EXPLICATING THE SALINITY TOLERANCE OF COWPEA (Vigna unguiculata L. Walp.) GENOTYPES AT SEED GERMINATING STAGE

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Abstract

Cowpea is an important food and fodder legume in the arid and semi-arid tropics of the world. Soil salinity adversely affects seed germination, which ultimately reduces crop yield. The present study was carried out to screen the cowpea germplasm and identify the salinity tolerant genotypes at the germination stage. Initially, an experiment with five salinity levels (0, 50, 100, 200, and 250 mM of NaCl) was conducted, and the results showed that 200 mM of NaCl concentration was found to be a sensible salinity stress to assess the salinity tolerance of cowpea genotypes. A total of 29 cowpea genotypes were evaluated under a 200 mM NaCl concentration. Four salinity tolerance indices such as absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) were calculated from germination percentage without stress (GC) and germination with 200 mM NaCl (GS). All the indices showed significant variation in responses to salinity stress among the cowpea genotypes. The results revealed that salinity stress significantly reduced the germinating percentage in cowpea. Total seven cowpea genotypes (G4, G9, G12, G15, G26, G27, and G32) were found to be salinity tolerant lines at the germination stage. The seven genotypes were clustered in the same group. which had the higher RST (G4: 0.58, G9: 0.80, G12: 0.50, G15: 0.60, G26: 0.50, G27: 0.60, and G32: 0.80) and STI (G4: 0.58, G9: 0.80, G12: 0.50, G15: 0.60, G26: 0.50, G27: 0.60, and G32: 0.79) under salinity stress. However, this is a preliminary screening against salinity at the germination stage, and further research is required at the seedling vegetative and reproductive stages for validation.

Keywords: Cowpea, Salinity tolerance index, Seed germination, Vigna unguiculata

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an important food and fodder legumes in the arid and semi-arid tropics of the world. The diploid chromosome number of cowpea is 2n = 22 and belongs to the family Fabaceae (Paudel *et al.*, 2021). This crop grows well in temperature of about 28°C (Craufurd *et al.*, 1997). Cowpea provides food for millions of people, mostly in developing countries, with an annual worldwide production is about 4.5 million metric tons (Animasaun *et al.*, 2015). The young leaves,

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immature pods, and peas are used as vegetables, whereas some appetizers and main dishes are prepared from the grain (Singh and Tarawali, 1997). It is not only grown for leafy vegetable and grain legumes for human consumption but also for livestock feed (Gerrano *et al.*, 2015). It is a good source of protein, vitamins, and minerals. In dry basis, cowpea grain contains about 23-32% protein, 50–60% carbohydrate and about 1% fat (Jayathilake, *et al.*, 2018). The consumption of cowpea exerts protective effects against several chronic diseases, including gastrointestinal disorders, cardiovascular diseases, hypercholesterolemia, obesity, diabetes and several types of cancer (Forta *et al.*, 2008, Rotimi *et al.*, 2013, Trehan *et al.*, 2015). Therefore, this crop is important for human nutrition. Cowpea requires less input to cultivate and able to fix atmospheric nitrogen to soil. It can be adapted to adverse environmental conditions. Although cowpea is nutritious and favorable for growing in the agro-climate of Bangladesh but production is still lower compared to other countries.

Salinity is one of the most serious limiting factors for cowpea production in the arid and semi-arid tropics of the world. In Bangladesh, coastal area constitutes 20% to 30% of the agricultural land (https://www.thedailystar.net/news-detail-145077). Salinity alters the morphology, physiology, and metabolism of plants and severely affects growth and yield of crop plants. Grain yield is frequently used in crops such as cowpea as the main criteria for salt tolerance. In the view of some researchers only physiological markers such as content of Na, K, the ratio of the potassium to sodium, and the proline accumulation pattern are less feasible and are not promising (Shannon, 1984). The physiological tolerance along with agronomic traits has been shown to be applicable and their relationship with salinity stress tolerance indices are considered strong enough to be exploited as a selection tool in the breeding of salinity tolerant cultivars. Therefore, the relationship of physiological markers with grain yield in saline conditions is very important.

