibition of metabolic processes such as photosynthesis and respiration. Theses were reported by [47].

Relative water content (RWC):
Data regarding in Table (2) and Fig (6) indicates that variety difference had significant effect. TVU 21 (15.140) genotype had maximum RWC followed by Blackeye Crowder (14.785). RWC decreased significantly with increasing salt levels from 153 to 4800 ppm, the most decreased due to 4800 application (11.116). [48], indicated that salinity, which is a result of osmotic pressure leads to reduction in water absorbance so there is reduction in cell division and differentiation leading to reduction of Plumule and radicle length which will be explainable. Also the

high sensitivity of seedling length to salinity stress may be attributed to the reduced transition of nutrients from cotyledons to caulicle under saline condition. Moreover, saline condition disturbs water uptake by seed, resulting in the reduction of plant hormones and enzymes production which consequently inhibits seedling's growth. Theses results were found by [48], [49].

Ion leakage (IL):

Data in Table (2) and Fig (7) indicted that the cell membrane stability was decreased by increasing salt levels to 4800 ppm, and significant under salt stress, the genotype Sudany followed by Chinese red had maximum IL values. On contrary, TVU 21 had the minimum value for this trait.

Chlorophyll content (CC):

Data presented in Table (2) and Fig (8) indicated that variety differences had significantly decreased in (CC). The highest (CC) (77.440) was related to control treatment, and the lowest was observed (69.936) for 4800 ppm application. On the other side, there was a significant effect among salt levels for (CC). [50] Pointed out that chlorophyll content and ion leakage were negatively affected by Na Cl stress. Membrane permeability increased in plants exposed to salinity as compared to non-stressed plants, which confirms by [51]

Correlation analysis:

Correlation coefficient between all possible combinations was estimated and is shown in Table (3). Data showed that there was negative correlation among salt levels and GP %, SL, RL, SFW, SDW and CC. -0.289, -0.335, -0.380, -0.238 and -0.345, respectively. On contrary, was positive with RWC and IL 0.052 and 0.424, respectively.

Generally, the decreases in the germination and seedling growth, due to the increase in the environment's salt concentration, are caused by physicochemical effects or by osmotic-toxic salts which exist in saline solutions. In fact, increasing the osmotic pressure (more negative osmotic pressure) resulted from the increased environment's salinity, on one hand disrupts the seed hydration, and on the other hand, the high concentrations of cations and anions especially Na^+ and Cl^-) in the environment impede the seed germination by imposing toxicity in seeds [52], [53]. Moreover, can be said that this might be due to NaCl affects the permeability of the plasma membrane and increases influx of external ions and efflux of cytosolic solutes in plant cells [54] Secondly NaCl causes hardening of the cell wall and a decrease in water conductance of the plasma membrane causing reduction in seedling length. [55]

Table 1: Analysis of variance for the studied traits in five cowpea genotypes in response to water salinity stress.

Source of variance	d.f	Germination Percentage %	Shoot Length	Root Length	Seedling Fresh weight	Seedling Dry weight	Relative Water Content	Ion Leakage	Chlorophyll Content
Genotypes (G)	4	NS	**	**	**	**	**	**	**
Salinity (S)	3	**	**	**	**	**	**	**	**
G × S	12	NS	NS	**	*	**	**	**	**

*NS = not significant *=significant **= highly significant*

Table 2: Mean values of studied traits for five cowpea genotypes response under different water salinity levels.

