



RESEARCH ARTICLE

Screening pigeon pea (*Cajanus cajan* L.) genotypes for salinity tolerance

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Abstract

Pigeon pea is a major pulse, whose productivity is significantly affected by salinity. Salinization of soil is a condition, in which ions like Na⁺, Cl⁻, Ca²⁺, Mg²⁺ accumulate in the soil through irrigation water. Salinity alters the metabolic pathway of the plant, due to accumulation of various salts and thereby contributing to yield loss. In this research, salinity stress in pigeon pea genotypes was imposed in all the genotypes at a concentration of 50, 100, 150 and 200 mM NaCl. Screening was carried out by observing early growth parameters like germination percentage, root length, shoot length, fresh weight, dry weight, total seedling length and vigour index. The pigeon pea genotypes G₅ (ICP 2947), G₂₀ (ICP 14961), G₃ (ICP 7380), G₂ (ICP 7024) and G₁₀ (ICP 16184) were identified as salt stress tolerant genotypes. They exhibited tolerance, despite subjecting the seedlings to varying salt concentration levels. The tolerance may be developed due to the osmotic regulation, ionic homeostasis or activation of Salt-Overly-Sensitive (SOS) pathway. As a result, the effect of induced salinity in pigeon pea genotypes would aid in selection of suitable lines for challenging saline environments. This research highlights the impact of salinity in early growth stages of pigeon pea and the response will differ with different stages of growth.

Keywords: germination percentage; osmotic regulation; pigeon pea; salinity; vigour index

Introduction

Soil salinization is one of the abiotic stresses that has a devastating impact on crop yield worldwide. Soil salinization has emerged as a major cause of some socio-economic and ecological concerns like crop failure. As per the Global map of Salt Affected Soils (GSAS) released by Food and Agriculture Organization (2024), various soil related parameters were tested across 2.5 lakh locations. This data reveals the magnitude of stress posed by excessive salt accumulation. Around 10 % of both irrigated and rainfed crop land is affected by salinity (1). The Soil Science Society of America defines 'saline soil' as non-sodic soil which contains sufficient number of soluble salts. The uptake of K⁺ ions from the soil water is reduced and the uptake of Na⁺ and Cl⁻ ions increases (2). Hence, this affects the ion homeostasis and can potentially reduce the crop yield and quality of many high-value crops.

Pigeon pea (*Cajanus cajan* L.) is an important food legume crop mostly cultivated in tropical and subtropical regions of the world (3). Leguminous crops are considered suitable for increasing bio-productivity and reclaiming marginal soils. Salinity stress has a negative impact on pigeon pea (*C. cajan* L.), a significant legume crop that is grown to meet the protein needs of the human population. Unlike cereals, pigeon peas are

naturally susceptible to salt stress (4). Because of particular ion toxicity, nutritional imbalance and disrupted hormone interactions, salinity produces osmotic imbalance to tissues, that impacts overall growth and development of plants.

The reduction in protein and starch production occurs due to higher salt concentration. Protein degradation is caused by oxidative stress, that disrupts the metabolic pathways. Starch production is lowered due to interruptions in the carbohydrate metabolic pathway. In grain legumes, salinity also causes more leaf necrosis and chlorosis, which results in lower photosynthesis and leaf senescence.

In salinity stress studies, initial stress is often imposed by soaking seeds in distilled water or NaCl solutions of varying concentrations (5). Initial stress imposition, like applying a controlled salinity level, allows researchers to study salinity tolerance by directly observing and quantifying plant responses to the stress, revealing mechanisms of tolerance and sensitivity (6). The effects of high salinity on plants can be observed at the seedling stage and adult stages, because the death of plants and/or decreases in productivity.

The salt tolerance capability of a plant differs among species and development stages, determining its capacity to

endure and finish its life cycle in saline environments (7). Utilising the genetic heterogeneity found in the germplasm, salt-tolerant lines can be found. The production of saline-tolerant cultivars will be a suitable strategy for reducing yield losses under salinity stress conditions (8). The first and most crucial phases that are negatively impacted by salinity are seed germination and seedling survival (9). Increases in NaCl have been shown to decrease dry weight, shoot and root length and germination percentage in a number of monocot and dicot crops (10). The major impact of excessive salt concentrations is the buildup of Na⁺ and Cl⁻ ions in plant tissues (11).

The primary goal for simple crop establishment to increase yield under salt stress, especially in pigeon pea, would be to screen and select genotypes that are salt tolerant at the germination and seedling stages (12). In order to explore the potential for salt tolerance, the current study was conducted by evaluating the genotypes of pigeon pea in terms of morphological parameters at various salinity regimes, ranging from 0 to 200 mM NaCl concentration at the germination and seedling stage.

Materials and Methods

Experimental site and design

The experiment was conducted in the laboratory of Genetics and Plant Breeding, in association with Seed Science and Technology, SRM College of Agricultural Sciences, SRM Institute of Science and Technology at Chennai, Tamil Nadu. The 99 pigeon pea genotypes were sourced from International Crop Research Institute for Semi-Arid Tropics (ICRISAT, Hyderabad). The genotypes were evaluated in the seedling stage by subjecting them to salinity stress.

The treatments are applied to all the 99 genotypes, to study the salinity tolerance among the genotypes. The treatments encompass a control group with dry seeds as T₀ and hydropriming was done using distilled water for T₁. At various concentrations of NaCl, the treatments T₂, T₃, T₄, T₅ denote 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl respectively. The sterilized sand is filled in plastic trays, arranged according to the Factorial Completely Randomized Design (FCRD) with four replicates.

The genotypes are exposed to initial stress by soaking the seeds for 3 hr in distilled water and NaCl solutions according to the treatment. For each genotype, 25 seeds are soaked for every treatment. The sterilized sand trays were subjected to solutions of different NaCl concentration and the trays were irrigated with NaCl solution every day. In the due course of the experiment, the germination data was recorded, which helped in deciphering the seed characters and their response to salt stress at the seedling stages. The parameters observed for studying the salt stress tolerance in this research include root length, shoot length, germination percentage, fresh weight, dry weight and vigour index.

Germination percentage

The experiment followed International Seed Testing Association (ISTA) guidelines (13). The daily germination data was noted for every genotype. The germination (%) was calculated on the 10th day of emergence, when the final counts were taken.

$$\text{Germination \%} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

Root length (cm)

On the 10th day of emergence, 10 seedlings in healthy condition (seedlings with properly developed root and shoot) were taken out from each replication, for measuring the root length. It is denoted in 'cm'. The root length was measured from the tip of the primary root of each healthy seedling.

Shoot length (cm)

On the 10th day of emergence, 10 seedlings in healthy condition were taken out from each replication, for measuring the shoot length. It is denoted in 'cm'. The shoot length was measured from the tip of the leaf to end of the shoot of each healthy seedling.

