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ASSESSMENT OF SEEDLING EMERGENCE, GROWTH TRAITS AND PHYSIOLOGICAL INDICES OF A MEDICINAL PLANT *CALOTROPIS GIGANTEA* (L.) AITON f. UNDER NaCl INDUCED SALINITY STRESS

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Abstract

Calotropis gigantea (L.) Aiton f. giant milkweed or *Aakanda* (in Bengali) is a branched large shrub, spreading in many countries all over the world and has important medicinal and fiber production values. With this research, we evaluate the effects of NaCl salinity stress on the seedling emergence, growth and physiological traits of *Calotropis gigantea*. Following a randomized complete block design with artificial four levels of saline soils *viz*. 6, 8, 10 and 12 dS m^{-1} alongside with control (0 dS m^{-1}) were studied with three replications. *Calotropis gigantea* seeds were tested in varying concentrations of artificial saline soil for 45 days. Analysis of variance showed that seedling emergence, growth and physiological attributes revealed to be severely impacted due to salt stress. Mean emergence time and proline content rose along with a rise in salinity level, but other characteristics declined with the upsurge of saline stress. Reduction of total chlorophyll content was more in 12 dS m^{-1} salinity treatment compared to control condition. Principal component analysis showed that first principal component contributed 87% of the total variation and noticeably distinguished *Calotropis gigantea* genotype for seedling emergence, growth and physiological traits under all salinity treatments (6, 8, 10 and 12 dS m^{-1}) from control condition (0 dS m^{-1}). Correlation study exhibited that mean emergence time and proline content had positive correlation but both were negatively correlated with other studied traits. Overall, our present investigation brought a new output for cultivating the wild shrub *Calotropis gigantea* in saline prone soil, especially in the coastal regions of the world.

Key words: *Calotropis gigantea*, Chlorophyll, Proline content, NaCl salinity, Seedling emergence, Trait association.

Introduction

Calotropis gigantean (L.) Aiton f., belonging to Apocynaceae family prefers to grow in very low (closer to 0 m) to high altitudinal ranges (up to 1300 m) and has orthodox seed storage capability (Francis, 2003; Orwa *et al*., 2009). *Calotropis* is considered as a multipurpose plant and both the species *C. gigantea* and *C. procera* have similar medicinal and pharmacological properties (Ahmed *et al*., 2005). In Bangladesh, China, and India *C. gigantea* is often practiced in conventional medicine (Parhira *et al*., 2016; Motaleb, 2011). Bangladesh is one of the native countries of this fast-growing large shrub or small tree (Li, 2015) where it is locally called as *Aakanda*. *Calotropis gigantea* grows on fallow lands all over Bangladesh (Islam *et al*., 2019). The giant milkweed plant that reaches up to 4 m tall with clusters of white or lavender waxy flowers is a salt tolerant, drought resistant plant and also can grow in disturbed soil (Sharma and Tripathi, 2009). However, though *Calotropis gigantea* is considered a high valuable plant, but still, this is undomesticated and there is an urgent need to develop new varieties. Presently, scientists are giving their intense attention to cultivate *Calotropis* plant for medicine, fiber and biodiesel production (Parhira *et al*., 2016; Kanchan and Atreya, 2016; Muriira, 2017). However, there are limited number of investigations available under adverse environmental conditions such as salinity and drought with regards to commercial production of *Calotropis gigantea*. Therefore, considering the key significances of this plant, it is also crucial to analyze the seed germination, seedling emergence and growth traits under different environmental conditions of the globe.

Climate change has caused an increase in soil salinity, from approximately 0.83 million ha in 1973 to 1.02 million hectares in 2000, and 1.05 million ha in 2009 in Bangladesh (Anonymous, 2010). Recently, it is reported that this salinity has spread over more than 150 km upstream in the rivers of the southern coast that seriously affected agricultural crops production, environment and public health (Uddin, 2021). In Bangladesh's saline regions, cropping intensity is generally lower (62–144%) than that of the country as a whole (159%), which corresponds to typical growing circumstances (Karim *et al*., 1990). As an emerging medicinal and fiber plant, the domestication, establishment and cultivation of *Calotropis gigantea* is very essential for effective use of the coastal saline areas of Bangladesh.

Numerous tiny seeds are produced by wild *Calotropis* species, which also reproduces asexually through root and stem suckers and by wind-dispersed seeds. The spread of seeds, germination, emergence, and the development of young seedlings are the main factors in the establishment of a species in a new location. Germination, emergence, and early seedling growth, all of which are particularly susceptible to the salt of the substrate make up the three stages of the plant's establishment (Jamil *et al.*, 2005). Extremely low percentage of germination and delayed germination are both effects of soil salt (Baskin & Baskin, 1997). Early seedling establishment is enhanced by rapid and homogeneous seed germination under salty circumstances, which ultimately enhances the output (Bradford, 1995). The amount of salt tolerance during germination and the level of tolerance during seedling growth are related to one another (Jhala, 1997). Therefore, the present investigation was undertaken with an aim to assess the seedling emergence, growth and physiological attributes of *Calotropis gigantea* under salinity stress condition.