Treatments	Germination percentage	Shoot Length (cm)	Root Length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Relative water content	Ion Leakage	Chlorophyll content
Salinity (S)								
Control (153)	97.800 ^{a}	10.644 ^{a}	5.884 ^{a}	8.616 ^{a}	7.127 ^{a}	13.092 ^{a}	32.196 ^{a}	77.440 ^{a}
2500 ppm	97.400 ^{a}	10.340 ^{ab}	5.132 ^{ab}	7.964 ^{ab}	6.444 ^{a}	12.448 ^{a}	31.160 ^{a}	75.320 ^{b}
3500 ppm	96.400 ^{ab}	9.804 ^{b}	5.032 ^{b}	7.326 ^{bc}	5.580 ^{b}	11.212 ^{b}	27.448 ^{a}	72.432 ^{c}
4800 ppm	94.800 ^{b}	8.936 ^{c}	3.808 ^{c}	6.908 ^{c}	5.128 ^{b}	11.116 ^{b}	11.352 ^{b}	69.936 ^{d}
Genotypes (G)								
Sudany	96.500 ^{a}	10.265 ^{b}	5.410 ^{a}	5.171 ^{c}	3.895 ^{c}	8.080 ^{c}	33.285 ^{a}	80.660 ^{a}
Chinese red	96.750 ^{a}	8.865 ^{c}	4.920 ^{a}	5.430 ^{c}	4.560 ^{c}	8.940 ^{c}	28.325 ^{ab}	81.385 ^{a}
Kaha 1	96.000 ^{a}	9.081 ^{c}	3.405 ^{b}	9.066 ^{b}	7.280 ^{ab}	12.890 ^{b}	22.960 ^{b}	73.350 ^{b}
TVU 21	96.750 ^{a}	12.410 ^{a}	5.320 ^{a}	8.375 ^{b}	6.590 ^{b}	15.140 ^{a}	21.900 ^{b}	62.970 ^{d}
Blackeye Crowder	97.000 ^{a}	9.055 ^{c}	5.765 ^{a}	10.475 ^{a}	8.024 ^{a}	14.785 ^{a}	22.960 ^{b}	70.510 ^{c}

Means with the same letters are not significantly different at 5%

Table 3: Correlation coefficients between cowpea seedling characteristics and respiration of cowpea genotypes grown under control and salt stress conditions.

Treatments	Salinity	Germination Percentage %	Shoot Length	Root Length	Seedling Fresh weight	Seedling Dry Weight	Relative Water Content	Ion Leakage	Chlorophyll Content
Salinity	1								
Germination rate	-0.289**								
Germination percentage	-0.289**	1							
Hypocotyl Length	-0.335**	0.205**	1						
Radicle Length	-0.380**	0.097	0.153	1					
Seedling fresh weight	-0.238**	0.136	0.129	-0.071	1				
Seedling dry weight	-0.345**	0.095	0.226 **	-0.013	0.791**	1			
Relative water content	0.052	0.042	0.209**	-0.115	0.644**	0.590**	1		
Ion Leakage	0.424	-0.144	-0.242	-0.119	-0.340**	-0.430**	-0.137	1	
Chlorophyll content	-0.349**	-0.011	-0.235	0.064	-0.383**	-0.310**	-0.660**	-0.006	1

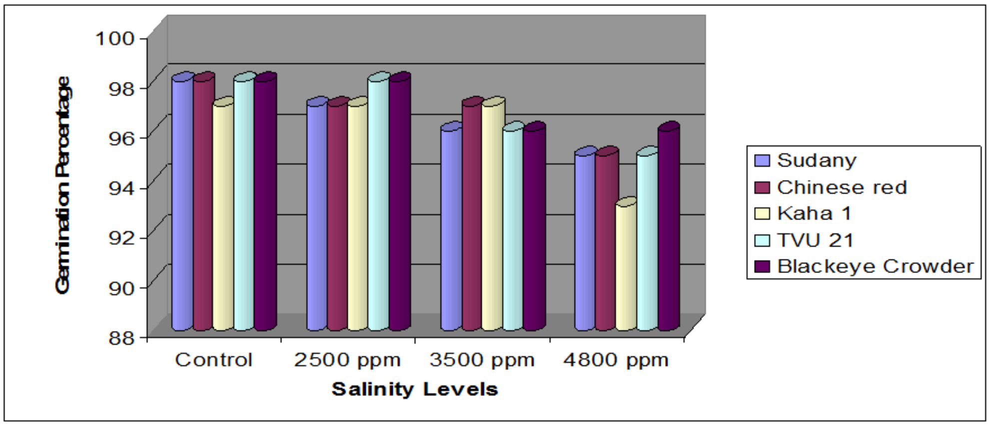

Fig1. Effect of different salinity levels on germination percentage of five cowpea genotypes