Fresh weight (mg seedling⁻¹)

On the 10th day of emergence, 10 healthy seedlings were taken out from each replication, for computing the fresh weight. It is denoted in 'mg seedlings⁻¹ (based on 10 seedlings)'. The seedlings were weighed as soon as they were uprooted from the sand tray. This would reveal the initial root and shoot biomass of the seedling.

Dry weight (mg seedling⁻¹)

The 10 healthy and uprooted seedlings were shade dried for 24 hr to compute the final root and shoot biomass. The dried seedlings were kept in hot air oven for 20 hr, at 70 °C and then weighed in a weighing balance. It is denoted in 'mg seedlings⁻¹ (based on 10 seedlings)'.

Total seedling length (cm)

On the 10th day of emergence, ten healthy seedlings were taken out from each replication, for computing the total seedling length. The total seedling length is calculated by adding the values of root length and shoot length. It is expressed in 'cm'.

Seedling vigour index

Vigor index values were determined using the given formula and the average values were represented (14).

Vigour index = Germination percentage × Total seedling length (cm)

Statistical analysis

Means and standard deviation were calculated for all traits. The experiment was set up using a Factorial Completely Random Design (FCRD). An analysis of variance (ANOVA) was carried out and comparison was checked using Duncan's Multiple Range Test (DMRT). Mean differences were deemed significant when the *p*-value was 0.05. The SPSS version 16.0 was used to carry out the Statistical analysis (SPSS Inc., Chicago, USA).

Results

Germination percentage

Application of NaCl at increasing concentrations significantly reduced germination percentage across pigeon pea genotypes. The maximum germination percentage recorded at 50 mM NaCl, was observed in G₅ (84 %), followed by G₂₀, G₃, G₂ and G₁₀. When the concentration was 100 mM NaCl, the genotypes G₅ (82 %),

subsequently G₂₀, G₃, G₂ and G₁₀ showed the highest germination percentage. At 150 mM NaCl, the genotypes G₅ (75 %), succeeded by G₃, G₂₀, G₂ and G₁₀ recorded the highest germination percentage. At the maximum concentration of 200 mM NaCl, supreme germination percentage was observed in the genotype G₅ with 68 % germination (Table 1). These results indicate negative correlation between NaCl concentration and germination percentage in pigeon pea genotypes.

Root length (cm)

The application of salinity significantly altered the root length of the seedlings at different concentration of NaCl. The genotype G₅ (28.9 cm) exhibited maximum root length at 50 mM NaCl and it was followed by G₂₀, G₂, G₃ and G₁₀. The highest root length was recorded in G₅ (25.6 cm) and subsequently G₂₀, G₃, G₂ and G₁₀ at 100 mM NaCl show higher root length. When the concentration was 150 mM NaCl, G₅ (21.3 cm) again recorded the maximum root length. At 200 mM NaCl, the genotypes G₅ (17.0 cm), followed by G₂₀, G₃, G₂ and G₁₀ showed topmost root length (Fig. 1). The result demonstrates that root length is reduced with increase in concentration of NaCl.

Shoot length (cm)

Shoot length was significantly reduced with increasing NaCl concentration. When the seedlings are observed at 50 mM NaCl, G₅ (14.3 cm) recorded highest shoot length, followed by G₂₀, G₂, G₃ and G₁₀. At concentration of 100 mM NaCl, the largest shoot length was observed in G₅ (12.7 cm), succeeded by G₂₀, G₃, G₂ and G₁₀. When the concentration was 150 mM NaCl, G₅ (10.5 cm) exhibited maximum shoot length, followed by G₂₀, G₂, G₃ and G₁₀. At 200 mM NaCl, notably highest shoot length was noted in G₅ (8.1 cm) (Fig. 2). Thus, shoot length declined progressively with increasing salinity.

Fresh and dry weight (mg 10 seedlings⁻¹)

The imposed salinity across all the genotypes exhibited significant effects on both fresh weight and dry weight of the uprooted seedlings by exposing the seedlings to varied concentration from 0-200 mM NaCl. At maximum concentration (200 mM NaCl), the highest fresh weight was seen in G₅ (698.23), followed by G₂₀ (686.74), G₃ (664.98), G₂ (657.89) and G₁₀ (632.76) (Fig. 3). At 200 mM NaCl, the top most dry weight was observed in G₅ (139.81), G₂₀ (138.39), G₃ (137.93), G₂ (136.48) and G₁₀ (135.78) where the genotype G₅ showed highest dry weight among all the other genotypes (Fig. 4). The root and shoot biomass are highly influenced by imposed levels of salinity.

Seedling vigour index

Seedling vigour index declined with increasing NaCl concentrations. At a concentration of 50 mM NaCl, 100 mM NaCl, 150 mM NaCl and 200 mM NaCl, the genotype G₅ consistently exhibited higher vigour index values (Table 2). The genotype G₅ recorded vigour index value of 1567, even when seedlings are exposed to 200 mM NaCl, followed by G₂₀ (1549), G₃ (1508), G₂ (1459) and G₁₀ (1417). A higher vigour index indicates better tolerance to environmental stress, suggesting that G₅ is the most salt-tolerant genotype.

Discussion

Germination is a process that initiates the growth of a seed into a

developed plant (15). Imbibition is a process that primarily decides early emergence of seedlings and high vigour among pulses (16), because they have hard seed coat. Salinity impacts the seed germination due to toxicity of salts, osmotic stress and ionic toxicity (17, 18). Several factors contribute to delayed germination of pulses under stress (19). The experiment conducted reveals that germination percentage reduces drastically with increase in exposure to salinity levels. Although the genotypes G₅, G₂₀, G₃, G₂ and G₁₀ continued to perform well under 200 mM NaCl, the germination percentage is decreased due to increase in solute concentration (20). Salt stress lowers the osmotic potential of soil solution and affects the embryo by Na⁺ and Cl⁻ toxicity (21). It is stated that poor seedling emergence may also be due to the hypocotyl weakness (22). The findings suggest that, germination percentage may be altered because of reactive oxygen species production, cell damage and destruction of bio-film structure (23).

When seeds are sown, roots will be the first emerging organ (24). Roots are highly prone to salinity stress (25). Salinity inhibits the elongation of root portion, thereby reducing the surface area of roots by around 60 %, which will in turn affect the uptake of nutrients. So, the normal growth potential of the plant is impacted negatively. The root length significantly increased upon hydropriming (T₁) in all genotypes. Root length declined significantly with increasing NaCl concentrations (26). It is reported that root anatomy is negatively impacted due to salt stress. The treatments with 150 mM NaCl and 200 mM NaCl showed a steep decrease in root length. In G₁₀, the control had 16.5 cm root length and it increased to 18.1 cm through hydropriming. But when it was exposed to 200 mM NaCl, the root length reduced to 8.9 cm which is almost 50 % of the root length of control group (T₀). G₅ and G₂₀ exhibited tolerance towards salinity, in terms of root length. The root injury is mainly due to differences in water potential and ionic imbalance. The results correlate root growth with levels of salinity imposed. It allows us to understand the need and develop cultivars with wider adaptability (27).