Material and Methods

Experimental design and layout: The experiment was carried out in a randomized complete block design with three replications at the laboratory and research field of Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during February–May, 2021. There were five NaCl treatments, used in the experiment *viz*. control (0), 6, 8, 10 and 12 dS m^{-1}. The seedlings were grown in 12 L plastic pots that had the following dimensions: 25 cm height, 27 cm opening, and 21 cm diameter base maintaining distances between pot to pot and replication to replication were 0.5 and 1.0 m, respectively.

Soil analysis: At first, the soil was collected from the land of the HSTU research farm. Weeds and stubble were removed, cleaned and crushed properly. For physiochemical analysis of used soil, three random samples were tested in Soil Resource Development Institute (SRDI), Dinajpur and the following mean results were found: texture sandy loam, pH 5.50, salinity 0.370 dS m^{-1}, organic carbon 0.240%, organic matter 0.413%, total N 0.021%, available P 29.22 µg/g and exchangeable K 0.117 meq/100g.

Development of artificial saline soil using standard curve: Artificial saline soil of different treatments (0, 6, 8, 10 and 12 dS m^{-1}) was carried out utilizing a specified quantity of NaCl in soil using standard curve (Fig. 1). The salts were fully combined with the air-dried soil after being dissolved in tap water. For every 10 kg soil, we applied NaCl as 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% solution in each dish (Capacity 15 L) which replicated three times followed completely randomized design (Table 1). After proper mixing, the dishes of soil were covered with polythene sheet for 7 days and everyday soil was mixed carefully. After passing seven days the dishes were uncovered and continued proper mixing of soil every day till it was completely dried. Rainfall was also controlled with proper management using polythene sheet. When the soils were dried (almost 15 days required), 100 g of soil from each bowl kept in a separate place. Then the soils were cleaned and sieved properly. The soil samples were packed in different polybags, tagged and sent to SRDI for determining the level of salinity. From the results (Table 1) a regression linear (*r*) graph was established using developed salinity (dS m^{-1}) *versus* added salt (g kg^{-1} soil) (Fig. 1). Further, the linear regression (*r*) was tested and it was found significant which was considered as a standard curve ($y = 1.4598x + 0.387$, $r = 0.985$, $p<0.001$). Using this significant standard curve, artificial soil salinity of 6, 8, 10 and 12 dS m^{-1} was developed at four different trays following mentioned procedures (Hussain *et al.*, 1989) and finally, again affirmed the salinity level by testing three soil samples from each tray.

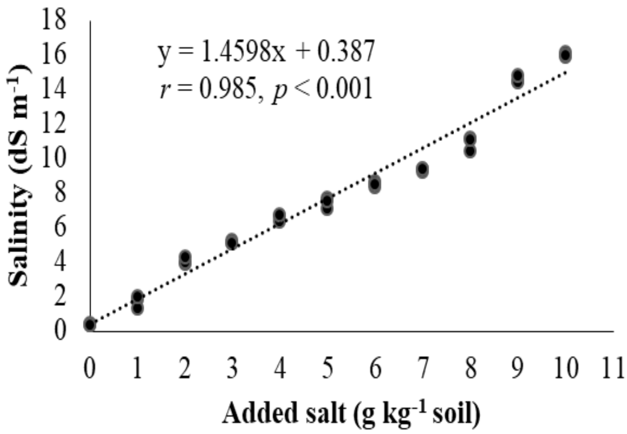

Fig. 1. Linear standard curve for developing various level of artificial salinity in studied soil for pot experiment.

Seed collection and sowing: The fruit color of *C. gigantea* is changed from dark green to green or yellowish green on maturation (El-Tantawy, 2000). Physiologically fruit riped with open capsules and brown seeds, was collected from fallow land of Nayanpur, Dinajpur Sadar, Bangladesh (25°39′12′′ N latitude and 88°39′14′′ E longitude) during August 2020. Ten pods were collected from three plants maintaining ≤ 2 m plant to plant distance. Previously, the distance between two sampling individuals of *C. gigantea* was studied as 20−50 m within a population and revealed the homogenous genetic diversity of the individuals within this distance (Islam *et al.*, 2019). *Calotropis gigantea* is a cross pollinated plant, bearing wind dispersal seeds with prominent vegetative propagation traits, hence the plants within ≤ 2m might be having homogenous genetic characters and mixing of all seeds from three plants was reasonable for conducting present investigation. Seeds were manually removed from the pod, mixed properly and put in the shade to dry. The dried seeds were preserved in a refrigerator at 5°C temperature until sowing at 1st February 2021. Pots were filled with 10 kg prepared saline soil (6, 8, 10 and 12 dS m^{-1}) from the respective trays along with the control. The soil was mixed properly before seed sowing and twenty seeds of *C. gigantea* were sown in each pot. In accordance with the suggestions from the chemical and physical analyses, soil was fertilized, 5% organic matter and 2.5 g of triple super phosphate applied at foundation, 10 g of potassium chloride and 10 g of urea applied at 30 days after sowing (DAS) of seed in each pot. Plants were irrigated by tap water in every 3 days interval, based on moisture availability of the pot soil. A Thiamethoxam-based product (8 g/ 20 L) was applied to control aphid infestation at 45 DAS.