The salinity-affected soils are predominant in the southern coastal areas of the country, including Khulna, Satkhira, Bagerhat, Pirozpur, Jhalakathi, Patuakhali, Chittagong, Cox's Bazar, Noakhali, Barguna and Bhola. The farming communities of these areas need to introduce salinity tolerant crop cultivars. Exploiting genetic variability of available cowpea germplasm which have the salinity tolerance mechanism is vital for crop yield. Moreover, very little research has been carried out on improvement of the cowpea in Bangladesh. Considering the above problem and prospects, the proposed research is planned to evaluate the potential for salinity tolerance of cowpea genotypes at seed germinating stage.

Materials and Methods Plant materials and experimental design

Twenty-nine cowpea genotypes were used in this study (Table 1) which were collected from Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University (SAU) and Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI). The experiment was conducted in a factorial experiment with two factors (genotype \times salinity stress) following completely randomized design (CRD) with three replications in the laboratory of Department of Genetics and Plant Breeding, SAU, Dhaka, Bangladesh during Rabi season December 2022 to February7 2023.

Salinity treatment and screening of tolerant genotypes

Initially, five genotypes comprised G1, G3, G5, G6 and G31 and the five salinity levels were 0, 50, 100, 200, and 250 mM were considered to find out the optimum seed germination under different salinity levels. Three replications for both the genotypes and salinity levels were maintained. Different salinity levels of 50, 100, 200 and 250 mM were prepared by dissolving 2.92, 5.84, 11.68 and 14.612 g of pure NaCl in one liter distilled water, respectively while distilled water (0 mM) was used as control for the in vitro experiment (Asfaw, 2011). The germination experiment was conducted in the laboratory at room temperature as previously described Mamo et al. (1996). Petri dishes with a diameter of 10 cm were lined with Whatman No. 3 filter paper supplied with 8 ml of each treatment and control solutions. The seeds were surface sterilized 60 s with 2% bleach followed by 70% ethanol and rinsed with distilled water (Ravelombola et al., 2017). The optimum NaCl concentration for assessing salinity tolerance at the germination stage was considered where cowpea seed germination percentage between genotypes were significantly different. Afterward, ten uniform seeds of each cowpea genotypes were placed on each petri dish consisted 8 ml of 100 mM. Moreover, the petri dishes were covered with a polyethylene sheet to avoid the loss of moisture through evaporation. The seed was considered as germinated when both the plumule and radicle had emerged 2 mm long. The germination percentage (GP) was determined using the following formula at day eight after sowing (Islam et al., 2019).

Sl. No.	Genotypes	Accession	Sl. No.	Genotypes	Accession
1	G1	Vu/19/GPB-0001	16	G16	Vu/19/GPB-0017
2	G2	Vu/19/GPB-0002	17	G17	Vu/19/GPB-0020
3	G3	Vu/19/GPB-0003	18	G18	Vu/19/GPB-0021
4	G4	Vu/19/GPB-0004	19	G19	Vu/19/GPB-0022
5	G5	Vu/19/GPB-0005	20	G20	Vu/19/GPB-0023
6	G6	Vu/19/GPB-0006	21	G21	Vu/19/GPB-0024
7	G7	Vu/19/GPB-0007	22	G22	Vu/19/GPB-0026
8	G8	Vu/19/GPB-0008	23	G25	Vu/19/GPB-0029
9	G9	Vu/19/GPB-0009	24	G26	Vu/19/GPB-0030
10	G10	Vu/19/GPB-0010	25	G27	Vu/19/GPB-0032
11	G11	Vu/19/GPB-0011	26	G28	Vu/19/GPB-0033
12	G12	Vu/19/GPB-0012	27	G30	Vu/19/GPB-0035
13	G13	Vu/19/GPB-0013	28	G31	Vu/19/GPB-0036
14	G14	Vu/19/GPB-0015	29	G32	Vu/19/GPB-0037
15	G15	Vu/19/GPB-0016			