The shoot length was reduced across treatments. It is reported that length of the shoot was reduced, due to the decrease in number of elongated cells (28). The addition of Na⁺ and Cl⁻, decreased the K⁺ content in the shoot and thus affected shoot length (29). The genotypes G₃, G₅ and G₄₈ showed good results, among other genotypes. It is also observed that there was 50 % reduction in the shoot length of T₅ (200 mM NaCl), when compared to control group (T₀). The hydropriming treatment (T₁) was effective and it increased the shoot length significantly. Thus, hydropriming is positively correlated with shoot length, because imbibed water would facilitate elongation of the cells more rapidly than the control group (30). Conversely, imposed salinity is negatively correlated with the shoot length. High saline levels will impact the internode elongation and limits the emergence of tissues, due to ionic toxicity under salinity conditions (31).

The biomass of the seedling will be fluctuating, in relation to the imposed stress (32). It is noted that the seedling height is positively correlated with the biomass of the seedling (33). As reported, nutrient stress and ionic toxicity decreased the shoot fresh weight (34). The genotype G₅ had a fresh weight of 698.25 mg 10 seedlings⁻¹ at 200 mM NaCl, whereas it weighed 1397.46 mg 10 seedlings⁻¹ when seeds were hydro primed. This downfall in biomass is attributed by increased salinity levels. The least fresh weight was observed in G₇₅ (302.6 mg 10 seedlings⁻¹),

Table 1. Effect of salinity stress on germination percentage of pigeon pea genotypes