Table 1. Development of artificial soil salinity in 10 kg soil at dishes for pot experiment.

NaCl solution (%) applied in soil	Amount of soil (kg)	Applied salt (g)	Developed salinity (dS m^{-1})
0.0	10	0	0.36
0.1	10	10	1.69
0.2	10	20	4.05
0.3	10	30	5.14
0.4	10	40	6.57
0.5	10	50	7.43
0.6	10	60	8.47
0.7	10	70	9.33
0.8	10	80	10.92
0.9	10	90	14.57
1.0	10	100	16.00

Table 2. Analysis of variance table for the studied seedling emergence, growth and physiological traits of *Calotropis gigantea* genotype.

Traits	MS
Seedling emergence percentage	3040.00**
Emergence vigor index	230.03**
Mean emergence time	4.73**
Total leaf area	39.88**
Number of leaves	18.51**
Stem diameter	0.192**
Shoot length	53.22**
Root length	51.17**
Total dry mass	1.10**
SPAD value	208.46**
Total chlorophyll content	0.175**
Proline content	3.34**

MS = Mean squares, **Indicates significant at 1% level of probability

Data collection: Seedling emergence and its associate traits were estimated till 30th days after seed placement into pots. After emergency evaluation, *C. gigantea* plants were thinned from each pot keeping 5 healthy seedlings and allowed to grow upto 45 days for further assessment of seedling growth and the physiological attributes. Seedling emergence percentage (SEP), one of the characteristics of seedling emergence, was estimated by subtracting the number of emerged seedlings from the total number of seeds put in each pot, then multiplying the result by 100 (Mitchell and Vogel, 2012). The emergence vigor index was calculated as: Emergence Vigor Index = $\sum(Et/Dt)$, Where Et is the number of seedlings emerged, Dt is the number of days after planting when the seedlings were counted (Yang *et al*., 2005; Copeland, 1976). Mean germination time (MGT) by Al-Ansari and Ksiksi (2016) was used to estimate the mean emergence time (MET): $\Sigma Fx/\Sigma F$, where F is number of seedlings emerged on day x.

Among seedling growth traits: total leaf area (TLA), number of leaves (NL), stem diameter (SD), shoot length (SL), root length (RL) and total dry mass (TDM) were collected at 45 DAS. Individual leaf area was calculated using the equation Leaf Area (cm^2) = W × L × 0.75, proposed by Moreira *et al*. (2007) for *C. procera*, a sister species of *C. gigantea*. Total Leaf Area was the sum of all leaf area measurements for each leaf (Almeida *et al*., 2019). A digital caliper was used to measure Stem Diameter (cm) at the base of the stem, corresponding to the lap of the plants. After 45 DAS three seedlings were sampled from each pot for taking shoot length and root length manually and finally, dried separately at 70°C for 72 h in an electric oven and weighed on an electrical balance for recorded total dry mass.

Physiological trait SPAD (Soil Plant Analyses Development) value was taken from middle portion of the youngest fully expanded leaf of the three tagged plants at 45 DAS using self-calibrating Minolta chlorophyll meter and the average of the three values were recorded. Total chlorophyll content of the apex leaf at 45 DAS of *C. gigantea* was estimated according to Witham *et al*. (1986) as Total Chlorophyll (mg g^{-1} FW) = $[20.2(D_{645}) + 8.02(D_{663})] \times [V/(1000 \times W)]$; Where, V = Final volume of filtrated extract, W = Weight of fresh leaf, D_{645} = Absorbance at 645 nm wavelength, D_{663} = Absorbance at 663 nm wavelength. The proline content was estimated at 45 DAS by following the protocol of Bates *et al*., (1973) as fresh weight basis using the formula below:

µmoles proline / g of fresh plant material = {(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample / 5).

Statistical analysis of data: Collected data were statistically analysed to find out the level of significance using Statistix 10 program (https://www.statistix.com/free-trial/). The mean differences were compared by Tukey's HSD Test at $p \leq 5\%$ level. Relative changes in stress condition over control for all studied parameters were measured using the formula as Relative Change = [(Treatment - Control) / Control] ×100. Minitab 17 statistical software package (Minitab Inc., State College, PA, USA) was used for principal component (PC) analysis and Pearson correlation analysis. ANOVA of the PC scores was performed for treatments following one way ANOVA model.

