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Germination and Physiological Responses of *Calotropis gigantea* (L.) Seeds from Different Habitats under Salinity Stress

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GIANT MILKWEED (*Calotropis gigantea*) is a fiber resource shrub, which spreads in many countries worldwide and has important medicinal values. To evaluate the germination, emergence and post-emergence responses of different habitats' seeds of *C. gigantea* under salinity stress, two experiments (sand and pot culture) were carried out following a completely randomized design. The treatments consisted of seven levels of NaCl-induced salinity, viz. 0 (control, S_0), 5 (S_1), 6 (S_2), 7 (S_3), 8 (S_4), 9 (S_5), and 10 (S_6) dS m^{-1}; and five natural habitats' seeds collected from road side (T_1), railway line (T_2), river bank (T_3), waste dump (T_4), and grazing land (T_5). Results showed that the salt stress remarkably diminished germination percentage (2.39 to 72.09%), emergence percentage (3.51 to 75.00%), seedling growth and photosynthetic pigments (0.72 to 35.23% and 0.82 to 41.39% for total chlorophyll and carotenoids, respectively), while increased the stress-responsive traits such as proline contents (8.90 to 305.00%). However, the attributes were fluctuated among different habitats' seeds at various saline environments, even at the same concentration. Based on the studied trait and salt tolerance index (STI), the superior performance of different habitats' seeds was revealed as $T_1 > T_3 > T_5 > T_2 > T_4$ in non-saline condition (S_0), while it was observed as $T_2 > T_4 > T_3 > T_1 > T_5$ under salinity stress (S_1-S_6). Therefore, we considered that the railway line habitats' seeds could be a revegetation material in degraded saline areas till 10 dSm^{-1}, for the production of medicinal inputs and ecological restoration.

Keywords: *Calotropis gigantea*, germination traits, natural habitat, chlorophyll and proline content, salinity stress.

Introduction

Calotropis (L.) W.T. Aiton is a multipurpose genus, and both of its species, *C. gigantea* and *C. procera*, have wide ranges of medicinal and pharmacological properties (Bairagi et al., 2018). They are rich in essential organic compounds such as cardenolides, calactin, asclepsin, calotoxin, uscharin, calotropin, triterpenoids, frugoside, terpenes, flavonoids, tannin and gofruside (Chan et al., 2016). In Bangladesh, China and India, these species are broadly used in traditional medicine due to their anti-influenzal (Parhira et al., 2014), larvicidal (Shreya et al., 2012), anti-bacterial, anti-fungal (Sankar et al., 2014; Sethi, 2014), anti-malarial and anti-cancerous properties (Tariq et al., 2017; Hoopes et al., 2018). Additionally, the stem fiber of *Calotropis* is commercially used for making bowstrings, ropes, carpets and fishing nets, while the seed fiber is used for creating soft, stuffy mattresses and pillows (Kanchan and Atreya, 2016). The fibers from seeds also have the potential to reinforce natural fiber composites in automotive and building industries (Babu et al., 2014). Moreover, *Calotropis* can adapt to arid to semi-arid environments, making it a promising source of biodiesel production in these regions (Barbosa et al., 2014). However, the emergence, growth and development of *Calotropis* seedlings are severely affected by the salinity (Sinthy et al., 2024) and the variations of seed sources (Latif et al., 2025).

Globally, about one billion ha of land accounting for 7% of the total land surface are salt-affected, while approximately 30% of the irrigated land is secondarily salinized by human activities (Hopmans et al., 2021). In Bangladesh, approximately 1.056 million ha (62.5%) of the 1.689 million ha of coastal land are impacted by different concentrations of soil salinity (Miah et al., 2020). Moreover, salinity

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intrusion in the rivers of the Barisal and Jhalokati regions, extending over 150 km upstream, has led to a decline in agricultural productivity (Uddin, 2021). Although cropping intensity in Bangladesh has raised from 143.0% in 1971-72 to 198.0% in 2019-20, with a projected increase to 211.03% by 2030, salinity constraints the intensity of progressiveness in coastal regions compared to optimal growing conditions (Saif et al., 2024). Addressing these challenges, it is crucial to understand the germination and establishment requirements of *C. gigantea* in saline conditions for its successful establishment in coastal areas.

Habitats are the normal growing conditions in which seeds are naturally developed. It significantly influences the viability and germination behavior of seeds (El-Keblawy et al., 2018; Tudela-Isanta et al., 2018; Xu et al., 2021). The effects of maternal habitats also determine the successful establishment of their descendants in various unfavorable conditions (Mohamed et al., 2020). The degrees of resistance mechanisms of different habitats' seeds vary from species to species. A broad range of tolerance levels was observed in *Calotropis procera* under different light intensity, salinity and water stress conditions collected from Caatinga and Restinga ecosystems, contributing to its wide adaptation to ecological distribution (Leal et al., 2013). The other investigations revealed that maternal habitat affects the germination traits of various plant taxa, such as open-habitat herbs (Brink et al., 2013), *Anabasis setifera* (El-Keblawy et al., 2016), *Stipa* spp. (Zhang et al., 2020) and alpine species (Tudela-Isanta et al., 2018).

Germination and emergence are two critical periods of plants, affected by elevated levels of salinity (El-Shaieny, 2015; Galal et al., 2015; Ahmed, 2024). Salinity induces osmotic stress by reducing soil water potential and impairing water uptake by seeds and seedlings (Ibrahim, 2013; Kumari et al., 2021). It adversely affects the germination percentage, root length, shoot length, and seedling growth traits (Jaleel et al., 2008; Taghvaei et al., 2012; Ibrahim, 2013; Elhag, 2023). High levels of sodium (Na^+) concentrations due to the salinity disrupt potassium (K^+) uptake, a primary nutrient for plant growth and transpiration, leading to reduced development and even to death in extreme cases (Kumari et al., 2021; Hussein and Sabbour, 2024). There were some studies that explored the effects of salinity on the germination and early seedling growth of *C. procera* (Taghvaei et al., 2012; Galal et al., 2015) as well as in *C. gigantea* (Sinthy et al., 2024). However, there is no study has been done yet to reveal the performance of different habitats' seeds of *C. gigantea* under salt stress conditions.

The knowledge of seed germination and post-germination responses to the environmental factors is indispensable for species adaptation and restoration strategies (Carrera-Castaño et al., 2020). Therefore, the study aimed to assess the influence of different habitats' seeds on germination, emergence, seedling growth and physiological traits of *Calotropis gigantea* under salt stress conditions. It was assumed that the outputs of the investigation can contribute to a better understanding of how the natural habitats' seeds of *C. gigantea* respond to various degrees of salinity. At the same time, it might reveal which habitats' seeds show higher salt tolerance capacity, useful for sustainable regeneration and ecological restoration of this native species in the coastal regions of Bangladesh, as well as around the globe.