Table 1.	List	of c	cowpea	genotypes	used	in	the study	

% G = (No. of seeds germinated/No. of total of seeds for germination) $\times 100$

The salinity tolerance of the cowpea genotypes under NaCl stress was determined by calculating absolute decrease (AD), inhibition index (II), relative salt tolerance index (RST), salinity tolerance index (STI) following the previously described formulas (Saad *et al.*, 2014; Ravelombola *et al.*, 2017).

$$AD = GC - GS$$

II = 100 × (GC - GS)/GC
RST = GS/GC
STI = (GS × GC)/(GC_{av})²

Where, GC = Germination percentage without NaCl stress, GS = Germination percentage under NaCl stress, and $GC_{av} = Average$ germination percentage of a cowpea genotype without NaCl stress.

Data analysis

Mean data for each variable were worked out by dividing the total corresponding number of observations. Differences between cowpea genotypes for different variables and treatments were tested for significance by using analysis of variance and mean separations were carried out by using Statistix 10.0 program.

Results

Optimum NaCl concentration at seed germination stage

At first, an experiment with five salinity levels consisted of 0, 50, 100, 200, and 250 mM of NaCl was conducted to determine the optimum NaCl concentration for assessing salinity tolerance of cowpea genotypes (Fig. 1). The germination percentage (GP) revealed a significant variation among cowpea genotypes as well as salinity levels. The results suggest that the seed germination (GP) was decreased with the increase of salinity levels (Figs. 2-3). The highest seed germination (over 80 %) was observed at 0 mM (control without NaCl stress) while lowest at 250 mM of NaCl. The seed germination of cowpea genotypes varied significantly at 200 mM of NaCl.

Germination percentage (GP) of cowpea genotypes

All cowpea genotypes differ significantly for GP at both non-stressed (F = 1.79 and P = 0.0321) and NaCl stressed (F = 1.79 and P = 0.000) condition (Table 4). The GP of cowpea genotypes ranged from 61.11% to 94.44% at non-stress condition (0 mM NaCl) while 8.33% to 75.00% at 200 mM of NaCl (Table 2).

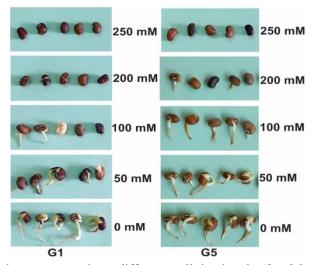


Fig. 1. Germinated cowpea seeds at different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl) at 48 h after sowing

The result showed that salinity stress significantly decreases the GP in cowpea genotypes. The cowpea genotypes G3 (86.11%), G5 (88.89%), G6 (88.89%) and G32 (94.44%) had the maximum GP, while G13 (61.11%) and G19 (66.67%) had the minimum GP at non-stressed condition (Table 2). On the other hand, the highest GP was recoded in G32 (75%) followed by G9 (66.67%) at salinity stress (200 mM NaCl) (Table 2). On the contrary, the lowest GP was observed in G1 (8.33%), G2 (8.33%), G3 (8.33%), G6 (8.33%), G11 (8.33%), G13 (8.33%), G19 (8.33%) and G20 (8.33%) at salinity stress (200mM NaCl) (Table 2).

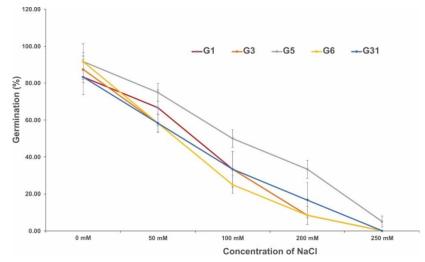


Fig. 2. Germination percentage of five cowpea genotypes under different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl)

Absolute decrease (AD) and inhibition index (II) of cowpea genotypes

The absolute decrease (AD) and inhibition index (II) of germination between the non-stressed and salinity stressed condition of cowpea genotypes were determined (Table 2). The result showed that salinity stress caused by 200 mM NaCl significantly decreased the AD (F = 4.38 and P = 0.0000) which ranged from 16.67% to 80.56%.