Results

Effects of different salinity treatment on seedling emergence traits of *Calotropis gigantea*: Analysis of variance revealed that several artificially developed saline soils (0, 6, 8, 10 and 12 dS m^{-1}) had a major impact on seedling emergence percentage, emergence vigor index and mean emergence time of *Calotropis gigantea* genotype at 30 days after sowing (Table 2). Due to salt stress condition emergence percentage and emergence vigor index of *C. gigantea* seedling were reduced vigorously in all salinity levels, whereas mean germination time of *C. gigantea* seedling was increased at salinity stress condition compared to control situation (Table 3). Highest emergence percentage was found at control (98.33) followed by 6 dS m^{-1} (65%) and 8 dS m^{-1} (60%). This reduction was not the same for all developed salinity for emergence percentage. The reduction was more pronounced in 12 dS m^{-1} (81.36%) and 10 dS m^{-1} (71.19%) than that of 8 dS m^{-1} (38.98%) and 6 dS m^{-1} (33.89%). For emergence vigor index, highest vigor index was detected at control (24.65) followed by 6 dS m^{-1} (11.88) and 8 dS m^{-1} (10.60). The reduction in relative changes over control were lower in 6 dS m^{-1} (51.81%) and 8 dS m^{-1} (56.99%) than 10 dS m^{-1} (81.99%) and 12 dS m^{-1} (90.91%). In case of 12 dS m^{-1} mean emergence, time was highest (23.09 days), which was followed by 10 dS m^{-1} (22.10 days). In control condition mean emergence time was decreased by 19.66 days. The increment for mean germination time was greater in 12 dS m^{-1} (17.45%) and 10 dS m^{-1} (12.41%) than 8 dS m^{-1} (9.16%) and 6 dS m^{-1} (8.44%).

Effects of different salinity treatment on seedling growth traits of *Calotropis gigantea*: Analysis of variance revealed that all the studied seedling growth traits like total leaf area, number of leaves, shoot length, stem diameter, root length and total dry mass were were extremely affected by the impact of various salinity levels (0, 6, 8, 10 and 12 dS m^{-1}) in soil culture (Table 2). It was observed that the studied growth traits in *C. gigantea* seedling declined with the increment of salt concentration in soil (Table 4), but the degree of reduction was not equal in different salinity stress. Greater leaf area degradation had been seen when exposed to salt stress in 12 dS m^{-1} (87.05%) and 10 dS m^{-1} (84.64%) than 8 dS m^{-1} (58.55%) and 6 dS m^{-1} (50.05%). The reduction in number of leaves over control were lower in 6 dS m^{-1} (37.05%), medium in 8 dS m^{-1} (51.10%) and 10 dS m^{-1} (53.11%) and higher in 12 dS m^{-1} (64.36%). The reduction of stem diameter was more pronounced in 12 dS m^{-1} (53.50%) and 10 dS m^{-1} (45.83%) than that of 8 dS m^{-1} (43.75%) and 6 dS m^{-1} (30.75%). The reduction for shot length was more pronounced in 12 dS m^{-1} (65.04%) and 10 dS m^{-1} (64.59%) than that of 8 dS m^{-1} (55.74%) and 6 dS m^{-1} (48.04%). Root length was decreased over control condition at 6, 8, 10 and 12 dS m^{-1} salinity levels by 46.91, 60.32, 63.64 and 65.56%, respectively. The reduction for total dry mass was more prominent in 12 dS m^{-1} (93.68%) and 10 dS m^{-1} (91.12%) than 8 dS m^{-1} (89.74%) and 6 dS m^{-1} (77.43%).

Effects of diverse salinity treatment on physiological traits of *Calotropis gigantea*: Analysis of variance exhibited that SPAD value, proline and total chlorophyll content of *Calotropis gigantea* seedling at 45 DAS were significantly influenced under various salinity levels (0, 6, 8, 10 and 12 dS m^{-1}) (Table 2). Due to salt stress condition SPAD value, total chlorophyll content of *C. gigantea* seedling was reduced dynamically in all salinity levels but the rising salt levels resulted in an increase in proline content (Table 5). The reduction of SPAD value was more pronounced in 12 dS m^{-1} (32.64%) and 10 dS m^{-1} (18.36%) than that of 8 dS m^{-1} (3.86%) and 6 dS m^{-1} (1.51%). The reduction in total chlorophyll content over control were lower in 6 dS m^{-1} (16.89%), medium in 8 dS m^{-1} (48.25%) and 10 dS m^{-1} (49.51%) and higher in 12 dS m^{-1} (51.65%). Under salt stress at 12 dS m^{-1} the highest amount of proline was found (2.70 µmole g^{-1} FW) and the control condition provided the lowest proline content (0.37 µmole g^{-1} FW). The degree of enhancement was more in 12 dS m^{-1} (629.73%) than 10 dS m^{-1} (624.32%), 8 dS m^{-1} (502.70%) and 6 dS m^{-1} (170.27%).