Genotypes (G)	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
G ₁	72 ± 0.44	77 ± 0.55	62 ± 0.21	60 ± 0.59	59 ± 0.38	48 ± 0.41
G ₂	82 ± 0.06	83 ± 0.88	80 ± 0.60	78 ± 1.30	72 ± 1.00	66 ± 0.70
G ₃	83 ± 0.11	86 ± 0.76	82 ± 0.17	81 ± 0.17	73 ± 0.65	67 ± 0.84
G ₄	79 ± 0.67	81 ± 0.39	75 ± 0.48	74 ± 0.78	67 ± 0.30	62 ± 0.19
G ₅	85 ± 0.61	88 ± 1.44	84 ± 0.11	82 ± 0.86	75 ± 0.77	68 ± 1.13
G ₆	73 ± 0.30	77 ± 0.71	71 ± 0.63	66 ± 0.92	61 ± 0.64	47 ± 0.10
G ₇	73 ± 0.27	73 ± 0.57	69 ± 0.52	63 ± 0.90	61 ± 0.15	56 ± 0.86
G ₈	78 ± 0.32	80 ± 0.82	72 ± 1.13	71 ± 0.92	65 ± 0.24	59 ± 0.64
G ₉	72 ± 1.15	78 ± 0.74	70 ± 0.81	65 ± 0.20	56 ± 0.93	51 ± 0.52
G ₁₀	81 ± 0.94	83 ± 0.93	79 ± 0.94	77 ± 1.26	70 ± 0.50	65 ± 0.77
G ₁₁	74 ± 0.23	74 ± 1.18	60 ± 0.45	59 ± 0.34	58 ± 0.39	57 ± 0.83
G ₁₂	73 ± 0.37	78 ± 0.85	62 ± 0.93	61 ± 0.46	55 ± 0.58	49 ± 0.67
G ₁₃	73 ± 0.82	75 ± 0.84	71 ± 0.05	66 ± 1.01	58 ± 0.57	53 ± 0.85
G ₁₄	75 ± 0.66	76 ± 0.52	68 ± 0.79	65 ± 0.40	56 ± 0.69	42 ± 0.13
G ₁₅	75 ± 0.64	75 ± 0.31	61 ± 0.98	60 ± 0.04	55 ± 0.88	52 ± 0.55
G ₁₆	74 ± 0.15	75 ± 0.74	67 ± 0.66	60 ± 0.27	58 ± 0.39	57 ± 0.52
G ₁₇	73 ± 0.79	73 ± 0.77	71 ± 0.19	70 ± 0.60	61 ± 0.19	55 ± 0.86
G ₁₈	71 ± 1.09	76 ± 0.08	67 ± 1.12	65 ± 0.27	54 ± 0.26	48 ± 0.34
G ₁₉	79 ± 1.32	80 ± 1.20	74 ± 0.23	73 ± 0.67	66 ± 0.09	60 ± 0.10
G ₂₀	84 ± 0.71	87 ± 0.12	83 ± 0.93	81 ± 0.69	74 ± 0.38	68 ± 0.16
G ₂₁	72 ± 0.83	79 ± 0.40	66 ± 0.22	65 ± 0.97	54 ± 0.22	52 ± 0.14
G ₂₂	72 ± 0.78	73 ± 1.14	63 ± 1.05	63 ± 0.06	55 ± 0.54	54 ± 0.17
G ₂₃	74 ± 0.08	75 ± 0.20	69 ± 0.94	62 ± 0.63	61 ± 0.02	48 ± 0.56
G ₂₄	74 ± 0.18	76 ± 1.27	61 ± 0.52	60 ± 0.33	59 ± 0.68	56 ± 0.50
G ₂₅	76 ± 0.10	73 ± 0.55	62 ± 0.97	61 ± 0.27	58 ± 0.26	51 ± 0.10
G ₂₆	71 ± 0.77	76 ± 0.93	67 ± 0.77	66 ± 0.38	59 ± 0.34	49 ± 0.80
G ₂₇	73 ± 1.12	73 ± 0.05	61 ± 0.95	70 ± 0.83	64 ± 1.02	46 ± 0.27
G ₂₈	73 ± 0.75	77 ± 1.15	71 ± 1.01	70 ± 0.83	63 ± 0.88	48 ± 0.65
G ₂₉	71 ± 0.12	76 ± 0.90	63 ± 0.00	61 ± 0.44	58 ± 0.00	56 ± 0.76
G ₃₀	73 ± 0.50	77 ± 1.05	70 ± 1.02	60 ± 0.39	64 ± 1.02	46 ± 0.56
G ₃₁	77 ± 1.02	73 ± 1.04	70 ± 0.76	61 ± 0.10	60 ± 0.08	48 ± 0.65
G ₃₂	73 ± 0.42	73 ± 1.04	69 ± 0.96	67 ± 0.09	58 ± 0.12	55 ± 0.62
G ₃₃	74 ± 0.45	75 ± 0.31	71 ± 0.87	64 ± 0.61	63 ± 0.92	57 ± 0.58
G ₃₄	76 ± 0.23	73 ± 0.25	62 ± 0.15	61 ± 0.50	56 ± 0.19	53 ± 0.47
G ₃₅	76 ± 0.08	78 ± 0.21	67 ± 0.87	62 ± 0.49	60 ± 0.41	49 ± 0.58
G ₃₆	75 ± 0.77	74 ± 0.48	63 ± 0.56	62 ± 0.02	60 ± 0.78	45 ± 0.58
G ₃₇	73 ± 0.35	76 ± 0.03	71 ± 0.17	63 ± 0.99	55 ± 0.82	48 ± 0.07
G ₃₈	72 ± 0.10	79 ± 0.73	62 ± 0.49	68 ± 0.19	56 ± 0.70	52 ± 0.30
G ₃₉	73 ± 0.42	77 ± 0.16	70 ± 1.02	68 ± 0.30	62 ± 0.72	58 ± 0.22
G ₄₀	76 ± 0.75	78 ± 1.19	65 ± 0.66	63 ± 0.79	57 ± 0.45	47 ± 0.64
G ₄₁	73 ± 1.07	74 ± 0.08	67 ± 0.68	64 ± 0.54	62 ± 0.49	58 ± 0.91
G ₄₂	76 ± 0.59	74 ± 0.18	65 ± 0.91	67 ± 0.43	55 ± 0.28	51 ± 0.14
G ₄₃	77 ± 0.86	78 ± 1.01	69 ± 0.87	63 ± 0.92	60 ± 0.80	46 ± 0.33
G ₄₄	73 ± 0.45	73 ± 0.12	66 ± 0.65	65 ± 0.42	56 ± 0.23	55 ± 0.41
G ₄₅	73 ± 1.07	78 ± 0.50	67 ± 0.05	66 ± 0.94	63 ± 0.79	54 ± 0.26
G ₄₆	75 ± 0.13	75 ± 0.18	65 ± 0.29	63 ± 0.69	58 ± 0.30	56 ± 0.13
G ₄₇	75 ± 0.08	74 ± 0.25	62 ± 0.82	60 ± 0.24	57 ± 0.37	50 ± 0.63
G ₄₈	81 ± 0.33	82 ± 0.03	78 ± 0.58	77 ± 0.73	69 ± 0.70	64 ± 0.98
G ₄₉	76 ± 0.72	76 ± 0.16	64 ± 0.61	63 ± 0.36	60 ± 0.82	45 ± 0.38
G ₅₀	80 ± 1.22	81 ± 0.11	77 ± 0.52	75 ± 0.71	68 ± 0.56	63 ± 0.06
G ₅₁	74 ± 0.88	78 ± 0.05	68 ± 0.14	61 ± 0.33	57 ± 0.66	52 ± 0.58
G ₅₂	76 ± 1.03	74 ± 0.98	67 ± 1.09	63 ± 0.75	61 ± 0.68	44 ± 0.63
G ₅₃	75 ± 1.07	79 ± 1.02	70 ± 0.71	66 ± 0.07	54 ± 0.00	48 ± 0.13
G ₅₄	77 ± 1.05	78 ± 0.16	68 ± 0.83	64 ± 0.13	60 ± 0.80	46 ± 0.39
G ₅₅	70 ± 0.90	77 ± 0.73	61 ± 0.02	65 ± 0.02	63 ± 0.21	56 ± 0.30
G ₅₆	72 ± 0.17	75 ± 0.26	68 ± 0.44	65 ± 0.02	54 ± 0.59	47 ± 0.05
G ₅₇	76 ± 0.39	79 ± 0.43	65 ± 0.11	64 ± 0.67	55 ± 0.30	50 ± 0.75
G ₅₈	73 ± 0.27	74 ± 0.23	68 ± 0.86	65 ± 0.20	61 ± 0.10	49 ± 0.43
G ₅₉	71 ± 0.43	79 ± 0.91	67 ± 0.50	64 ± 0.65	63 ± 0.73	49 ± 0.47
G ₆₀	75 ± 0.82	76 ± 0.65	70 ± 0.21	69 ± 0.70	55 ± 0.73	50 ± 0.32
G ₆₁	70 ± 0.62	75 ± 0.36	68 ± 0.56	66 ± 0.63	54 ± 0.75	47 ± 0.22
G ₆₂	70 ± 0.02	75 ± 0.92	68 ± 0.42	62 ± 0.13	59 ± 0.66	47 ± 0.45
G ₆₃	73 ± 0.50	75 ± 0.82	66 ± 0.34	62 ± 0.91	59 ± 0.16	54 ± 0.53
G ₆₄	74 ± 0.60	74 ± 1.18	70 ± 0.98	68 ± 0.46	62 ± 0.80	55 ± 0.80
G ₆₅	72 ± 0.49	73 ± 1.02	65 ± 0.71	61 ± 0.48	59 ± 0.58	50 ± 0.70
G ₆₆	77 ± 1.10	79 ± 1.26	69 ± 0.07	67 ± 0.66	60 ± 0.24	43 ± 0.67
G ₆₇	75 ± 0.97	74 ± 0.23	69 ± 0.12	64 ± 0.07	60 ± 0.22	46 ± 0.00
G ₆₈	71 ± 0.27	74 ± 0.20	65 ± 0.22	60 ± 0.90	62 ± 0.91	57 ± 0.89
G ₆₉	75 ± 0.41	74 ± 0.91	69 ± 0.47	69 ± 1.06	58 ± 0.24	54 ± 0.64
G ₇₀	77 ± 0.79	78 ± 0.29	67 ± 0.41	66 ± 0.81	59 ± 0.60	54 ± 0.29
G ₇₁	73 ± 0.12	73 ± 0.82	69 ± 0.56	68 ± 0.60	57 ± 0.54	51 ± 0.28
G ₇₂	73 ± 0.45	74 ± 0.48	71 ± 0.41	61 ± 0.52	57 ± 0.14	44 ± 0.43
G ₇₃	73 ± 0.02	75 ± 0.89	68 ± 0.62	67 ± 0.73	57 ± 0.76	46 ± 0.31
G ₇₄	76 ± 0.18	74 ± 0.48	69 ± 0.09	68 ± 0.99	59 ± 0.80	51 ± 0.75
G ₇₅	77 ± 0.34	74 ± 0.83	67 ± 0.07	63 ± 0.24	60 ± 0.31	42 ± 0.59

