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Appraisal of Salt Sensitivity among Elite Genotypes of Chickpea (*Cicer arietinum* L.) At Seedling Stage

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ABSTRACT

Background: The cool-season grain legume chickpea (*Cicer arietinum* L.) crop is sensitive to salt stress. Especially, NaCl salt inhibits both the rate and extent of seed germination and plant establishment.

Methods: The 20 *Desi* chickpea genotypes were investigated under different levels of NaCl salt stress (0, 4, 6, 8, and 10 dS/m) at the seedling stage with three replications during *Rabi* 2020-21. Observations were recorded at 15 and 30 DAS (Days after sowing) for seedling growth, vigor, and physiological performance.

Result: The seedling length (cm), fresh weight (g), and relative water content (RWC%) exhibited decreasing trends and reversibly increasing trends by the relative stress injury (RSI%) with increasing NaCl salinity levels. The values of the length of coleoptile and radicle (cm) at control to 10 dS/m ranged from 15.9-8.6 cm and 29.6-18.3 cm, respectively at 15 DAS and from 20.5-12.8 cm and 30.3-18.3 cm, respectively at 30 DAS. The chickpea genotypes identified as the source of salinity tolerance were HC5, HC3, HC1, H12-22, H14-14, and H03-56 as they maintained higher growth, water status, and fewer membrane injuries at the seedling stage, recognized as screening tools under salt stress which could be used to enhance/develop a new salt-tolerant variety in chickpea breeding programme.

Key words: Chickpea, NaCl, Salt-stress, RWC, RSI.

INTRODUCTION

The cool-season grain legume chickpea (*Cicer arietinum* L.) crop, a self-pollinated diploid plant (2n= 2x= 16) ranked third after common bean and field pea (Janghel *et al*., 2021), is vulnerable to salt stress than other crops (Flowers *et al*., 2015; Kumar *et al*., 2018). Soil salinity is an ever-increasing threat around the world with the use of saline water for irrigation under current climatic conditions (Dias *et al*., 2016). Now, the time has come up to think about it seriously with an alarming rate of transforming agricultural land into saline soil for various reasons including low precipitation, high evaporation rate and high solar balance. Chickpea is typically farmed in arid and semi-arid regions of the world, where soil salinization is a problem (Flowers *et al*., 2010). India now leads the globe in both area and production of chickpeas, accounting for 70.57 percent of the worldwide area and 69.21 percent of global production followed by Australia (Sharma *et al*., 2020). It is an essential component of crop rotations (Zawude and Shanko, 2017), improves soil health by fixing atmospheric nitrogen through a symbiotic relationship with Rhizobia, and is gradually becoming accepted as a future staple food crop due to its high nutritional content, market value, and adaptability (Kaashyap *et al*., 2017).

Especially salinity in chickpea, NaCl salt inhibits the rate and extent of seed germination, as well as plant establishment (Flowers *et al*., 2010). Plant growth is hampered by salts due to an increase in soil osmotic pressure and interference with plant nourishment (Machado and Serralheiro, 2017). Salt stress slows seed imbibition, radicle elongation, seed germination, and seedling growth due to osmotic effects (Kumar *et al*., 2018; Pereira *et al*., 2019). The high salt concentration in the soil solution limits the plant's ability to absorb water (Acosta-Motos *et al*., 2017), carbon dioxide absorption by promoting stomata closure (Osakabe *et al*., 2014), and the photosynthetic ability of dry matter accumulation (Silva *et al*., 2019). Chickpea germplasm has been tested for salt sensitivity and genetic diversity in seed production and component properties (Pushpavalli *et al*., 2020), but more extensive research work at the seedling stage is required. Thus, it is a pelion to manage the saline soil, so there is a sturdy need to screen the genotypes and develop the salt tolerant cultivars, which

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is the simplest and most cost-effective strategy to manage the soil salinity stress. The purpose of this study was to determine the salt sensitivity of chickpea genotypes at the seedling stage under varying levels of salt stress based on seedling growth, vigor, and physiological performance.

MATERIALS AND METHODS

The 20 *Desi* chickpea genotypes shown in Table 1 were investigated for salt sensitivity during *Rabi* 2020-21 in the growth chamber of the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar (Haryana), India. The seedling growth, vigor, and physiological performance were examined in a completely randomized design (CRD) with two factors (genotypes and salinity levels) in three replications at varied levels of NaCl salt (0, 4, 6, 8, and 10 dS m^{-1}). The homogeneous and healthy seeds of chickpea genotype were surface sterilized with a 1% sodium hypochlorite solution. Then, the 50 seeds (10 seeds at each level) of each genotype were allowed to sprout in the germination paper dipped with desired salinity levels of NaCl salt using the between paper (BP) method of seed germination under normal room temperature conditions (Temp 25±3°C and 60-70% RH). The following observations were recorded at 15 and 30 DAS (days after sowing) are as follows:

Seedling growth

Length (cm) of coleoptile and radicle measured in centimetre scale, whereas, fresh weight (FW) weighed in gram using digital balance immediately after separation of coleoptile and radicle.