Phenotypic correlation among studied seedling emergence, growth and physiological traits: Correlation study revealed the mutual relationship among the seedling emergence, growth and physiological traits (Table 6). The traits under study showed both strong positive and negative correlations. Proline content indicated substantial negative association with all other features excluding mean emergence time, and mean emergence time also exhibited strong negative correlation with proline content. Mean emergence time displayed significant positive correlation with proline content (r = 0.826). Remaining attributes showed significant positive correlation with each other.

Table 3. Effect of different salinity levels on seedling emergence traits of *Calotropis gigantea* genotype.

Salinity level (dS m^{-1})	SEP		EVI		MET	
	%	RC (%)	Values	RC (%)	Days	RC (%)
0	98.33 a	–	24.65 a	–	19.66 c	–
6	65.00 b	-33.89	11.88 b	-51.81	21.32 b	8.44
8	60.00 b	-38.98	10.60 b	-56.99	21.46 b	9.16
10	28.33 c	-71.19	4.44 c	-81.99	22.10 ab	12.41
12	18.33 c	-81.36	2.24 c	-90.91	23.09 a	17.45
CV (%)	**12.93**		**17.61**		**2.00**	

Mean followed by same letter(s) in column did not significant at 5% level of probability under Tukey HSD, RC (%) = Relative changes over control (%), SEP= Seedling emergence percentage (%), EVI= Emergence vigor index, MET= Mean emergence time

Table 4. Effect of different salinity levels on growth traits of *Calotropis gigantea* genotype.

Salinity level (dS m^{-1})	TLA		NL		SD		SL		RL		TDM	
	cm^2	RC (%)	No.	RC (%)	cm	RC (%)	cm	RC (%)	cm	RC (%)	g	RC (%)
0	10.35 a	–	9.96 a	–	1.20 a	–	15.59 a	–	15.07 a	–	1.52 a	–
6	5.17 b	-50.05	6.27 b	-37.05	0.831 b	-30.75	8.10 b	-48.04	8.00 b	-46.91	0.343 b	-77.43
8	4.29 b	-58.55	4.87 bc	-51.10	0.675 bc	-43.75	6.90 bc	-55.74	5.98 bc	-60.32	0.156 c	-89.74
10	1.59 c	-84.64	4.67 bc	-53.11	0.650 bc	-45.83	5.52 c	-64.59	5.48 c	-63.64	0.135 c	-91.12
12	1.34 c	-87.05	3.55 c	-64.36	0.558 c	-53.50	5.45 c	-65.04	5.19 c	-65.56	0.096 c	-93.68
CV (%)	**15.61**		**11.50**		**9.75**		**7.80**		**10.87**		**13.09**	

Mean followed by same letter(s) in column did not significant at 5% level of probability under Tukey HSD, RC (%) = Relative changes over control (%), TLA = Total leaf area, NL = Number of leaves, SD = Stem diameter, SL = Shoot length, RL = Root length, TDM = Total dry mass

Table 5. Effect of different salinity levels on physiological traits of *Calotropis gigantea* genotype.

Salinity level (dS m^{-1})	SPAD value		Total chlorophyll content		Proline content	
	SPAD unit	RC (%)	mg g^{-1} FW	RC (%)	µmole g^{-1} FW	RC (%)
0	59.53 a	−	1.030 a	−	0.37 c	170.27
6	58.63 a	-1.51	0.856 a	-16.89	1.00 bc	502.70
8	57.23 a	-3.86	0.533 b	-48.25	2.23 ab	624.32
10	48.60 ab	-18.36	0.520 b	-49.51	2.68 a	629.73
12	40.10 b	-32.64	0.498 b	-51.65	2.70 a	170.27
CV (%)	**8.23**		**9.41**		**31.98**	

Mean followed by same letter(s) in column did not significant at 5% level of probability under Tukey HSD, RC (%) = Relative changes over control (%)

Table 6. Correlation coefficients among seedling emergence, growth and physiological traits of *Calotropis gigantea* genotype.

Traits	SEP	EVI	MET	TLA	NL	SD	SL	RL	TDM	SPAD	TC	Proline
SEP	1.00											
EVI	0.972***	1.00										
MET	-0.920***	-0.954***	1.00									
TLA	0.935***	0.969***	-0.937***	1.00								
NL	0.888***	0.925***	-0.893***	0.940***	1.00							
SD	0.847***	0.896***	-0.872***	0.928***	0.954***	1.00						
SL	0.894***	0.961***	-0.892***	0.951***	0.926***	0.924***	1.00					
RL	0.850***	0.908***	-0.839***	0.939***	0.940***	0.924***	0.969***	1.00				
TDM	0.830***	0.923***	-0.862***	0.930***	0.935***	0.926***	0.984***	0.969***	1.00			
SPAD	0.786***	0.735**	-0.825***	0.725**	0.605*	0.576*	0.590*	0.546*	0.516*	1.00		
TC	0.834***	0.851***	-0.813***	0.893***	0.886***	0.896***	0.874***	0.895***	0.858***	0.634*	1.00	
Proline	-0.794***	-0.809***	0.826***	-0.873***	-0.805***	-0.823***	-0.804***	-0.821***	-0.722***	-0.677**	-0.899***	1.00