G ₇₆	76 ± 0.62	75 ± 0.51	71 ± 0.31	70 ± 1.10	57 ± 0.66	45 ± 0.40
G ₇₇	73 ± 0.70	77 ± 0.76	68 ± 0.19	61 ± 0.98	58 ± 0.37	55 ± 0.30
G ₇₈	71 ± 1.18	77 ± 0.79	69 ± 0.96	63 ± 0.79	56 ± 0.69	54 ± 0.90
G ₇₉	74 ± 0.05	75 ± 0.84	68 ± 0.49	66 ± 0.18	57 ± 0.52	49 ± 0.52
G ₈₀	72 ± 0.69	73 ± 1.17	71 ± 0.72	65 ± 1.00	55 ± 0.82	47 ± 0.43
G ₈₁	74 ± 0.13	77 ± 0.94	68 ± 0.05	65 ± 0.33	60 ± 0.27	49 ± 0.58
G ₈₂	71 ± 1.16	79 ± 1.29	66 ± 0.81	64 ± 0.63	59 ± 0.60	53 ± 0.16
G ₈₃	76 ± 1.27	77 ± 0.21	67 ± 0.25	66 ± 0.04	64 ± 0.57	47 ± 0.02
G ₈₄	74 ± 0.18	74 ± 1.01	67 ± 0.27	63 ± 1.05	54 ± 0.28	53 ± 0.54
G ₈₅	72 ± 0.00	77 ± 0.37	70 ± 0.17	68 ± 0.42	61 ± 0.87	50 ± 0.75
G ₈₆	73 ± 0.77	78 ± 1.27	68 ± 0.16	66 ± 0.56	59 ± 0.12	55 ± 0.58
G ₈₇	76 ± 0.54	74 ± 1.21	66 ± 0.47	65 ± 0.95	54 ± 0.33	49 ± 0.75
G ₈₈	77 ± 1.23	76 ± 0.65	70 ± 0.21	62 ± 0.06	59 ± 0.60	52 ± 0.50
G ₈₉	70 ± 0.81	76 ± 1.01	70 ± 1.02	64 ± 0.57	57 ± 0.89	48 ± 0.44
G ₉₀	70 ± 1.10	76 ± 0.72	69 ± 0.87	64 ± 0.63	56 ± 0.65	50 ± 0.75
G ₉₁	77 ± 0.21	75 ± 0.56	61 ± 0.19	60 ± 0.37	55 ± 0.51	53 ± 0.41
G ₉₂	71 ± 0.46	76 ± 1.11	69 ± 0.52	68 ± 0.09	60 ± 0.90	46 ± 0.74
G ₉₃	74 ± 0.08	74 ± 1.21	71 ± 1.18	68 ± 0.86	60 ± 0.53	41 ± 0.60
G ₉₄	75 ± 1.02	76 ± 0.44	69 ± 0.05	68 ± 0.07	54 ± 0.18	50 ± 0.02
G ₉₅	74 ± 0.03	76 ± 0.26	66 ± 1.01	64 ± 0.81	56 ± 0.55	43 ± 0.20
G ₉₆	74 ± 0.88	73 ± 0.55	65 ± 0.82	61 ± 0.17	60 ± 0.41	45 ± 0.26
G ₉₇	72 ± 0.73	79 ± 0.48	62 ± 0.78	61 ± 0.21	54 ± 0.55	51 ± 0.35
G ₉₈	74 ± 0.93	76 ± 1.22	71 ± 0.68	67 ± 0.68	55 ± 0.04	49 ± 0.53
G ₉₉	77 ± 1.26	78 ± 0.08	66 ± 1.10	65 ± 1.08	58 ± 0.24	47 ± 0.29

Seed germination (Mean ± SE, n = 4) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl. The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5 % significance level.

Table 2. Effect of salinity stress on vigour index of pigeon pea genotypes

Genotypes (G)	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
G ₁	2404±1.68	2702±24.74	1857±24.13	1556±17.43	1284±1.80	840±13.19
G ₂	2952±30.25	3245±40.82	2736±14.04	2496±34.01	2081±19.72	1459±3.39
G ₃	3013±28.58	3388±0.00	2829±22.21	2596±4.33	2146±21.76	1508±21.52
G ₄	2765±36.74	3062±32.29	2475±16.09	2264±6.12	1809±29.52	1265±14.29
G ₅	3188±51.03	3502±18.00	2957±17.02	2700±18.37	2273±37.11	1567±11.55
G ₆	2415±31.31	2732±26.90	2108±9.27	1682±4.45	1296±15.96	812±7.80
G ₇	2446±12.49	2606±22.21	2085±0.71	1668±22.70	1359±9.27	957±10.21
G ₈	2691±6.37	2976±31.31	2318±22.82	2109±19.31	1684±5.19	1168±9.96
G ₉	2425±1.68	2799±18.13	2096±30.62	1673±7.92	1207±12.25	892±1.35
G ₁₀	2900±18.74	3220±38.42	2686±2.74	2441±18.37	2002±22.45	1417±0.41
G ₁₁	2423±39.48	2547±0.00	1765±28.21	1503±21.52	1240±0.41	944±14.66
G ₁₂	2371±11.31	2638±19.72	1803±15.39	1534±14.98	1163±5.88	775±7.06
G ₁₃	2402±3.39	2640±43.11	2190±4.45	1757±29.27	1297±6.12	902±8.53
G ₁₄	2492±1.68	2653±23.39	2037±29.27	1682±8.16	1218±16.57	706±6.82
G ₁₅	2483±15.23	2691±10.21	1829±18.62	1562±25.43	1201±11.55	922±6.49
G ₁₆	2442±30.62	2678±0.95	2054±17.43	1579±5.88	1273±3.84	1019±5.88
G ₁₇	2367±15.23	2479±5.88	2046±1.35	1770±21.76	1297±16.70	876±6.12
G ₁₈	2306±33.76	2561±15.64	1930±11.15	1613±23.56	1126±15.39	805±0.71
G ₁₉	2733±30.62	2992±40.82	2412±14.04	2212±6.12	1749±3.39	1212±2.74
G ₂₀	3074±31.31	3445±37.44	2897±28.58	2635±10.90	2205±33.03	1549±19.47
G ₂₁	2287±34.33	2638±22.45	1886±5.19	1610±4.33	1122±14.53	829±5.02
G ₂₂	2326±34.01	2455±14.29	1822±12.94	1576±24.49	1157±10.58	843±12.00
G ₂₃	2449±36.74	2585±14.98	2040±20.17	1583±26.54	1308±2.29	741±8.16
G ₂₄	2464±36.05	2707±6.37	1862±17.68	1582±2.74	1307±1.80	977±7.92
G ₂₅	2592±7.06	2611±0.95	1854±10.21	1583±23.15	1263±0.00	902±10.21
G ₂₆	2322±34.01	2637±23.39	2065±21.76	1746±20.78	1302±14.29	881±3.72
G ₂₇	2478±26.17	2596±20.41	1837±0.71	1847±9.51	1421±1.35	760±10.58
G ₂₈	2329±13.60	2628±17.02	2010±8.16	1708±18.00	1291±20.66	790±1.68
G ₂₉	2322±17.43	2690±34.01	1925±21.76	1601±22.45	1276±0.95	1000±5.88
G ₃₀	2336±27.88	2740±1.80	2086±5.02	1538±18.74	1372±21.11	798±8.86
G ₃₁	2526±2.74	2577±7.06	2149±34.46	1620±6.12	1338±18.37	823±12.62
G ₃₂	2402±7.47	2562±38.42	2142±20.41	1797±25.19	1308±13.92	925±6.37
G ₃₃	2420±31.97	2490±37.44	2204±14.29	1714±2.98	1418±22.70	960±8.16
G ₃₄	2437±14.04	2460±18.37	1796±21.35	1528±4.08	1178±6.37	880±10.45
G ₃₅	2489±17.02	2667±13.60	1918±6.49	1542±2.04	1251±19.07	828±10.90
G ₃₆	2412±16.33	2554±14.66	1821±8.16	1539±23.56	1241±4.78	756±1.11
G ₃₇	2452±37.44	2674±3.72	2116±2.74	1646±15.64	1209±4.45	779±12.49
G ₃₈	2326±4.78	2730±29.68	1777±2.98	1679±3.39	1162±19.07	867±13.92
G ₃₉	2372±6.37	2667±37.11	2026±21.35	1691±0.00	1292±14.53	978±0.25
G ₄₀	2502±4.33	2731±21.35	1906±12.94	1590±24.86	1203±4.45	802±5.19
G ₄₁	2381±17.02	2537±40.58	1963±15.39	1625±7.23	1320±12.94	980±13.60
G ₄₂	2474±33.60	2508±6.12	1894±17.43	1689±28.21	1166±5.19	818±9.84
G ₄₃	2487±25.43	2778±42.62	2104±17.02	1658±25.43	1327±19.72	764±9.96
G ₄₄	2416±39.48	2460±28.58	2061±13.35	1752±26.78	1269±19.72	995±6.82
G ₄₅	2451±4.08	2735±32.66	1965±7.47	1681±22.45	1345±14.98	936±8.53