Materials and Methods

Experimental design and treatment

There were two experiments carried out following a two-factor completely randomized design (CRD) with three replications for each treatment at the Laboratory and Research farm of Crop Physiology and Ecology department in Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh. The first factor included seven levels of NaCl-induced saline conditions viz. 0 (S_0, control), 5 (S_1), 6 (S_2), 7 (S_3), 8 (S_4), 9 (S_5), and 10 (S_6) dS m^{-1}; while the second factor composed of five different habitats' seeds collected from natural growing sites such as road side (T_1), railway line (T_2), river bank (T_3), waste dump (T_4) and grazing land (T_5)

Preparation of saline solution and development of artificial saline soil

Saline solutions (0, 5, 6, 7, 8, 9, and 10 dS m^{-1}) were prepared by dissolving the calculated amount of NaCl (0.00, 7.81, 9.38, 10.94, 12.50, 14.06 and 15.63 g NaCl L^{-1}, respectively) in tap water. Artificial saline soil for pot culture was developed by incorporating NaCl solutions into air-dried soil, following the methods described by Zheng et al. (2008) and Islam et al. (2024). The initial nutrient status of the sandy loam soil was: pH 5.25, organic carbon 0.97%, organic matter 1.67%, nitrogen 0.084%, available phosphorus 42.68 µg g^{-1} and exchangeable potassium 0.33 meq 100 g^{-1} soil

Seed collection, sowing and growth of plants

Seeds of giant milkweed were collected from their natural habitats in Dinajpur, Bangladesh (**Fig. 1**) and surface-sterilized by 1% mercuric chloride solution for 5 minutes, followed by rinsing with distilled water. The sand culture experiment related to the germination and early seedling growth was conducted in petri dishes (9 cm diameter, 1 cm height) from November to December 2021. It was carried out at the laboratory to facilitate controlled environmental conditions for all replications, which helped in initial screening and precise comparisons of germination traits of different habitats' seeds of *C. gigantea*. The mean maximum temperature, minimum temperature, and relative humidity inside the laboratory were 30.63 °C, 11.69 °C, and 84.43%, respectively. On the other hand, the experiment engaged with emergence, seedling growth and physiological traits was done in pot (capacity: 12 L, height: 25 cm, diameter: 27 and 18 cm at opening and base, respectively) from January to April 2022. The pots were arranged in open field conditions to allow the expression of whole-plant traits influenced by real-time environmental factors for the evaluation of emergence, morphology and physiological parameters. During the growing period of plants, average maximum temperature, minimum temperature, relative humidity, and rainfall were recorded as 32.91 °C, 11.64 °C, 88.57%, and 6.65 mm, respectively. Before seed sowing, each of the pots was filled with 10 kg of saline soil mixed with 5% organic matter and 2.5 g of triple super phosphate. The plant of each pot was fertilized by muriate of potash (10 g) and urea (10 g) at 30 days after sowing for their optimum growth and development (Latif et al., 2025). Twenty-five seeds were used in both experiments, where the petri dishes and pots were irrigated with the required amount of tap water and saline solutions according to treatments. Extra seedlings were thinned out, keeping only 10 in each petri dish and allowed to grow till 20 DAS for further evaluations, while for the pot experiment, seedling removal was done at 30 DAS, leaving 5 healthy seedlings pot^{-1}, permitted to grow for 60 DAS for further physiological evaluation.

Fig. 1 Seed sampled locations (habitats) of *Calotropis gigantea* at Dinajpur Sadar Upazilla, Bangladesh .

Data collection:

Determination of germination attributes

Germination data were recorded at 24-hour intervals for 15 days, where the seeds were considered germinated once the radicle exceeded 2 mm in length. The germination percentage (GP) was calculated using the following formula (El-Shaieny, 2015).

GP = (Germinated seeds / Total number of seeds) × 100

Mean germination time (MGT) is the measure of the rate and time spread of germination. It was calculated using the following formula, previously described by Al-Mudaris (1998).

$MGT = \Sigma Fx / \Sigma F$

Where F = Number of seeds germinated on day x, and Σ F = Final germination.

Germination rate index (GRI) was calculated using the following formula (Al-Mudaris, 1998)

$GRI = G_1 / 1 + G_2 / 2 + ... + G_i / i$

Where G_1 = Germination percentage on day 1, G_2 = Germination percentage at day 2, and so on.

The co-efficient of velocity of germination (CVG) is the measure of the quickness of seed germination in a sample. It was calculated using the following formula (Al-Mudaris, 1998).

$CVG = [100 (A_1 + A_2 + \text{---------} + A_n) / (A_1T_1 + A_2T_2 + \text{---------}A_nT_n)]$

Where A = Number of seeds germinated, T = Time (days) corresponding to A, n = Number of days to final count.Timson germination index (TGI) has been reported as a good indicator of seed germination rate, which combines both the germination speed and completeness into a single value. The equation used to calculate the TGI is as follows (Khan and Ungar, 1998).

TGI = Σ G / T

Where, G = Percentage of seed germinated day^{-1}, T = Germination period

Assessment of seedling growth traits

Shoot length (SL) and root length (RL) were taken using a measuring scale at 20 days after sowing (DAS) from five randomly selected seedlings of each petri dish, while these data were measured at 60 DAS from three plants pot^{-1} for the pot experiment. Dry weight seedling^{-1} (DWS) and dry weight plant^{-1} (DWP) were recorded with an electrical balance after drying at 70 °C for 72 hours in an electric oven (Model E28# 03-54639, Binder, Germany) at 20 and 60 DAS for laboratory and pot experiments, respectively.

Evaluation of emergence parameters

Seedling emergence was recorded daily up to 30 days, where the seedling emergence percentage (SEP) was calculated following the methods described by Mitchell and Vogel (2012).

SEP = (ES / NS) × 100

Where ES = Emerged seedlings pot^{-1}, NS = Number of seeds placed in each pot

Mean emergence time (MET) was determined by the following formula (Mao et al., 2013).

MET = Σ Fx / Σ F

Where, F = Number of newly emerged seedlings on day x and Σ F = Total emergence

Measurement of plant morphology

The number of fully expanded leaves plant^{-1} was counted manually from the randomly selected three plants of each pot, and the mean was calculated. Leaf length (L) and width (W) were measured for three leaves from the lower, middle and upper sections of the three plants for each pot, and single leaf area was obtained using the following equation (Moreira et al., 2007).

Single leaf area (cm²) = W × L × 0.75

Quantification of physiological traits

Photosynthetic pigments, such as chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{a+b}) and total carotenoids (C_{x+c}) were determined at 60 DAS. Exactly, 0.25 g fresh leaf of *C. gigantea* grown in pots was taken into a brown colored bottle, and 25 ml of 80% (v / v) acetone was poured into it. Then the bottles were stored in a dark place for 48 hours to facilitate the extraction of the pigments. After the period, the extracted solution was taken into the cuvette, and the absorbance reading was measured by using a spectrophotometer. The following formulae were used to calculate the pigment levels (Lichtenthaler, 1987).

$C_a = 12.25A_{663.2} - 2.79A_{646.8}$

$C_b = 21.50A_{646.8} - 5.10A_{663.2}$

$C_{a+b} = 7.15A_{663.2} + 18.71A_{646.8}$

$C_{x+c} = (1000A_{470} - 1.82C_a - 85.02C_b) / 198$

For proline estimation, 0.5g of fully expanded young fresh leaf was taken, and the level of proline was determined according to Bates's (1973) method.

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

Salt tolerance index (STI) for both experiments was determined based on the DWS and DWP, respectively, following the method of Goudarzi and Pakniyat (2008).