*, ** and *** = significant at $P \leq 5$, ≤ 1% and ≤ 0.1% level of probability, SEP = Seedling emergence percentage (%), EVI = Emergence vigor index, MET = Mean emergence time (days), TLA = Total leaf area (cm^2), NL = Number of leaves (No.) , SD = Stem diameter (cm), SL = Shoot length (cm), RL = Root length (cm), TDM = Total dry mass (g), SPAD = SPAD value (SPAD unit), TC = Total chlorophyll content (mg g^{-1} fresh weight), Proline = Proline content (µmole g^{-1} fresh weight).

Table 7. Coefficients of PCs from PCA and mean PC scores with standard deviation of *Calotropis gigantea* genotype.

Variable	PC1
Seedling emergence percentage (%)	0.292
Emergence vigor index	3.02
Mean emergence time (days)	-0.294
Total leaf area (cm^2)	3.05
Number of leaves (No.)	0.297
Stem diameter (cm)	0.294
Shoot length (cm)	0.299
Root length (cm)	0.299
Total dry mass (g)	0.292
SPAD value (SPAD unit)	0.224
Total chlorophyll content (mg g^{-1} fresh weight)	0.286
Proline (µmole g^{-1} fresh weight)	-0.274
% Variation explained	87.00
***P* value**	<0.001
Mean PC scores with standard deviation	
Treatment	**PC1**
0 dS m^{-1}	5.55 ± 0.14 a
6 dS m^{-1}	0.86 ± 0.56 b
8 dS m^{-1}	-0.80 ± 0.45 c
10 dS m^{-1}	-2.30 ± 0.15 d
12 dS m^{-1}	3.30 ± 0.30 e

Principal component analysis (PCA): PCA is a dimensionality-reduction method that lessens the dimensionality of the large data sets. In this experiment, first principal component (PC1) explained 87.00% of the total data variation for the studied seedling emergence, growth and physiological traits of genotype *Calotropis gigantea* (Table 7). This PC was acknowledged on the basis of an eigen value higher than unity. Positive coefficients of total leaf area (3.05), emergence vigor index (3.02) and negative coefficient of mean emergence time (-0.294), proline content (-0.274) were mostly contributed to the variation explained by PC1. PC scores clearly separated all salinity treatments (6, 8, 10 and 12 dS m^{-1}) from control (0 dS m^{-1}) condition for *C. gigantea* genotype by their opposing scenery in PCA-biplot (Fig. 2) and by their differing mean PC scores (Table 7). The PCA-biplot clearly revealed that PC1 scores for control (0 dS m^{-1}) were completely separated from the PC1 scores of other salinity treatments (6, 8, 10 and 12 dS m^{-1}) used in this experiment (Fig. 2, Table 7).