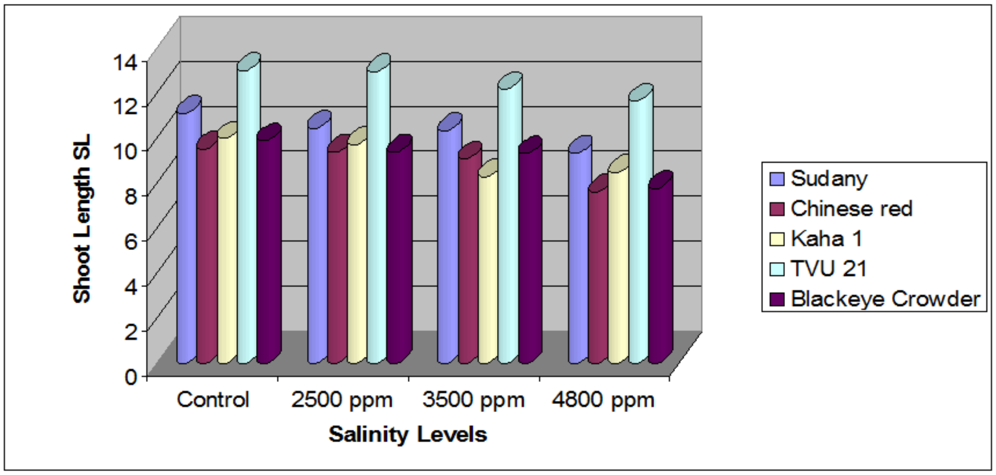

Fig2. Effect of different salinity levels on shoot length of five cowpea genotypes

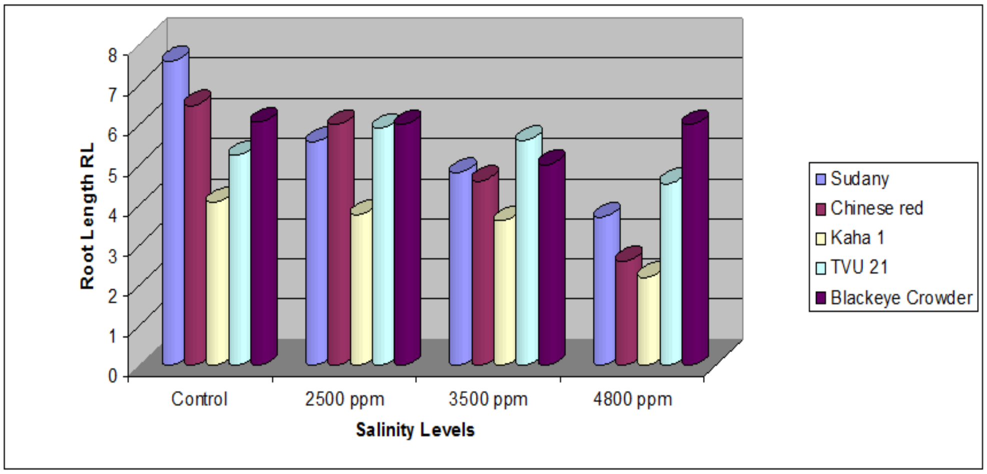

Fig3. Effect of different salinity levels on root length of five cowpea genotypes

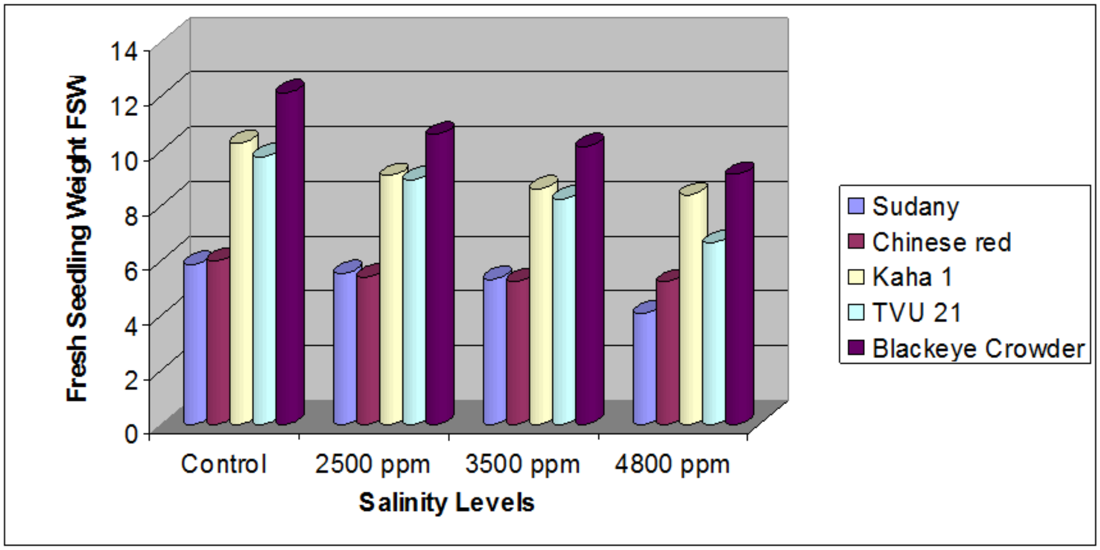

Fig4. Effect of different salinity levels on fresh seedling weight of five cowpea genotypes