The highest AD was recorded in G6 (80.56%) while the lowest in G9 (16.67%) (Table 2). The II of germination was varied significantly among the cowpea studied genotypes (F = 5.74, P = 0.0000) (Table 4). The II varied from 20% to 91.11% (Table 2) demonstrating a wide range of variation among the cowpea studied genotypes against salinity stress. The higher II was exhibited in cowpea genotypes, including G1 (91.11%), G3 (91.11%), G2 (90%), G6 (90%) and G20 (90%) while lower II was recorded in G32 (20%), G9 (20%), G27 (40%), G15 (40%), G4 (41%), G26 (50%) and G12 (50%) under salinity stress (Table 2).

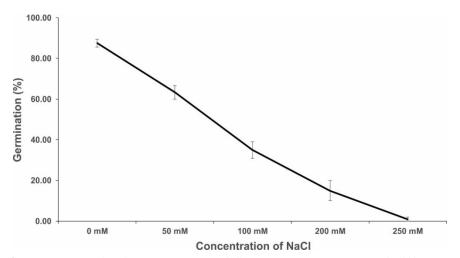


Fig. 3. Pooled germination percentage of five cowpea genotypes of different salinity levels (0 mM, 50 mM, 100 mM, 200 mM and 250 mM of NaCl)

Salinity stress tolerance of cowpea genotypes

The relative salinity tolerance (RST) and salinity tolerance index (STI) of cowpea genotypes which are commonly to measure salinity tolerance in plant were determined. The RST differed significantly for the studied genotypes (F = 5.71 and P = 0.0000) (Table 4). The higher RST was detected in G32 (0.80), G9 (0.80), G27 (0.60), G15 (0.60), G4 (0.58), G26 (0.5) and G12 (0.5) whereas the lower RST was found in G1 (0.09), G2 (0.10), G3 (0.09), G6 (0.10) and G20 (0.10) under salinity stress (200 mM NaCl) (Table 2). The cowpea genotypes showed a significant variation for STI (F = 6.46, P = 0.0000). The maximum STI was recorded in G9 (0.80), G32 (0.79), G27 (0.60), G15 (0.60), G4 (0.58), G26 (0.5) and G12 (0.5) while minimum in G6 (0.09) followed by G1 (0.11), G2 (0.11), G3 (0.11) and G20 (0.11) salinity stress (200 mM NaCl) (Table 2).

Clustering of different cowpea genotypes

The cowpea genotypes were further clustered using 'ClustVis: a web tool for visualizing clustering of multivariate data' available at https://biit.cs.ut.ee/clustvis/. The result showed that there were two cluster and cluster I exhibited a total of seven genotypes, including G27, G15, G4, G26, G12, G32 and G9 which were shown to be salinity tolerant at 200 mM NaCl (Fig. 4).

Discussion

In the first study, five different salinity levels were used to find out optimal salinity stress level in cowpea (Figs. 1-2). Gogile *et al.* (2013) also used four different concentrations of NaCl (0, 50, 100 and 200 mM) to screen salinity tolerant cowpea genotypes. The highest seed germination percentage (GP) was recorded in control (0 mM NaCl) and the lowest at 250 mM of NaCl as anticipated (Figs. 2-3). Therefore, no salinity tolerance can be assessed at those salinity levels. Besides, the GP was decreased with the increase of salinity levels in cowpea (Figs. 2-3). Thiam *et al.* (2013) and Ravelombola *et al.* (2017) also previously reported a similar finding in cowpea. Ravelombola *et al.* (2017) showed that 150 mM Concentration of NaCl to analyze the performance of cowpea genotypes against salinity stress at seed germination stage. However, the present study revealed that 200 mM of NaCl concentration could be a sensible salinity stress to assess the salinity tolerance of cowpea genotypes. Thus, 200 mM NaCl concentration was used for evaluation salinity tolerance of 29 cowpea genotypes in screening study.