G ₄₆	2475±24.49	2655±14.29	1925±6.49	1602±1.68	1229±2.98	966±15.39
G ₄₇	2475±0.71	2649±22.45	1878±15.96	1593±0.00	1273±17.27	867±13.35
G ₄₈	2876±37.11	3157±52.71	2621±31.97	2418±20.41	1939±26.90	1376±7.47
G ₄₉	2470±1.68	2633±31.31	1852±14.98	1567±16.57	1250±10.21	759±5.88
G ₅₀	2832±21.11	3102±29.52	2564±31.31	2333±22.21	1877±15.96	1326±21.76
G ₅₁	2376±6.49	2633±31.31	1963±8.86	1526±16.57	1196±0.41	823±7.06
G ₅₂	2508±11.15	2479±0.00	1978±32.90	1608±1.68	1309±19.72	707±9.84
G ₅₃	2531±23.15	2777±10.45	2108±10.90	1716±14.53	1179±6.37	762±4.78
G ₅₄	2526±29.27	2781±19.72	2077±10.58	1689±16.09	1331±21.35	771±12.62
G ₅₅	2378±37.11	2731±4.78	1809±25.19	1674±12.49	1361±14.98	982±2.04
G ₅₆	2387±27.64	2666±3.72	2023±28.82	1660±23.15	1149±3.15	814±7.80
G ₅₇	2554±41.76	2820±37.44	1964±4.08	1694±15.64	1226±8.86	855±0.95
G ₅₈	2365±19.31	2642±36.74	2055±11.88	1691±23.56	1334±20.41	864±4.08
G ₅₉	2343±26.29	2844±4.78	2060±10.45	1690±10.90	1395±18.13	881±14.29
G ₆₀	2430±23.15	2523±7.80	2133±34.70	1809±28.33	1206±13.60	888±0.71
G ₆₁	2328±11.15	2623±39.15	2030±13.19	1706±13.92	1171±9.96	763±7.06
G ₆₂	2317±17.27	2513±10.21	2124±7.92	1673±18.74	1340±0.95	796±7.06
G ₆₃	2431±35.64	2655±9.10	2043±6.12	1656±10.21	1324±12.25	890±12.25
G ₆₄	2471±24.49	2597±9.84	2107±0.71	1768±9.51	1355±6.82	896±14.66
G ₆₅	2427±24.74	2569±24.49	1913±3.84	1559±7.92	1265±13.60	870±7.80
G ₆₆	2510±11.15	2773±23.56	2130±12.25	1776±22.45	1326±4.08	775±5.02
G ₆₇	2528±41.19	2641±31.31	2085±28.95	1694±14.98	1337±0.95	749±2.74
G ₆₈	2338±28.58	2605±41.52	1897±20.66	1508±12.25	1310±17.43	969±0.71
G ₆₉	2527±30.86	2657±6.37	2067±2.74	1776±12.25	1251±8.16	945±10.58
G ₇₀	2503±12.00	2668±39.89	2054±32.90	1741±27.23	1302±15.96	965±5.19
G ₇₁	2351±3.15	2504±4.33	2128±0.71	1815±6.82	1277±12.25	915±4.78
G ₇₂	2460±35.07	2634±14.29	2167±8.86	1609±10.45	1265±6.37	704±2.74
G ₇₃	2404±27.88	2573±38.54	2002±25.19	1704±5.19	1217±17.43	720±0.25
G ₇₄	2485±10.21	2627±10.90	2099±0.71	1787±19.47	1303±17.02	826±2.74
G ₇₅	2508±24.74	2516±7.80	1908±2.74	1559±9.51	1244±16.57	706±7.23
G ₇₆	2432±26.54	2678±10.90	2152±12.49	1821±0.71	1236±10.45	795±0.00
G ₇₇	2386±7.23	2636±13.60	1974±12.94	1554±15.96	1243±1.35	796±7.23
G ₇₈	2333±35.64	2706±24.86	2010±27.88	1581±6.37	1181±2.42	916±14.04
G ₇₉	2417±19.07	2535±16.33	1978±30.37	1650±7.92	1194±8.16	831±13.60
G ₈₀	2333±10.21	2517±24.74	2051±18.74	1617±23.15	1143±15.64	789±11.55
G ₈₁	2429±4.08	2657±27.88	2005±23.15	1660±19.07	1286±6.49	725±4.08
G ₈₂	2363±16.09	2737±9.27	1964±29.27	1646±1.11	1276±12.62	865±6.49
G ₈₃	2538±40.58	2678±37.44	1998±22.45	1700±20.41	1384±1.35	756±0.00
G ₈₄	2413±32.90	2486±2.42	1972±22.21	1602±1.68	1154±10.90	778±2.74
G ₈₅	2326±33.35	2769±41.52	2104±4.33	1774±4.78	1335±5.02	889±6.82
G ₈₆	2358±9.51	2644±6.37	1974±8.16	1645±10.21	1225±9.96	928±5.43
G ₈₇	2452±20.05	2500±5.19	1892±0.71	1636±5.43	1143±6.12	786±7.80
G ₈₈	2587±1.80	2729±28.82	2083±32.66	1588±12.49	1271±14.66	902±2.04
G ₈₉	2331±19.07	2675±23.80	2165±17.02	1700±23.15	1269±10.90	868±11.55
G ₉₀	2317±38.78	2614±2.74	2111±23.15	1684±7.47	1233±2.04	891±2.42
G ₉₁	2537±12.94	2597±13.19	1804±26.90	1537±8.41	1182±19.31	841±1.80
G ₉₂	2400±8.16	2622±7.06	2154±32.29	1834±13.60	1361±14.98	787±6.37
G ₉₃	2503±13.60	2607±43.56	2143±13.19	1772±0.00	1313±20.41	644±5.02
G ₉₄	2468±34.46	2546±37.11	2153±2.98	1832±23.80	1223±13.35	849±2.04
G ₉₅	2502±28.21	2684±15.64	1949±27.88	1641±24.49	1204±10.21	750±9.10
G ₉₆	2412±4.78	2613±40.82	1991±17.02	1604±6.49	1316±11.15	804±2.04
G ₉₇	2347±6.37	2844±30.99	1889±29.52	1628±21.11	1213±6.49	864±11.15
G ₉₈	2405±10.58	2569±15.64	2046±28.58	1663±18.13	1147±2.74	822±3.39
G ₉₉	2596±28.33	2802±40.13	1979±16.70	1674±27.23	1252±2.74	823±1.11