STI = (Variable measured under stress condition / Variable measured under normal condition)

Statistical analyses

All the data were analyzed using Statistix 10 program to determine statistical significance. Mean differences were compared by Tukey’s HSD test at $P \leq 5\%$ level. Correlation analysis was conducted to assess the relationships among germination and seedling growth parameters.

Results

Effects of habitats and salinity levels on germination and early seedling growth traits of *Calotropis gigantean*

The data presented in **Table 1** show the gradual reduction of germination attributes of *C. gigantea* with the increase of salt concentrations. The significant values of GP and TGI in salt conditions ranged from -72.09% to +14.29% and -7.75% to -88.11%, respectively, when compared to their relative controls. The least decline of GP and TGI was found in T_2 habitats’ seeds in combination with different salt levels (S_1-S_6). However, a slight increment of GP was found in $S_1 \times T_2$ (14.29%), followed by a neutral $S_4 \times T_2$ (0.00%), than those of their respective control. In case of MGT, GRI and CVG, there were no significant interactions between salt concentrations and different habitats’ seeds; nonetheless, the negativity of salt stress was increased at higher levels of concentrations.

The minimal reductions of SL, RL and DWS were observed in $S_2 \times T_2$ (0.62%), $S_3 \times T_2$ (1.84%) and S_2

× T_4 (12.52%), respectively, than those of their relative control (**Table 2**). Moreover, the seedlings germinated from T_2 habitats' seeds provided substantial increments of DWS associated with the S_1-S_5 levels of salt stress over the control (S_0). In case of STI, the highest value was observed in $S_2 \times T_2$ (1.71), followed by $S_3 \times T_2$ (1.37), $S_1 \times T_4$ (1.26), $S_1 \times T_2$ (1.19), $S_5 \times T_2$ (1.18) and $S_4 \times T_2$ (1.17). Oppositely, the lowest STI were found in $S_6 \times T_1$ (0.29), followed by $S_5 \times T_5$ (0.45), $S_5 \times T_1$ (0.48), $S_4 \times T_1$ (0.50), $S_5 \times T_4$ (0.53) and $S_2 \times T_1$ (0.54). Based on the observations, the seeds collected from the railway line habitat (T_2) exhibited superior performance under saline conditions, and the results were statistically comparable to T_4 (waste dump habitat) in some cases.

Table 1. Influence of various habitats' seeds of *Calotropis gigantea* on germination traits under salt stress conditions.

Treatments		GP		MGT		GRI		CVG		TGI	
		%	RC (%)	days	RC (%)	-	RC (%)	-	RC (%)	-	RC (%)
S_0	T_1	81.67 a	-	11.27	-	33.55	-	6.59	-	25.80 a-d	-
	T_2	70.00 ab	-	9.96	-	36.02	-	6.90	-	27.36 abc	-
	T_3	81.67 a	-	9.44	-	42.74	-	6.99	-	27.26 abc	-
	T_4	70.00 ab	-	8.87	-	39.91	-	7.14	-	30.64 a	-
	T_5	71.67 ab	-	11.52	-	40.27	-	6.67	-	28.68 ab	-
S_1	T_1	51.67 b-g	-36.73	13.60	+20.67	20.38	-39.25	6.27	-4.86	19.71 d-h	-23.60
	T_2	80.00 a	+14.29	11.34	+13.86	23.32	-35.26	6.45	-6.52	22.56 b-f	-17.54
	T_3	60.00 a-e	-26.53	12.15	+28.71	11.95	-72.04	6.23	-10.87	13.52 h-l	-50.40
	T_4	63.33 a-d	-9.53	10.22	+15.22	28.06	-29.69	6.81	-4.62	24.17 a-e	-21.12
	T_5	33.33 g-j	-53.50	11.11	-3.56	14.78	-63.30	6.55	-1.80	8.95 j-n	-68.79
S_2	T_1	40.00 e-j	-51.02	12.93	+14.73	15.91	-52.58	6.07	-7.89	9.92 j-n	-61.55
	T_2	68.33 abc	-2.39	8.25	-17.17	27.08	-24.82	7.01	+1.59	20.01 d-h	-26.86
	T_3	43.33 d-i	-46.95	12.68	+34.32	25.43	-40.50	6.45	-7.73	15.93 f-j	-41.56
	T_4	56.67 b-f	-19.04	10.80	+21.76	31.06	-22.17	6.72	-5.88	21.48 c-g	-29.90
	T_5	40.00 e-j	-44.19	11.27	-2.17	10.85	-73.06	6.42	-3.75	12.15 i-n	-57.64
S_3	T_1	40.00 e-j	-51.02	13.24	+17.48	13.87	-58.66	6.10	-7.44	12.50 i-m	-51.55
	T_2	63.34 a-d	-9.51	12.64	+26.91	22.16	-38.48	6.35	-7.97	25.24 a-d	-7.75
	T_3	51.67 b-g	-36.73	10.84	+14.83	13.53	-68.34	6.42	-8.15	15.35 g-k	-43.69
	T_4	56.67 b-f	-19.04	11.59	+30.67	23.00	-42.37	6.25	-12.46	13.62 h-l	-55.55
	T_5	30.00 g-j	-58.14	11.43	-0.78	10.41	-74.15	6.47	-3.00	5.13 n	-82.11
S_4	T_1	45.00 d-i	-44.90	12.20	+8.25	16.01	-52.28	6.28	-4.70	8.58 k-n	-66.74
	T_2	70.00 ab	0.00	11.66	+17.07	31.11	-13.63	6.53	-5.36	17.50 e-i	-36.04
	T_3	46.67 c-h	-42.86	10.51	+11.33	18.12	-57.60	6.65	-4.86	11.79 i-n	-56.75
	T_4	51.67 b-g	-26.19	11.52	+29.88	25.61	-35.83	6.56	-8.12	17.63 e-i	-42.46
	T_5	20.00 j	-72.09	12.37	+7.38	6.10	-84.85	6.14	-7.95	8.36 k-n	-70.85
S_5	T_1	28.33 hij	-65.31	13.66	+21.21	5.86	-82.53	5.93	-10.02	6.33 mn	-75.47
	T_2	63.34 a-d	-9.51	12.74	+27.91	21.70	-39.76	6.37	-7.68	19.71 d-h	-27.96
	T_3	35.00 f-j	-57.14	11.98	+26.91	12.60	-70.52	6.30	-9.87	7.31 lmn	-73.18
	T_4	35.00 f-j	-50.00	11.06	+24.69	10.17	-74.52	6.33	-11.34	8.38 k-n	-72.65
	T_5	26.67 hij	-62.79	14.23	+23.52	5.98	-85.15	5.84	-12.44	7.14 lmn	-75.10
S_6	T_1	23.33 ij	-71.43	13.59	+20.59	7.80	-76.75	5.94	-9.86	5.06 n	-80.39
	T_2	51.67 b-g	-26.19	11.84	+18.88	24.37	-32.34	6.39	-7.39	17.30 e-i	-36.77
	T_3	41.67 d-j	-48.98	13.41	+42.06	8.17	-80.88	5.96	-14.74	10.85 i-n	-60.20
	T_4	46.67 c-h	-33.33	11.68	+31.68	9.37	-76.52	6.33	-11.34	9.17 j-n	-70.07
	T_5	20.00 j	-72.09	12.05	+4.60	8.59	-78.67	6.26	-6.15	5.44 mn	-81.03
CV (%)		13.81	-	14.36	-	54.96	-	3.88	-	14.24	-

Here, S_0 = 0 dSm^{-1}, S_1 = 5 dSm^{-1}, S_2 = 6 dSm^{-1}, S_3 = 7 dSm^{-1}, S_4 = 8 dSm^{-1}, S_5 = 9 dSm^{-1}, S_6 = 10 dSm^{-1}, T_1 = Road side, T_2 = Railway line, T_3 = River bank, T_4 = Waste dump, T_5 = Grazing land, GP = Germination percentage, MGT = Mean germination time, GRI = Germination rate index, CVG = Co-efficient of velocity of germination, TGI = Timson germination index.