Genotypes	Germination without stress (GC)	Germination with 200 mM of NaCl (GS)		II (%)	RST	STI
G1	83.33	8.33	75.00	91.11	0.09	0.11
G2	77.78	8.33	69.44	90.00	0.10	0.11
G3	86.11	8.33	77.78	91.11	0.09	0.11
G4	72.22	41.67	30.56	41.67	0.58	0.58
G5	88.89	33.33	55.56	62.22	0.38	0.38
G6	88.89	8.33	80.56	90.00	0.10	0.09
G7	72.22	16.67	55.56	76.67	0.23	0.23
G8	83.33	16.67	66.67	80.00	0.20	0.20
G9	83.33	66.67	16.67	20.00	0.80	0.80
G10	72.22	16.67	55.56	76.67	0.23	0.23
G11	72.22	8.33	63.89	88.33	0.12	0.12

Table 2. Germination percentage (without stress, GC; with 100 mM of NaCl, GS, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST) and salt tolerance index (STI) in cowpea genotypes

Genotypes	Germination without stress (GC)	Germination with 200 mM of NaCl (GS)		II (%)	RST	STI
G12	83.33	41.67	41.67	50.00	0.50	0.50
G13	61.11	8.33	52.78	85.56	0.14	0.14
G14	83.33	16.67	66.67	80.00	0.20	0.20
G15	83.33	50.00	33.33	40.00	0.60	0.60
G16	72.22	25.00	47.22	65.56	0.34	0.36
G17	77.78	25.00	52.78	68.33	0.32	0.33
G18	72.22	16.67	55.56	75.56	0.24	0.23
G19	66.67	8.33	58.33	88.33	0.12	0.14
G20	77.78	8.33	69.44	90.00	0.10	0.11
G21	77.78	16.67	61.11	80.00	0.20	0.23
G24	77.78	16.67	61.11	78.33	0.22	0.21
G25	83.33	16.67	66.67	80.00	0.20	0.20
G26	83.33	41.67	41.67	50.00	0.50	0.50
G27	83.33	50.00	33.33	40.00	0.60	0.60
G28	83.33	16.67	66.67	80.00	0.20	0.20
G30	66.67	16.67	50.00	71.11	0.29	0.23
G31	83.33	16.67	66.67	80.00	0.20	0.20
G32	94.44	75.00	19.44	20.00	0.80	0.79

The result indicated that genotype G32 and G9 exhibited higher GP 75% and 66.67%, respectively (Table 2). The highest GP of these genotypes suggesting that they were tolerant to salinity stress (200 mM NaCl) at seed germination stage (Table 2). Furthermore, the lowest GP (8.33%) was detected in eight cowpea genotypes, including G1, G2, G3, G6, G11, G13, G19 and G20 at salinity stress (Table 2). The result demonstrating that these genotypes were very susceptible to 200 mM NaCl salinity stress () at seed germinating stage. Genotypes with higher AD value indicated higher susceptibility to salinity stress while with the lower II value indicated higher level of salinity tolerance (Ravelombola *et al.*, 2017). Five cowpea genotypes including G1, G2, G3, G6, and G20 showed over 90% II values (Table 2) indicated that these genotypes were highly susceptible to salinity stress at seed germination stage. On the other hand, seven cowpea genotypes such as G32, G9, G27, G15, G4, G26 and G12 exhibite lower II under salinity stress (200 mM NaCl). This results suggested that they could be utilized as salinity tolerant cowpea genotypes at germination stage for further genetic improvement

Salinity tolerance of cowpea genotypes

program for cultivation in the salinity prone areas. The present results of AD and II were in agreement with the previous findings reported by Ravelombola *et al.* (2017) for cowpea genotypes under salinity stress.