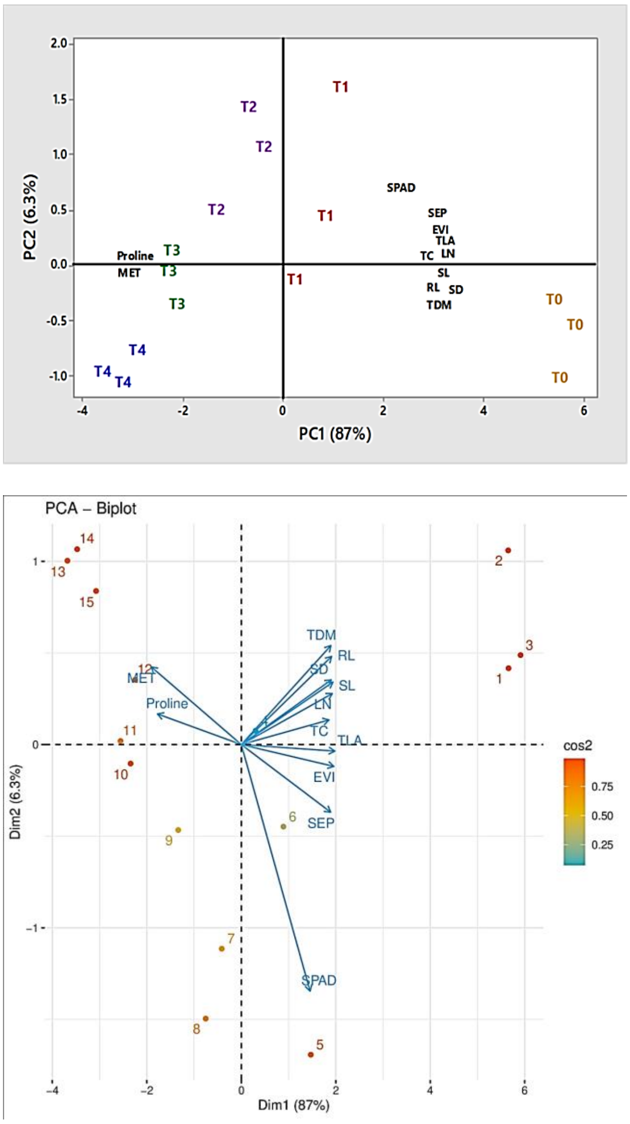

Fig. 2. Biplot for studied seedling emergence, growth and physiological traits of *Calotropis gigantea* genotype under different salinity (NaCl) levels; SEP = Seedling emergence percentage (%), EVI = Emergence vigor index, MET = Mean emergence time (days), TLA = Total leaf area (cm^2), NL = Number of leaves (No.) , SD = Stem diameter (cm), SL = Shoot length (cm), RL = Root length (cm), TDM = Total dry mass (g), SPAD = SPAD value (SPAD unit), TC = Total chlorophyll content (mg g^{-1} fresh weight), Proline = Proline content (µmole g^{-1} fresh weight). 1, 2 and 3 = T0 = 0 dS m^{-1} salinity treatment, 4, 5 and 6 = T1 = 6 dS m^{-1} salinity treatment, 7, 8 and 9 = T2 = 8 dS m^{-1} salinity treatment, 10, 11 and 12 = T3 = 10 dS m^{-1} salinity treatment, 13, 14 and 15 = T4 = 12 dS m^{-1} salinity treatment.

Discussion

Seedling emergence evaluation under salinity stress: The stages of emergence and germination are essential for plant establishment (Song *et al*., 2008). The majority of plant species are extremely vulnerable to high salt during the germination and seedling stages of development (Gulzar *et al*., 2003). Most plants are vulnerable to ion stress during germination and seedling emergence (Catalan *et al*., 1994, Chauhan and Johnson, 2009). In this experiment, all salinity levels vigorously reduced the emergence percentage and emergence vigor index of *C. gigantea* seedling, whereas an increase in mean germination time of *C. gigantea* seedling was observed at different salinity levels over control (Table 3). More distinct reduction of emergence percentage and emergence vigor index was noticed at 12 and 10 dS m^{-1} salinity treatments compared to 6 and 8 dS m^{-1} salinity treatments (Table 3). Similarly increase of mean emergence time was also more prominent in 12 dS m^{-1} compared to 6 dS m^{-1} (Table 3). This result of emergence percentage showed conformity with the findings of Guo *et al*., (2020) who reported that greater salt concentrations decreased the rate of emergence of seedlings, with a drop of 26.00 % (when cultivated in 200 mM NaCl) and a fall of 46.00 % (in 400 mM NaCl) for third generation seeds of *Suaeda salsa*. Again Dehnavi *et al*., (2020) also found that seedling vigor index of different genotypes of sorghum was decreased 55.5-88.4% at 200 mM NaCl saline stress. Gill *et al*., (2003) found that salt stress delayed the germination of seeds due to developing osmotically enforced dormancy. It might be a mechanism used by seeds to avoid germination under challenging conditions and ensure optimal seedling establishment. These outputs were in support with the present findings.

Seedling growth evaluation under salinity stress: Plants are predominantly sensitive in early stages of seedling development by elevated salinity (Jamil *et al*., 2005). Salinity stress also tempts the incidence of water shortage stress through reduction of osmotic potential of soil solutes thus crop roots face difficulties for water uptake from soil (Heidarpour *et al*., 2009). This causes harmful effects on the metabolism of plants (James *et al*., 1983). Salinity stress decreased root length, callus size, coleoptile length, and seedling growth (Agnihotri *et al*., 2006; Bera *et al*., 2006). Numerous plants are susceptible to ion stress caused by salinity condition during early seedling growth (Alom *et al*., 2016) Additionally, a high Na+ concentration prevents the absorption of K+ ions, a crucial component for growth and development, which lowers productivity and may even cause plant death (James *et al*., 1983). In present experiment, all the studied growth traits in *C. gigantea* seedling were decreased when the percentage of salt rises in soil (Table 4). The reduction of total leaf area, number of leaves, stem diameter, shoot length and root length were more noticeable in higher salinity level compared to control condition (Table 4). When the impact of saline water stress on *Calotropis procera* seedling growth and characteristics was examined in a greenhouse setting, it was discovered that the control treatment had the highest rates of root length, shoot and root dry weight, seedling height, collar diameter, and number of leaves (Bahmani and Kartoolinejad, 2017). Bilkis *et al*., (2016) established that shoot height, root length, shoot and root dry weight were greatly diminished as a result of an increase in salt concentrations in wheat. Matsuura *et al*., (2005) found that the leaf area was reduced by the salt treatment to 72% and 52% is under control in Tsushima and Pontivy buckwheat, respectively. These outputs were in parallel with the present findings. Reduction in the number of leaves per plant over control was found in polyembryonic mango