Correlation co-efficient (r) among the germination and seedling growth attributes at 20 days after seed placement is presented in **Table 3**. All the germination parameters, including GP, GRI, CVG, TGI, RL, SL and DWS of *C. gigantea*, maintained a significantly positive correlation among themselves, except MGT. The most positive significant correlation co-efficient was found between GP × TGI (0.8392^{***}), followed by GP × SL (0.7797^{***}), whereas the highest neative was found in MGT × CVG (-0.8492^{***}), trailed by MGT × DWS (-0.4192^{***}). MGT, by far, showed the negative significant relations with all germination and seedling growth traits, whereas a non-significant correlation was found with GRI.

Table 2. Influence of various habitats' seeds on early seedling growth traits of *Calotropis gigantea* under saline conditions.

Treatments		SL		RL		DWS		STI
		cm	RC (%)	cm	RC (%)	mg	RC (%)	
S_0	T_1	6.40 a	-	6.00 ab	-	8.31 ab	-	-
	T_2	4.82 b-e	-	5.44 a-e	-	5.48 b-g	-	-
	T_3	5.63 ab	-	5.32 a-f	-	7.51 a-d	-	-
	T_4	4.76 b-e	-	4.91 a-g	-	6.39 a-f	-	-
	T_5	4.89 b-e	-	5.60 a-d	-	6.09 a-g	-	-
S_1	T_1	4.51 c-f	-29.53	5.06 a-g	-15.67	5.93 a-g	-28.64	0.71
	T_2	6.40 a	+32.78	4.98 a-g	-8.46	6.51 a-f	+18.80	1.19
	T_3	4.70 b-e	-16.52	4.76 b-g	-10.53	5.51 b-g	-26.63	0.73
	T_4	5.11 bcd	+7.35	6.50 a	+32.38	8.06 abc	+26.13	1.26
	T_5	3.89 e-j	-20.45	4.64 b-g	-17.14	6.89 a-e	+13.14	1.13
S_2	T_1	4.61 b-e	-27.97	4.42 b-g	-26.33	4.45 c-g	-46.45	0.54
	T_2	4.79 b-e	-0.62	5.75 abc	+5.70	9.37 a	+70.99	1.71
	T_3	4.67 b-e	-17.05	4.47 b-g	-15.98	5.43 b-g	-27.70	0.72
	T_4	4.69 b-e	-1.47	4.70 b-g	-4.28	5.59 a-g	-12.52	0.87
	T_5	3.97 e-i	-18.81	4.71 b-g	-15.89	5.16 b-g	-15.27	0.85
S_3	T_1	4.32 c-g	-32.50	4.35 c-g	-27.50	5.02 b-g	-39.59	0.60
	T_2	5.26 bc	+9.13	5.34 a-f	-1.84	7.49 a-d	+36.68	1.37
	T_3	4.52 c-f	-19.72	4.84 b-g	-9.02	4.91 b-g	-34.62	0.65
	T_4	4.64 b-e	-2.52	4.29 c-g	-12.63	4.96 b-g	-22.38	0.78
	T_5	3.27 g-k	-33.13	4.24 c-g	-24.29	3.94 d-g	-35.30	0.65
S_4	T_1	3.91 e-j	-38.91	3.88 efg	-35.33	4.15 d-g	-50.06	0.50
	T_2	4.47 c-f	-7.26	4.90 a-g	-9.93	6.42 a-f	+17.15	1.17
	T_3	4.11 d-h	-27.00	4.56 b-g	-14.29	5.11 b-g	-31.96	0.68
	T_4	4.40 c-f	-7.56	4.27 c-g	-13.03	4.20 d-g	-34.27	0.66
	T_5	3.90 e-j	-20.25	4.34 c-g	-22.50	3.91 d-g	-35.80	0.64
S_5	T_1	3.14 h-k	-50.94	4.16 c-g	-30.67	3.96 d-g	-52.35	0.48
	T_2	4.45 c-f	-7.68	4.62 b-g	-15.07	6.48 a-f	+18.25	1.18
	T_3	3.53 f-k	-37.30	3.86 efg	-27.44	5.08 b-g	-32.36	0.68
	T_4	4.04 d-i	-15.13	3.86 efg	-21.38	3.39 efg	-46.95	0.53
	T_5	3.03 ijk	-38.04	3.61 g	-35.54	2.76 fg	-54.68	0.45
S_6	T_1	2.74 k	-57.19	3.74 fg	-37.67	2.40 g	-71.12	0.29
	T_2	3.95 e-j	-18.05	3.53 g	-35.11	4.75 b-g	-13.32	0.87
	T_3	2.89 jk	-48.67	3.93 efg	-26.13	4.06 d-g	-45.94	0.54
	T_4	2.98 ijk	-37.39	4.12 d-g	-16.09	4.88 b-g	-23.63	0.76
	T_5	2.50 k	-48.88	3.91 efg	-30.18	4.37 c-g	-28.24	0.72
CV (%)		7.65	-	10.81	-	21.75	-	-

Here, S_0 = 0 dSm^{-1}, S_1 = 5 dSm^{-1}, S_2 = 6 dSm^{-1}, S_3 = 7 dSm^{-1}, S_4 = 8 dSm^{-1}, S_5 = 9 dSm^{-1}, S_6 = 10 dSm^{-1}, T_1 = Road side, T_2 = Railway line, T_3 = River bank, T_4 = Waste dump, T_5 = Grazing land, SL = Shoot length, RL = Root length, DWS = Dry weight $seedling^{-1}$, STI = Salt tolerance index based on DWS

Effects of habitats and salt stress on emergence and plant growth traits of *Calotropis gigantea*