Vigour index (Mean ± SE, n = 4) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl. The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5 % significance level.

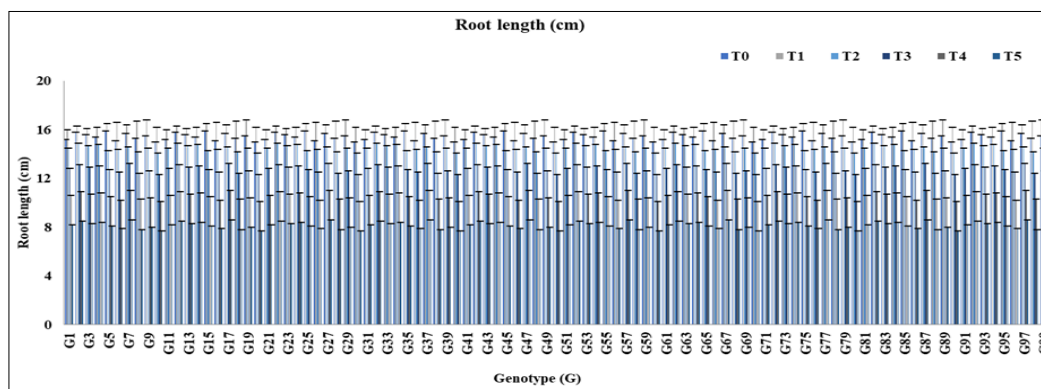


Fig. 1. Effect of imposed salinity stress on root length (cm) of pigeon pea genotypes in seedling stage.

Root length (Mean ± SE, n = 4) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl. The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5 % significance level.

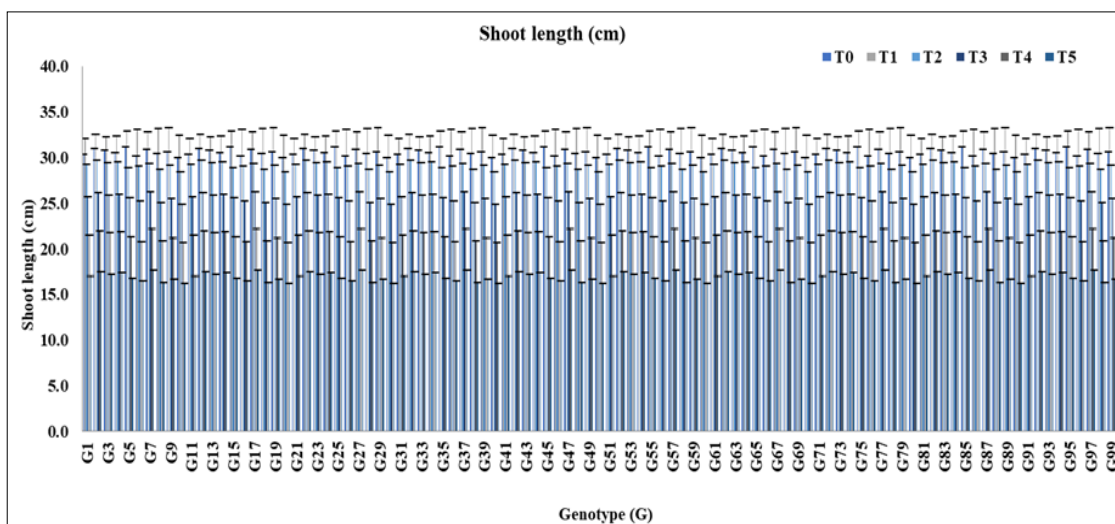


Fig. 2. Effect of imposed salinity stress on shoot length (cm) of pigeon pea genotypes in seedling stage.

Shoot length (Mean \pm SE, $n = 4$) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl). The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5% significance level.

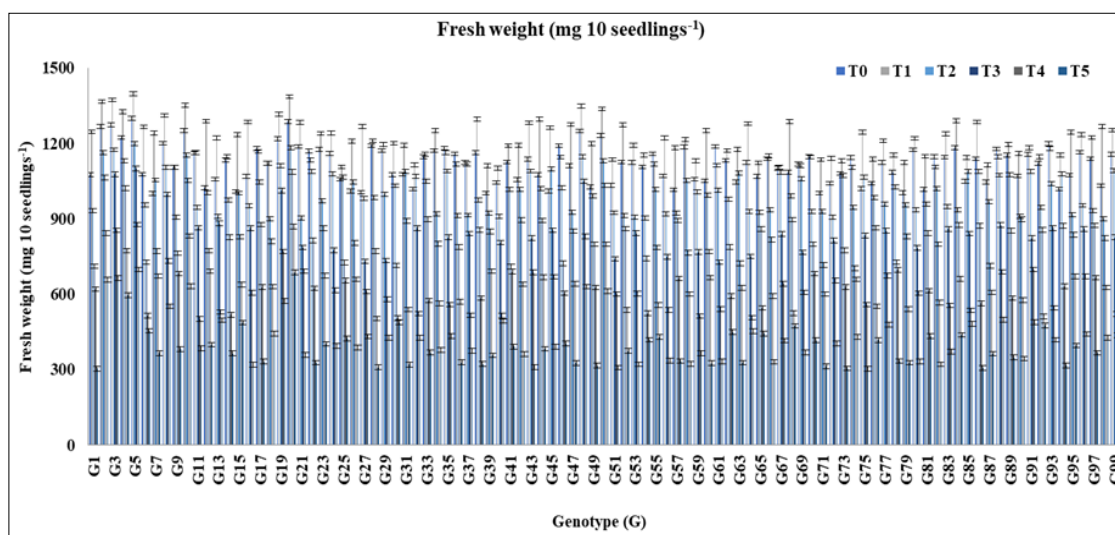


Fig. 3. Effect of imposed salinity stress on fresh weight (mg 10 seedlings⁻¹) of pigeon pea genotypes in seedling stage.