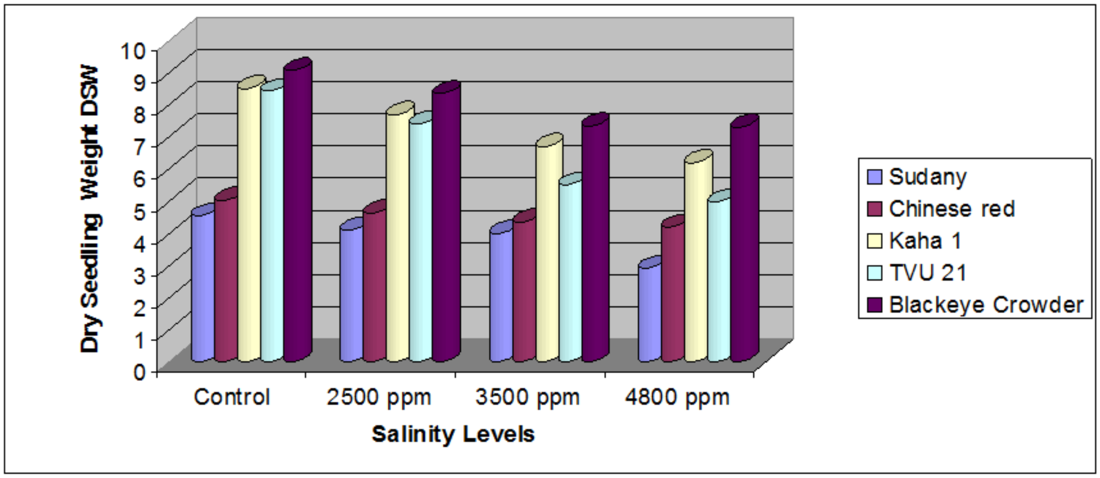

Fig5. Effect of different salinity levels on dry seedling weight of five cowpea genotypes

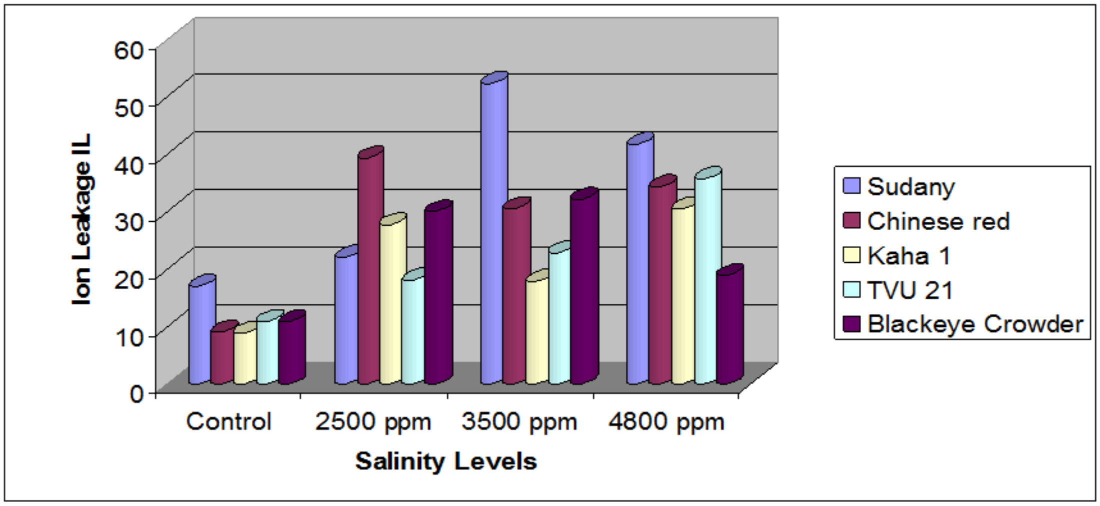

Fig6. Effect of different salinity levels on ion leakage of five cowpea genotypes