Plants grown under artificially developed saline soil exhibited reduced emergence, growth and developmental traits, where the degree of reduction varied with seeds' habitats and salinity levels (**Table 4**). The least reduction in SEP was found in $S_1 \times T_2$ (3.51%) followed by $S_2 \times T_2$ (21.05%), $S_5 \times T_4$ (21.43%), $S_4 \times T_4$ (28.58%) and $S_6 \times T_4$ (35.72%) compared to their respective control in different levels of salinity (S_1-S_6). Surprisingly, an increment level of SEP (21.43%) was found in $S_1 \times T_4$ treatment, while $S_2 \times T_4$ showed no relative change (0.00%). There were no significant interaction effects between salinity levels and habitat on MET, although the higher level of salinity generally increased the time required for emergence. Regarding growth traits, the lowest values for LN $plant^{-1}$ (3.33), single leaf area (2.47 cm^2), SL (3.42 cm), RL (3.50 cm) and DWP (0.052 g) were recorded under high saline conditions in ($S_6 \times T_5$), ($S_5 \times T_5$), ($S_6 \times T_5$), ($S_6 \times T_1$) and ($S_5 \times T_5$) treatment combinations, respectively. However, the reduction levels were lowest in $S_1 \times T_2$ (3.67%) for LN plant^{-1}, $S_2 \times T_2$ (59.38%) for single leaf area, $S_3 \times T_2$ (35.17%) for SL, $S_2 \times T_4$ (2.72%) for RL and $S_2 \times T_2$ (59.73%) for DWP. Besides, there were some treatment combinations such as $S_3 \times T_4$, $S_2 \times T_5$, $S_1 \times T_2$ and $S_4 \times T_2$, showed increasing relative performance for root length (23.25%, 8.82%, 8.80% and 5.54%, respectively). For salt tolerance index, the highest STI value was found in $S_2 \times T_2$ (0.403), followed by $S_1 \times T_2$ (0.384) and $S_3 \times T_2$ (0.310), whereas the lowest STI values were recorded in $S_5 \times T_5$ (0.026), $S_6 \times T_1$ (0.028) and $S_6 \times T_3$ (0.049).

Table 3. Correlation co-efficient (*r*) among the germination and early seedling growth attributes of *Calotropis gigantea* under NaCl-induced salinity.

	GP	MGT	GRI	CVG	TGI	RL	SL	DWS
GP	1							
MGT	-0.3608^{***}	1						
GRI	0.6559^{***}	-0.1164^{NS}	1					
CVG	0.5505^{***}	-0.8492^{***}	0.5203^{***}	1				
TGI	0.8392^{***}	-0.3717^{***}	0.6666^{***}	0.6042^{***}	1			
RL	0.6088^{***}	-0.3579^{***}	0.5335^{***}	0.5453^{***}	0.6435^{***}	1		
SL	0.7797^{***}	-0.2993^{**}	0.5128^{***}	0.4332^{***}	0.7497^{***}	0.603^{***}	1	
DWS	0.6407^{***}	-0.4192^{***}	0.4834^{***}	0.5864^{***}	0.5757^{***}	0.725^{***}	0.6016^{***}	1

Here, GP = Germination Percentage, MGT = Mean Germination Time, GRI = Germination Rate Index, CVG = Co-efficient of velocity of germination, TGI =Timson Germination Index, RL = Root Length, SL = Shoot Length, DWS = Dry weight seedling^{-1}, '^{NS}' = Non-significant, '***' and '**' are significantly different at $P \leq 0.1\%$ and $\leq 1.0\%$, respectively.

Effects of habitats and salinity levels on photosynthetic pigments and proline contents of *Calotropis gigantea* plants

Fig. 2 represents the physiological traits of giant milkweed plants, emerged from the seeds of different habitats and nurtured in seven levels of salinity. At increasing levels of salt concentrations, the maximum reduction of photosynthetic pigments (C_a, C_b, C_{a+b} and C_{x+c}) was found in T_3 habitats' seeds, compared to others. The smallest decrements of C_a (1.50%), C_b (1.09%), C_{a+b} (0.72%) and C_{x+c} (0.82%) were revealed in $S_3 \times T_4$, $S_2 \times T_5$, $S_2 \times T_1$ and $S_2 \times T_1$ treatments, respectively (**Sup. Table 1**). Surprisingly, the level of C_{a+b} was increased by 31.68% in treatment $S_1 \times T_4$ compared to the respective control. Again, a significant increment of C_{x+c} was found in $S_1 \times T_4$ (60.54%), followed by $S_3 \times T_4$ (16.05%) and $S_5 \times T_4$ (1.34%) compared to their control treatment ($S_0 \times T_4$). Regarding of proline contents, the highest level of increment was found in $S_5 \times T_1$ (305.00%), followed by $S_4 \times T_1$ (248.00%) $S_3 \times T_1$ (163.00%) and $S_6 \times T_1$ (117.00%) than those of their relative controls, whereas the greatest reductions were revealed in $S_5 \times T_2$ (41.18%), succeeded by $S_6 \times T_5$ (37.72%), $S_4 \times T_5$ (32.34%), $S_1 \times T_2$ (23.53%), $S_3 \times T_2$ (22.88%) and $S_2 \times T_3$ (19.18%).

Discussion

The abiotic and biotic factors greatly influenced the efficiency of seed germination and seedling emergence traits under climate-driven changes (Lamichhane et al., 2018). Habitat stimulus effects on *C. gigantea* seeds under seven levels of salt concentrations were explored in the current study. The findings revealed that the maternal habitats of collected seeds significantly played an important role in the germination, growth and physiological development of *C. gigantea*, which varied with the changes of salt concentrations. El-Keblawy et al.(2018) and Xu et al. (2021) found that the germination of different habitats' seeds was influenced by both genetic and environmental factors. The maternal growing conditions of seeds in their natural habitats significantly determine the germination and fitness traits in their successive generations (Galloway et al., 2005; Tudela-Isanta et al., 2018). Various germination parameters (GP, MGT, GRI, CVG and TGI) of five different habitats' seeds of *C. gigantea* were evaluated by Latif et al. (2025), and observed that the traits were varied significantly with the sources of seeds. Besides variations of habitats, the salinity problem in coastal regions substantially affects the germination, emergence and growth of plants through the formation of reactive oxygen species (ROS), nutrition and osmotic imbalance and ion toxicity due to the piling up of sodium and chloride ions (Rai et al., 2019; Hussein and Sabbour, 2024). In *C. Procera*, Taghvaei et al. (2012) and Galal et al. (2015) found the superiority of germination traits under the control condition compared to the saline conditions (ranging from -0.01 to -0.15 Mpa $NaCl/CaCl_2$ potentials and 500 to 4000 mg g^{-1} NaCl concentrations, respectively), where the traits were deteriorated with the increment of salt concentrations. It was observed that the time required for the germination of seeds was less in non-saline conditions than in saline one (Ibrahim, 2013; Tsegay and Gebreslassie, 2014; Galal et al., 2015). It means that the higher the salt

Table 4. Effects of different habitats on emergence and plant growth traits of *Calotropis gigantea* under saline conditions