(*Mangifera indica* L.) genotypes at saline stress (Nimbolkar *et al*., 2018). Guo *et al*., (2020) revealed that stem diameter was reduced due to salinity stress in *S. salsa*. Dehnavi *et al*., (2020) found that shoot length of different genotypes of sorghum was decreased over control 40–70.4% whereas root length was decreased over control 48.8–73.7% at saline stress. Dehnavi *et al*., (2020) also found that dry weight of PAYAM genotypes of sorghum was decreased over control 72% at saline stress. Similar declines in growth metrics were recorded in *Catharanthus roseus* (Jaleel *et al*., 2008a, b), *Withania somnifera* (Jaleel *et al*., 2008c) and in *Salvodora persica* (Dagar and Kumar, 2004) under saline conditions. All these outputs displayed conformity with the present findings.

Physiological traits evaluation under salinity stress: Soil salinity became a critical problem worldwide among various environmental stresses because of its intense effects on physiology and enactments (Flowers *et al*., 1997). All environmental stresses directly or indirectly effect the osmotic pressure of the plants (Hannan *et al*., 2020; Nupur *et al*., 2020). Reduced photosynthetic activity and a reduced stomatal aperture are two physiological changes brought on by osmotic stress at the early stages of salinity stress (Rahnama *et al*., 2010). For revealing the salinity impact on photosynthetic responses, researchers quantify the amount of chlorophyll in the leaf. The use of Soil and plant analysis development (SPAD) meter is also used for determination of greenness (indicated chlorophyll content indirectly) for leaf under stress condition (Negrão *et al*., 2017). Salinity hampers photosynthesis by destroying chlorophyll pigments and hindering the activity of photosystem II (Saddiq *et al*., 2021). Within the current study, SPAD value and total chlorophyll content of *C. gigantea* seedling were reduced dynamically in all salinity levels but proline content was increased with increasing salinity levels (0, 6, 8, 10 and 12 dS m^{-1}) (Table 5). Like all the studied seedling emergence and growth traits, reduction of SPAD value and total chlorophyll content were more in 12 dS m^{-1} salinity treatment compared to control. However, with the increase of saline level, proline content was increased and it was observed that the highest amount of proline for *C. gigantea* found at 12 dS m^{-1} compared to other saline conditions (Table 5). Proline content increases with the rise of salt concentration also reported in *Salvadora persica* (Dagar and Kumar, 2004), *Catharanthus roseus* (Osman *et al*., 2007), wheat (Bilkis *et al*., 2016) and *Calotropis procera* (Alaish *et al*., 2016). One of the most common alterations caused by salt stress in plants is accumulation of the amino acid proline, which is assumed to be involved in stress tolerance pathways, but its exact function is still controversial (Lutts *et al*., 1999). Ali *et al*., (2004) reported reduced chlorophyll concentrations in 18 modern rice genotypes with artificial salinization (EC = 8.5 dS m^{-1}) soil conditions. It was reported that the growth and chlorophyll levels of *Calotropis procera* plant were badly affected by NaCl stress (Al-Sobhi *et al*., 2006). Ibrahim (2013) also found that the salinity reduced chlorophyll content in *Calotropis procera* at 100% sea water as compared to control. Jaleel *et al*., (2008b) reported about 14% and 34% total chlorophyll content reduction under low and high salinity, respectively in *Catharanthus roseus*. These outputs are in agreement with the present findings.

Trait association: In this experiment, principal component analysis revealed the most suitable blend of the studied traits through the mean values, whereas the magnitude of described variation by particular attributes and treatment were displayed by vector length on PCA-biplot. A negative association of mean emergence time and proline content with other growth traits was found. PC1 clearly separated the performance of *C. gigantea* genotype under all salinity treatments (6, 8, 10 and 12 dS m^{-1}) from control condition (0 dS m^{-1}) (Fig. 2, Table 7). From this, it could be inferred that different salinity levels affected the growth and physiological performance of *C. gigantea* genotype. On the biplot, scattered position of the same treatment might be due to heterogeneity within the treatments. From correlation analysis, it was confirmed that mean emergence time and proline content was negatively correlated with other studied traits, whereas they showed positive correlation between them (Table 6). Earlier studies also reported significant correlation among germination, growth and physiological traits of various plant species (Majid *et al*., 2013; Saddiq *et al*., 2021).

Conclusion

At artificially developed saline soil, seedling emergence, growth traits (shoot and root length, total dry mass, stem diameter, leaf number and area) and chlorophyll content were decreased as a result of NaCl salt stress. Mean emergence time and salinity levels rose along with a rise in proline content. This study concluded that *C. gigantea* is a salinity tolerant plant and it has a potential scope on commercial cultivation in coastal saline regions of the globe.

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