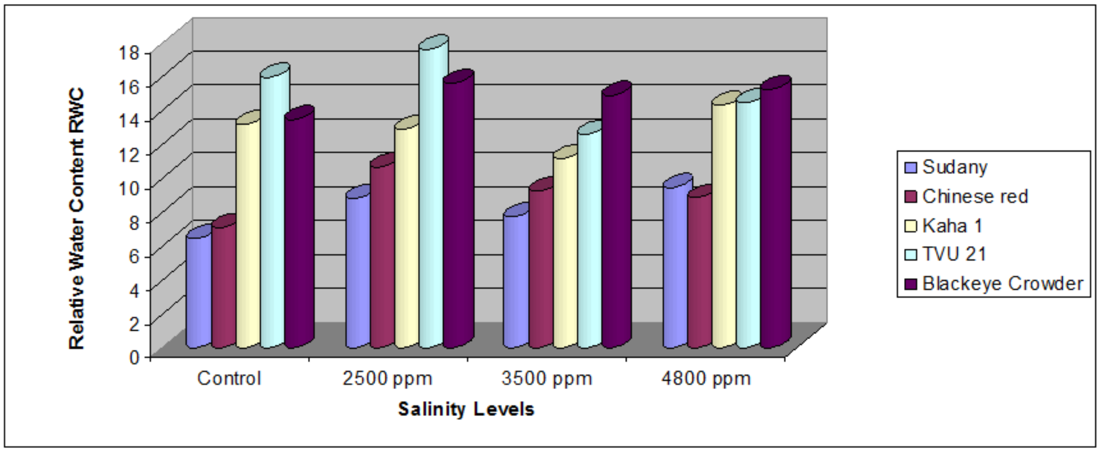

Fig7. Effect of different salinity levels on relative water content of five cowpea genotypes

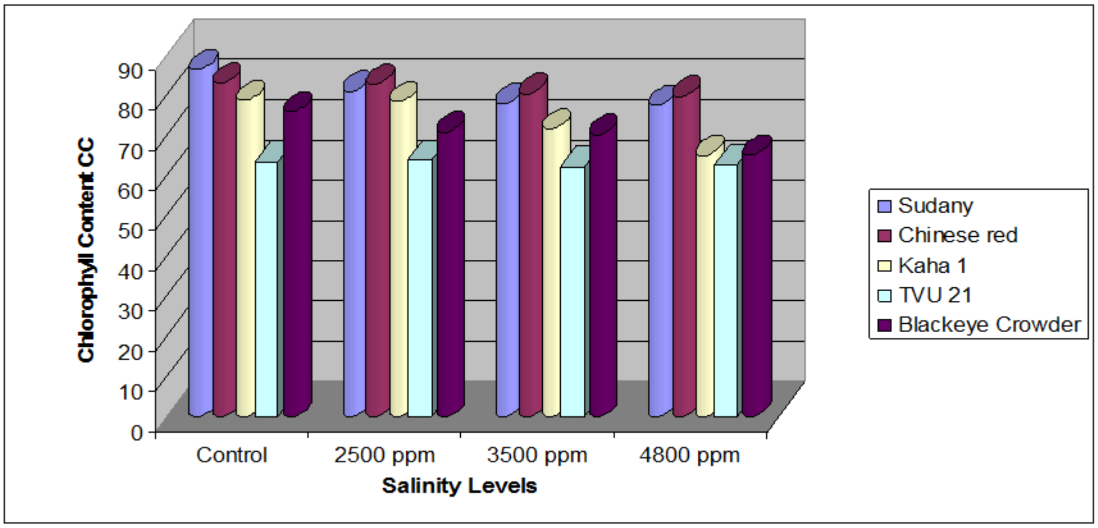

Fig8. Effect of different salinity levels on chlorophyll content of five cowpea genotypes

CONCLUSION

Cowpea genotypes showed significant differences in their tolerance to salinity. The results indicated that under all evaluated conditions Blackeye Crowder genotype was found to be most tolerant to salt stress among the genotypes. Water salt concentrations of 4800 ppm affect the germination of cowpea seeds, as well as the cowpea seedlings growth and chlorophyll content. Finally, it can be concluded that salinity stress significantly decreased all studied traits.

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