Treatments		SEP		MET		LN Plant^{-1}		Single leaf area		SL		RL		DWP		STI
		%	RC (%)	days	RC (%)	no.	RC (%)	cm^2	RC (%)	cm	RC (%)	cm	RC (%)	g	RC (%)	
S_0	T_1	88.89 a	-	17.57	-	11.67 a	-	36.17 a	-	18.67 ab	-	12.22 a-d	-	2.915 a	-	-
	T_2	63.33 bcd	-	16.99	-	9.00 bc	-	27.72 c	-	18.00 abc	-	10.11 a-e	-	1.532 d	-	-
	T_3	73.33 ab	-	14.75	-	11.56 a	-	32.47 b	-	17.33 a-d	-	15.50 a	-	2.483 b	-	-
	T_4	46.67 d-i	-	15.49	-	7.11 c-f	-	22.22 d	-	13.94 b-e	-	10.28 a-e	-	1.706 cd	-	-
	T_5	66.67 bc	-	16.93	-	10.56 ab	-	31.94 b	-	20.83 a	-	12.25 a-d	-	2.000 c	-	-
S_1	T_1	50.00 c-h	-43.75	29.43	67.50	4.67 g-j	-59.98	7.45 fgh	-79.40	6.00 f-i	-67.86	6.17 b-e	-49.51	0.266 e-h	-90.87	0.091
	T_2	61.11 b-e	-3.51	26.42	55.50	8.67 bcd	-3.67	9.91 ef	-64.25	11.33 d-g	-37.06	11.00 a-e	8.80	0.588 ef	-61.62	0.384
	T_3	53.33 c-g	-27.27	29.57	100.47	6.33 d-h	-45.24	8.06 efg	-75.18	10.33 e-h	-40.39	10.50 a-e	-32.26	0.267 e-h	-89.25	0.108
	T_4	56.67 b-f	+21.43	27.37	76.69	6.67 c-g	-6.19	8.09 efg	-63.59	7.17 f-i	-48.57	9.00 a-e	-12.45	0.475 efg	-72.16	0.278
	T_5	46.67 d-i	-30.00	30.14	78.03	5.00 f-j	-52.65	5.23 g-m	-83.63	11.00 d-h	-47.19	10.00 a-e	-18.37	0.253 fgh	-87.35	0.127
S_2	T_1	36.67 g-l	-58.75	28.03	59.53	4.56 g-j	-60.93	7.20 f-i	-80.09	7.00 f-i	-62.51	6.08 b-e	-50.25	0.191 gh	-93.45	0.066
	T_2	50.00 c-h	-21.05	28.21	66.04	9.00 bc	0.00	11.26 e	-59.38	10.50 e-h	-41.67	6.33 b-e	-37.39	0.617 e	-59.73	0.403
	T_3	40.00 f-k	-45.45	27.28	84.95	6.00 e-i	-48.10	7.22 f-i	-77.76	10.50 e-h	-39.41	7.67 a-e	-50.52	0.304 e-h	-87.76	0.122
	T_4	46.67 d-i	0.00	24.90	60.75	6.22 e-h	-12.52	7.83 fgh	-64.76	7.17 f-i	-48.57	10.00 a-e	-2.72	0.472 efg	-72.33	0.277
	T_5	26.67 j-m	-60.00	29.24	72.71	5.50 e-j	-47.92	4.83 g-m	-84.88	9.00 e-i	-56.79	13.33 ab	8.82	0.227 fgh	-88.65	0.114
S_3	T_1	36.67 g-l	-58.75	28.83	64.09	5.56 e-j	-52.36	4.89 g-m	-86.48	7.33 f-i	-60.74	6.39 b-e	-47.71	0.351 e-h	-87.96	0.120
	T_2	44.44 e-j	-29.83	29.90	75.99	7.67 cde	-14.78	8.04 efg	-71.00	11.67 c-f	-35.17	9.50 a-e	-6.03	0.475 efg	-68.99	0.310
	T_3	36.67 g-l	-49.99	28.94	96.20	5.67 e-j	-50.95	5.12 g-m	-84.23	9.11 e-i	-47.43	6.83 b-e	-55.94	0.368 e-h	-85.18	0.148
	T_4	36.67 g-l	-21.43	29.14	88.12	6.56 d-g	-7.74	7.33 f-i	-67.01	7.75 e-i	-44.40	12.67 abc	23.25	0.386 e-h	-77.37	0.226
	T_5	20.00 lm	-70.00	28.80	70.11	5.50 e-j	-47.92	4.65 h-m	-85.44	8.11 e-i	-61.07	5.33 cde	-56.49	0.186 gh	-90.70	0.093
S_4	T_1	33.33 h-m	-62.50	25.09	42.80	4.33 g-j	-62.90	3.62 j-m	-89.99	5.56 f-i	-70.22	5.33 cde	-56.38	0.154 gh	-94.72	0.053
	T_2	38.89 f-k	-38.59	29.80	75.40	5.33 e-j	-40.78	6.82 f-j	-75.40	8.00 e-i	-55.56	10.67 a-e	5.54	0.335 e-h	-78.13	0.219
	T_3	33.33 h-m	-54.55	26.80	81.69	4.44 g-j	-61.59	4.96 g-m	-84.72	6.83 f-i	-60.59	10.89 a-e	-29.74	0.245 fgh	-90.13	0.099
	T_4	33.33 h-m	-28.58	26.04	68.11	5.00 f-j	-29.68	5.16 g-m	-76.78	8.00 e-i	-42.61	5.63 b-e	-45.23	0.271 e-h	-84.11	0.159
	T_5	26.67 j-m	-60.00	26.04	53.81	4.00 hij	-62.12	3.41 klm	-89.32	6.00 f-i	-71.20	7.00 b-e	-42.86	0.153 gh	-92.35	0.077
S_5	T_1	23.33 klm	-73.75	28.62	62.89	3.67 ij	-68.55	2.98 lm	-91.76	4.67 hi	-74.99	7.67 a-e	-37.23	0.088 h	-96.98	0.030
	T_2	37.78 g-l	-40.34	26.88	58.21	6.00 e-i	-33.33	6.57 g-k	-76.30	5.17 ghi	-71.28	6.17 b-e	-38.97	0.307 e-h	-79.96	0.200
	T_3	33.33 h-m	-54.55	26.60	80.34	3.70 ij	-67.99	3.03 lm	-90.67	7.17 f-i	-58.63	6.17 b-e	-60.19	0.211 gh	-91.50	0.085
	T_4	36.67 g-l	-21.43	27.82	79.60	4.00 hij	-43.74	4.13 i-m	-81.41	5.00 ghi	-64.13	4.63 de	-54.96	0.216 gh	-87.34	0.127
	T_5	16.67 m	-75.00	26.22	54.87	3.33 j	-68.47	2.47 m	-92.27	6.67 f-i	-67.98	6.67 b-e	-45.55	0.052 h	-97.40	0.026
S_6	T_1	23.33 klm	-73.75	26.41	50.31	4.00 hij	-65.72	2.97 lm	-91.79	5.00 ghi	-73.22	3.50 e	-71.36	0.082 h	-97.19	0.028
	T_2	33.33 h-m	-47.37	27.88	64.10	5.00 f-j	-44.44	6.15 g-l	-77.81	4.67 hi	-74.06	5.00 cde	-50.54	0.192 gh	-87.47	0.125
	T_3	26.67 j-m	-63.63	29.10	97.29	4.00 hij	-65.40	3.36 klm	-89.65	6.50 f-i	-62.49	8.75 a-e	-43.55	0.122 gh	-95.09	0.049
	T_4	30.00 i-m	-35.72	26.20	69.14	4.17 hij	-41.35	3.75 j-m	-83.12	5.75 f-i	-58.75	8.00 a-e	-22.18	0.144 gh	-91.56	0.084
	T_5	20.00 lm	-70.00	27.67	63.44	3.33 j	-68.47	2.49 m	-92.20	3.42 i	-83.58	4.67 de	-61.88	0.077 h	-96.15	0.039
CV (%)		13.67	-	8.45	-	12.15	-	10.94	-	22.33	-	29.28	-	20.86	-	-