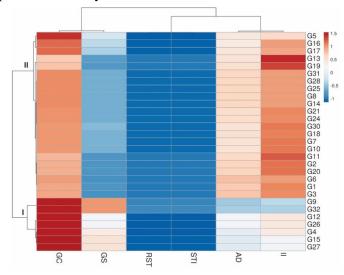


Fig. 4. Heatmap represents clustering cowpea genotypes in respect of six parameters. GC: Germination without stress (No stress), GS: Germination with 100 mM of NaCl (stress), AD: absolute decrease, II: inhibition index, RST: relative salt tolerance and (STI) salt tolerance index. This heatmap was generated using online tool freely available at https://biit.cs.ut.ee/clustvis/

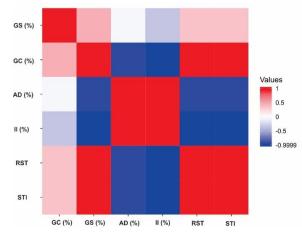


Fig. 5. Correlation matrix showing correlation between different parameters of 29 cowpea genotypes. The blue and red colors in the legend indicates highest and lowest values, respectively. GC: Germination without stress (No stress), GS: Germination with 100 mM of NaCl (stress), AD: absolute decrease, II: inhibition index, RST: relative salt tolerance and (STI) salt tolerance index. This heatmap was generated using online tool freely available at http://www.heatmapper.ca/

The genotypes with higher the RST and STI values are considered as salinity tolerant the genotypes (Saad *et al.*, 2014). The higher RST (0.5 or over) of G32, G9, G27, G15, G4, G26 and G12 (Table 2) under salinity stress (0 Mm NaCl) indicating that they were salinity tolerant genotypes at seed germination stage. On the other hand, the lower RST of G1, G2, G3, G6 and G20 (Table 2) indicating that they were susceptible to salinity stress at seed germination stage. A similar result for RST and STI was reported in cowpea by Ravelombola *et al.* (2017) under salinity stress.

Furthermore, the correlation studies of six parameters were performed using online correlation matrix visualization tool available at http://www.heatmapper.ca/. The result indicated that there was a lower relationship between the germination percentages (GC) under non-stressed condition (Fig. 5). Thus revealed that salinity tolerance at seed germination stage had a poor relationship with the GP non-stressed conditions. Conversely, the germination percentage under salinity stress (GS) had a strong negative correlation with AD and II. Nonetheless, GS had a very strong positive correlation RST and the STI indicating that salinity tolerance at seed germination stage is greatly associated with GS (Fig. 5). Seven cowpea genotypes such as G27, G15, G4, G26, G12, G32 and G9 clustered in the same cluster (I) which had the highest RST and STI which shown to be salinity tolerant at 200 mM NaCl (Table 2 and Fig. 4). The results revealed that the stress tolerance parameters like RST and STI could be very important parameters for evaluation of salinity tolerance at seed germination stage in cowpea.

Conclusion

Significant variation in responses to salinity stress was observed for all variables among the cowpea genotypes. Salinity stress significantly affected the germination percentage of the cowpea genotypes. Considering all six variables including germination without stress (GC), germination with 200 mM of NaCl (GS), absolute decrease (AD), inhibition index (II), relative salt tolerance (RST) and salt tolerance index (STI), total seven cowpea genotypes, including G4, G9, G12, G15, G26, G27 and G32 were found to be salinity tolerant under salinity stress (200 mM NaCl) at seed germination stage. These genotypes although showed tolerance to salinity at germination stage needs further screening against salinity stress at seedling, vegetative and reproductive stages to confirm the salinity tolerance. However, this screening technique may be used for preliminary screening for salinity tolerance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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