Fresh weight (Mean \pm SE, $n = 4$) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl). The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5% significance level.

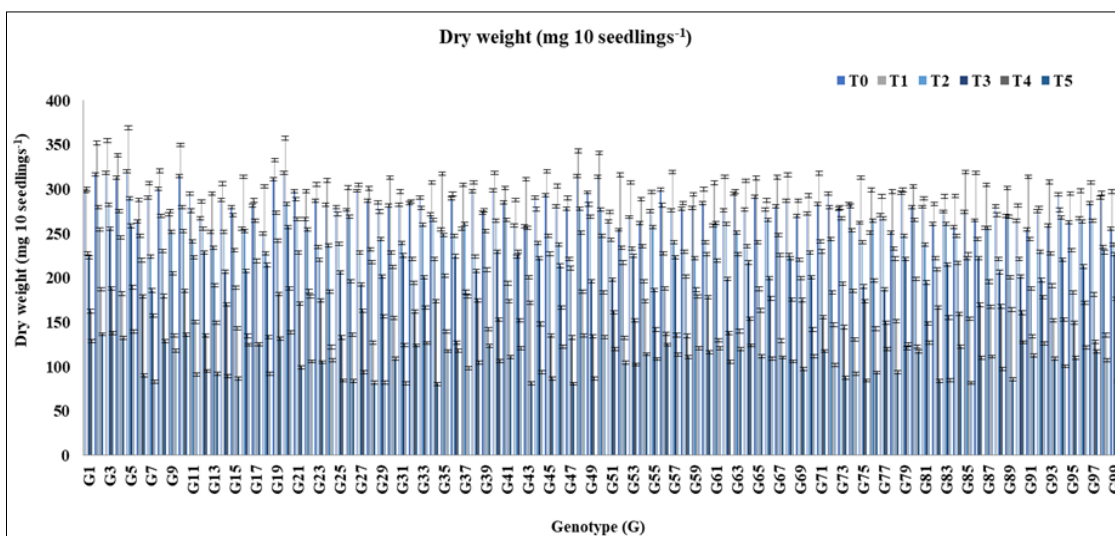


Fig. 4. Effect of imposed salinity stress on dry weight (mg 10 seedlings⁻¹) of pigeon pea genotypes in seedling stage.

Dry weight (Mean \pm SE, $n = 4$) of different treatments (T): (T₀ – Control, T₁ – Hydropriming, T₂ – 50 mM of NaCl, T₃ – 100 mM of NaCl, T₄ – 150 mM of NaCl, T₅ – 200 mM of NaCl). The analysis of variance was carried out and compared by Duncan's multiple range test (DMRT) at 5% significance level.

which reveals the degree of sensitivity to salt stress. Hydropriming can impact fresh weight positively because, seeds have already imbibed water before sowing. Thus, the metabolic processes start sooner than the control group (35). The osmotic potential is disturbed under saline conditions, leading to a striking reduction in seedling fresh weight (36). The findings prove that the fresh weight is affected by imposition of salinity and there is a need to address salt sensitivity among pigeon pea genotypes.

Dry weight eliminates variation due to water content and reflects parameters like absorption and stress response (37). The genotype G₅ has a dry weight of 139.81 mg 10 seedlings⁻¹ at 200 mM NaCl, which is only 20 % of its fresh weight at same concentration. The genotypes G₄₇ and G₃₄ exhibited more sensitivity with 80.73 mg 10 seedlings⁻¹ and 80.54 mg 10 seedlings⁻¹ dry weight respectively. In case of dry matter production, the seeds soaked in distilled water (T₁) showed higher values. As root is very sensitive to salinity, it limits the availability of O₂ and increases ethylene accumulation (38). This is the reason for lowered root dry weight. Root injury due to salt stress affects water uptake and it leads to decline in shoot dry weight (39). Hence, dry weight is also affected due to ionic toxicity and osmotic disequilibrium (40). This research reveals that dry weight was impacted due to increase in salt concentration.

The seedling vigour index showcases a link between high salt concentration and seedling establishment (41). The genotypes G₅ and G₂₀ performed well throughout all the salinity levels, whose vigour indices are 1567 and 1549 respectively, even at 200 mM NaCl. The G₅ and G₂₀ exhibited higher vigour index values in control group also. The increased salinity levels cause osmotic imbalance and affection homeostasis of the seedlings (42). Higher salt concentration can also cause inactivation and/or deactivation of enzymes (43). At cellular level, the compartmentation of ions is disrupted and stomatal conductance is decreased (44). Therefore, membrane permeability is increased and membrane stability is reduced. These physiological changes collectively reduce seedling vigour. This research proclaim that salinity condition would significantly contribute to decline in vigour index of the seedlings.

Conclusion

The imposed salinity significantly impacted on pigeon pea, with respect to the concentration of NaCl. The genotypes G₅ (ICP2947), G₂₀ (ICP14961), G₃ (ICP7380), G₂ (ICP 7024) and G₁₀ (ICP 16184) demonstrated tolerance to the increasing salt levels. The tolerance exhibited can be possibly due to the osmotic adjustment, activation of SOS (Salt-Overly-Sensitive) pathway or due to genetic factors. The SOS pathway mediates calcium signalling and consequently maintains ion homeostasis. This research provides insight on different growth parameters at seedling stage. The pigeon pea responds to salt stress in different ways, at different stages. The upcoming research should be potentially directed towards combating various abiotic stresses like drought and temperature stress. Addressing the sensitivity of crops to both biotic and abiotic stresses, will pave the way for sustainable and climate-smart agriculture.

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Authors' contributions

SS, TA and VR performed the experiments. VN, MH and SS analysed data. TA, SS and VR statistically analysed results. TK, SS and MS wrote the draft of the manuscript. VN and MH conducted the critical revision of the manuscript. VN, TK, MS and MH worked out the concept and design, supervised and funded the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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