Here, S_0 = 0 dSm^{-1}, S_1 = 5 dSm^{-1}, S_2 = 6 dSm^{-1}, S_3 = 7 dSm^{-1}, S_4 = 8 dSm^{-1}, S_5 = 9 dSm^{-1}, S_6 = 10 dSm^{-1}, T_1 = Road side, T_2 = Railway line, T_3 = River bank, T_4 = Waste dump, T_5 = Grazing land, SEP = Seedling emergence percentage, MET = Mean emergence time, LN plant^{-1} = Leaf number plant^{-1}, , SL = Shoot length, RL = Root length, DWP = Dry weight plant^{-1}, RC = Relative change over the respective control and STI = Salt tolerant index based on DWP.

concentration, the longer the germination time until seeds develop tolerance and start to germinate by overcoming the germination time delay. We found that all habitats' seeds provided higher levels of GP, GRI, CVG and TGI, but reduced MGT under the control condition (S_0) than salt stress (**Table 1**).

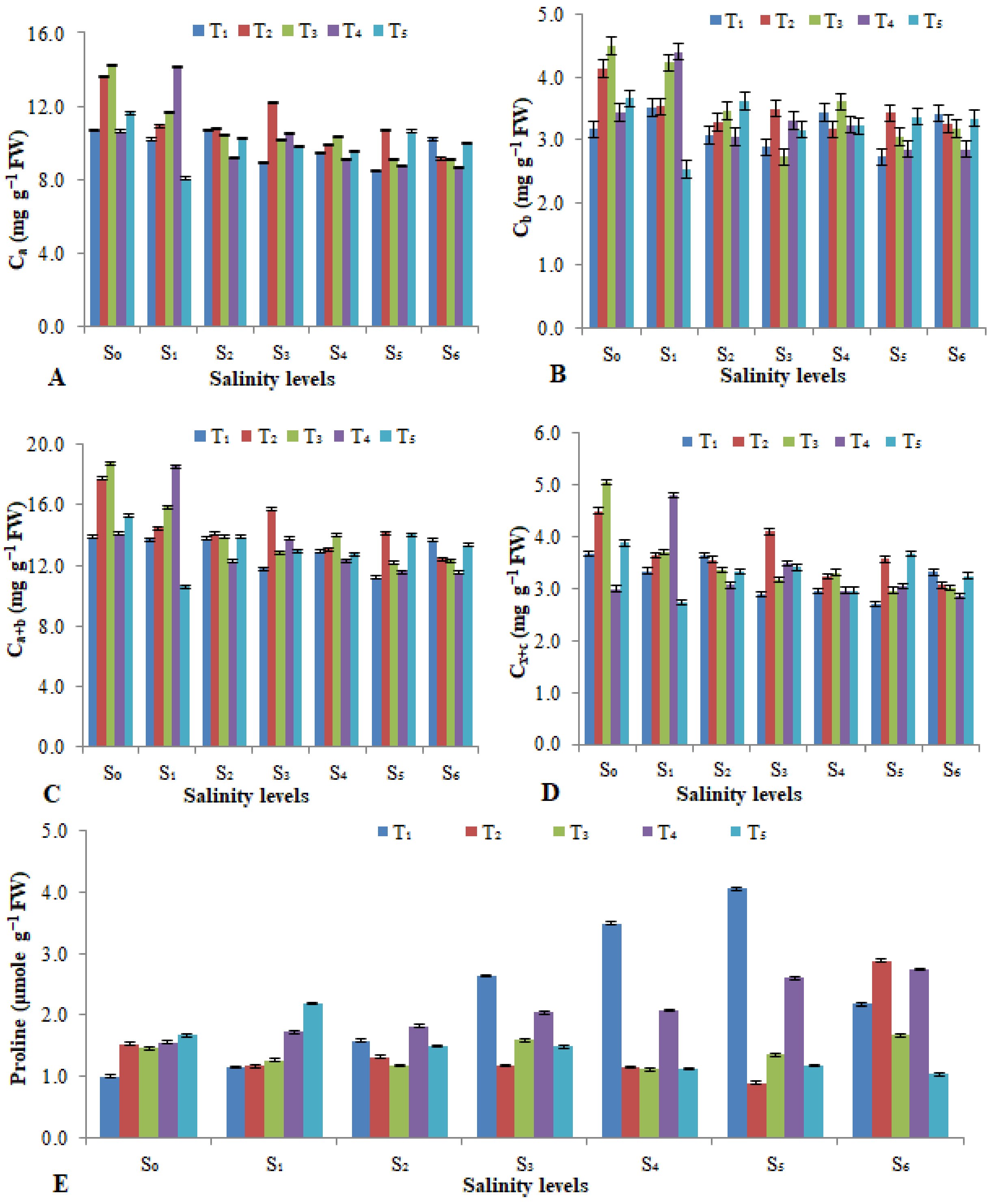

Fig. 2. Photosynthetic traits and proline contents of *C. gigantea* influenced by salinity and various habitats' seeds, where A: Chlorophyll a, B: Chlorophyll b, C: Total chlorophyll, D: Total carotenoids and E: Proline contents, S_0 = 0 dS m^{-1}, S_1 = 5 dS m^{-1}, S_2 = 6 dS m^{-1}, S_3 = 7 dS m^{-1}, S_4 = 8 dS m^{-1}, S_5 = 9 dS m^{-1}, S_6 = 10 dS m^{-1}, T_1 = Road side, T_2 = Railway line, T_3 = River bank, T_4 = Waste dump, T_5 = Grazing land, C_a = Chlorophyll a, C_b = Chlorophyll b, C_{a+b} = Total Chlorophyll, C_{x+c} = Total carotenoids .

However, seeds from the railway line habitat (T_2) revealed the lowest reduction of GP, GRI, CVG and TGI than the other habitats' seeds. This could have happened due to their comparatively higher adaptation capacity to stress conditions than other habitats' seeds, acquired through ample sunlight and minimal competition for nutrients in their growing environment. The above mentioning stidies were in parallel with our findings relevent to various germination traits of *C. gigantea*.

The study revealed that the increasing levels of salt concentrations (S_1-S_6) considerably decreased the early seedling growth traits, i.e. SL, RL and DWS (**Table 2**). This phenomenon happens due to ionic imbalances, which are responsible for reduced photosynthesis and stunted plant growth in salt stress conditions (Taghvaei et al., 2012; Elhag, 2023; Islam et al., 2024). Among the different sources (habitats) of seeds, the lower decrement of SL, RL and DWS and higher STI were observed in railway line habitats' seeds (T_2) under salinity stress. This supremacy of railway line habitats' seeds (T_2) might occur because of their resilient characteristics against the abiotic stress, inherited from their mother plants (Latif et al., 2025).

Correlation analysis revealed a negative relationship between MGT and other germination traits. The most negative association was found in relationship between MGT and other germination traits. The most negative association was found in MGT and DWS at $P \leq 0.001$ (r = -0.4192). The more time required for germination could be responsible for decreasing the accumulation of photosynthates in the plant's body and reducing their biomass production. On the other hand, positive correlations among the other traits (GP, GRI, CVG, TGI, RL, SL and DWS) indicated the synergistic association among them (**Table 3**). These findings are in parallel with the results of Islam et al. (2024) and Sinthy et al. (2024) for *C. gigantea*.

Different habitats' seeds of *C. gigantea* grown on artificially developed saline soil faced gradual harmful effects on the emergence and plant growth traits with the increasing level of salt concentrations (**Table 4**). The variations of reduction in emergence and post-emergence parameters influenced by different habitats seeds were previously found in *Saussurea salsa*, *Conyza sumatrensis* and *Calotropis gigantea* species (Xu et al., 2021; Latif et al., 2025). We observed that the seedling emergence and morphological growth traits of *C. gigantea* were reduced under saline conditions, and it was severe at 10 dS m^{-1}. Guo et al. (2020) found that higher salt concentration notably lowers the seedling emergence rates of *Suaeda salsa*, with a 26% and 46% decrease when grown in 200 mM and 400 mM of NaCl concentrations, respectively. The increasing level of salinity is also responsible for the reduction of SL, RL and DWP (Jaleel et al., 2008; Islam et al., 2024). Railway line habitats' seeds showed higher SEP, LN, single leaf area, SL, RL and DWP in artificially developed saline soils over the others. This supremacy of railway line habitats' seeds may have been governed by the high salt tolerance capacity of *C. gigantea* due to its genetic makeup and adaptive ecological restoration. Besides, the exacerbation of MET in saline conditions over the control for all habitats' seeds was due to the salt stress conditions, which delayed the normal physiological processes for seed emergence. Other than the *Calotropis gigantea* (Islam et al., 2024), the lower number of leaves and leaf area was also found in cereals (Matsuura et al., 2005) as well as in ornamental plants (Jaleel et al., 2008) under saline conditions. These outputs support our findings.

Gradual reduction of photosynthetic pigments (C_a, C_b, C_{a+b}, and C_{x+c}) and increment of proline content with the increasing level of salt concentrations (S_1-S_6) confirmed the adverseness of salt stress in *C. gigantea* physiology (**Fig. 2**). Salinity leads to oxidative stress by generating ROS and accumulation of proline contents, which reduces the photosynthetic pigments and plant productivity (Soliman et al., 2020; Singh et al., 2022; Hussein and Orabi, 2025). As well as, maternal habitats determine the levels of chlorophyll, total carotenoids, proline contents and leaf color of emerged plants (Brand, 1997). It was observed that variation of habitats had significant effects on C_a; however, the effect was not significant for C_b (Mohamed et al., 2020). The highest reduction of C_a, C_b, C_{a+b}, and C_{x+c} in river bank habitats' seeds (T_3) at 6, 7, 9 and 10 dS m^{-1} indicated its high susceptibility to salt stress. The increment of total carotenoids at 5, 6, 7 and 9 dS m^{-1} salt concentrations was an insight that revealed the adjustability of waste dump habitats' seeds (T_4), which might be led by their inherited characteristics from their mother plants. Similar to our results, the reduction of chlorophyll contents was observed in *C. procera* under 100% seawater (Ibrahim, 2013) and in *Catharanthus roseus*, where total chlorophyll was reduced by 14% and 34% under low and high salinity, respectively (Jaleel et al., 2008). In our experiment, the greater amount of proline was revealed by the road side habitats' seeds (T_1) at 9 dS m^{-1}, trailed by 8, 7, 10 and 6 dS m^{-1}, indicating its vulnerability to stress conditions. Thus, the plants living in road side habitat tried to survive by releasing higher proline content. However, the lowest level of proline revealed by railway line habitats' seeds (T_2) at 9, 7 and 5 dS m^{-1} salt levels validated its higher level of resistance capacity under salt stress conditions. Salt stress triggers to accumulate the proline in plant cells to combat the harshness of unfavorable conditions (Soliman et al., 2020). Ibrahim (2013) found that the proline contents were enhanced by 194-307% in *C. procera* and *Suaeda aegyptiaca* under saline conditions. Similar to that, Islam et al. (2024) observed a wide range of proline increments (170.27-629.73%) in *C. gigantea* when subjected to 6-12 dS m^{-1} salt stress. These results are in parallel with the present findings.

The plant communities are gradually decreasing due to rapid climate change, increasing levels of salinity, and various anthropogenic activities, which leads to diminish species richness in coastal areas (Woods et al., 2020). To restore the soil health, improve biodiversity and enhance ecosystem functions, it is indispensable to revegetate degraded saline areas with salt tolerant and economically valuable plants (Xu et al., 2016; Villagra et al., 2021). Native salt tolerant plants can thrive easily the harsh environmental conditions of coastal regions. Begam et al. (2017) observed the usefulness of four native grasses (*Porteresia coarctata*, *Myriostachya wightiana*, *Paspalum vaginatum*, and *Sporobolus virginicus*) to manage the salty region, which showed 80–90% survival in degraded mudflats of the Sundarbans mangrove forest. In this point of view, as a native key taxon to the Indian subcontinent, including Bangladesh, *C. gigantea* may serve as an effective promising genetic resource for ecological restoration and coastal revegetation (Barbosa et al, 2014).

In the present investigation, we were unable to conduct molecular analysis due to funding limitations. Based on the availability of species, the seed populations were collected from five natural habitats, maintaining 1.76 to 21.08 km geographic distance, which might affirm the genetic variations among the populations, and it was supported by Islam et al. (2019), who revealed greater genetic diversity of eleven *C. gigantea* population in Bangladesh. Overall, the differential responses among diverse habitats' seeds indicated varying degrees of salt tolerance, where the railway line habitats' seeds exhibited the highest resilience. This finding might be effective in understanding the adaptive mechanism of *C. gigantea* under salt stress conditions for ecological restoration and sustainable land management in coastal regions.

Conclusion

The various natural habitats (road side, grazing land, river bank, railway line and waste dump) seeds of *C. gigantea* showed diverse variation in tolerance to salt stress conditions (S_1-S_6). It was revealed that with the increment of salinity level, the germination, emergence and seedling growth traits were decreased in all the habitats' seeds. However, based on the studied traits and salt tolerance index (STI), the best performance was occupied by railway line habitats' seeds (T_2), followed by waste dump (T_4), riverbank (T_3), and grazing land (T_5) under the saline conditions. Therefore, the seeds of *C. gigantea* from railway line habitats could be used as a regeneration material for restoring the vegetation in saline areas. We suggest further molecular research using more natural populations of *C. gigantea* from different habitats for observing greater insight related to the germination, growth, and physiological responses against salinity stress of this key plant.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

Md. Rabiul Islam: conceptualization, funding acquisition, supervision, project administration, final draft preparation, reviewing and editing. Md. Azgar Ali Liton: methodology, data collection, interpretation, formal analysis, reviewing and editing. Md. Abdul Momin: initial draft preparation, reviewing and editing. Md. Abu Hasan: validation, visualization, reviewing and editing. Most. Mohoshena Aktar: project administration, reviewing and editing. A.K.M.M. Bari Chowdhury: co-upervision, reviewing and editing. Md. Hafizur Rahman Hafiz: visualization, reviewing and editing.

Conflicts of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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