Seedling vigor index % (SVI %)

SVI (%) was assessed under different salinity levels as formulae suggested by Abdul-Baki and Anderson (1973):

$$\text{SVI \%} = \text{Seeding lenght (cm)} \times \text{Germination \%}$$

Relative water content % (RWC %)

RWC % of coleoptile and radicle of chickpea seedling estimated as per the method suggested by Weatherley (1950) and calculated by using the formula:

$$\text{RWC \%} = \frac{\text{(FW-DW)}}{\text{(TW-DW)}} \times 100$$

Where,
FW= Fresh weight (g).
DW= Dry weight (g).
TW= Turgid weight (g).

Relative stress injury % (RSI %)

RSI % of coleoptile and radicle of chickpea seedling measured as per the method suggested by Sullivan and Ross (1979) and calculated by the formula:

$$\text{RSI \%} = \frac{\text{1-ECa}}{\text{ECb}} \times 100$$

Where,
ECa= Electrical conductivity before heat treatment.
ECb= Electrical conductivity after heat treatment.

Statistical analysis

To assess the significant difference between the three treatments, the data was statistically analysed for ANOVA (analysis of variance) at 5% level of significance using OPSTAT online software (http://14.139.232.166/opstat/).

RESULTS AND DISCUSSION

For seedling growth, vigour, and physiological performance under varied salt levels (0, 4, 6, 8, and 10 dS m^{-1}), the two factors ANOVA were shown to be substantially different at the 5% level of significance.

Seedling growth performance

At both 15 and 30 DAS, seedling growth performance of chickpea genotypes, *i.e.* length of both coleoptile and radicle, decreased with increment of salt stress (Fig 1). With increasing salt levels, coleoptile length (cm) decreased, but this trend was less pronounced at 30 DAS than at 15 DAS. The minimum reduction (%) in the length of coleoptile at 10 dS m-1 as compared to control recorded for genotype H 14-01 (22.73), H 14-14 (27.59), H 12-63 (28.13), H 12-64 (29.69), H 13-02 (30.00), H 13-01 (31.58), H 14-21 (32.14), H 14-04 (32.73), H 12-55 (33.17), H 12-22 (34.78), H 15-25 (36.84), HC 5 (37.04) and ICCV 92944 (38.78) at 30 DAS (Fig 2A). However, at both 15 and 30 DAS, radicle length (cm) varied somewhat between genotypes and was modestly reduced with increasing salt stress (Fig 1 and 2A). At 30 DAS, the chickpea genotypes H 14-21 (6.98), H 14-01 (16.09), H 13-01 (19.78), H 14-14 (22.58), HC 5 (24.27), H 15-25 (25.00), H 12-55 (25.32), and HC 1 (25.56) showed the least reduction of radicle length (Fig 2A).

Fresh weight (FW) of coleoptile (g) in chickpea genotypes has shrunk in tandem with rising salt levels at both stages (15 and 30 DAS) (Fig 2B). After 30 days at 10 dS m^{-1}, the genotypes H 03-56 (36.99 g), HC 5 (44.25 g), HC 1 (43.92 g), H 12-64 (43.84 g), H 12-63 (30.56 g), H 12-55 (40.68 g), H 12-22 (27.27 g), H 13-02 (36.43 g), H 14-14 (36.36 g), H 14-04 (37.58 g), H 14-01 (30.82 g) and H 15-25 (44.07 g) had the highest fresh weight (Fig 2B). The FW of radicle (g) in different chickpea genotypes had shown slight variation, however, minimum reduction for FW of radicle (g) was found in HC 1 (35.43), H 12-63 (35.86), HC 5 (43.90), H 13-02 (44.83), H 14-01 (45.77), H 12-55 (45.93) and H 12-64 (46.48) at 30 DAS (Fig 2B). The above results showed that increasing salt concentrations up to 10 dS m-1 decreased seedling growth performances, such as coleoptile length, radicle length, coleoptile fresh weight, and radicle fresh weight, when compared to control, due to a decrease in osmotic potential caused by ionic and osmotic effects of NaCl salt that hinders water absorption, facilitates ion penetration into cells, deactivate enzymes, and inhibit protein synthesis, compared to control (Silva *et al.*, 2019). However, the genotypes HC 5, HC 1, H 12-64, H 12-63, H 12-55, H 13-02, H 14-14, H 14-01, and H 15-25 performed better for seedling growth under 10 dS m-1 of salt stress than other genotypes, which could be used to further screen chickpea genotypes for salt stress tolerance to seed yield level. The results are in agreement with the reports of Kandil *et al*. (2012), Mann *et al*. (2019), Kafi *et al*. (2021), Kaur *et al*. (2021), Nabati *et al*. (2021), Mergeb (2021), Shtaya *et al*. (2021) and Ismail *et al*. (2022).

Seedling vigour index (%)

The seedling vigor index (SVI%) declined in chickpea genotypes in the same way that seedling length and fresh weight did (FW). Although, 30 DAS the chickpea genotypes H 14-21 (16.90), H 14-01 (18.32), H 13-01 (24.32), H 14-14 (24.50), H 12-55 (28.77), H 12-64 (29.05), H 12-63 (29.33), H 15-25 (29.66), HC 5 (29.89), H 14-04 (30.88), H 12-22 (32.69) and HC 1 (35.54) performed better and showed minimal reduction for SVI at 10 dS m^{-1} of salt stress compared to control (Fig 3). Salt stress is likely to prolong the germination process by increasing the seed moisture imbibition phase, reducing the osmotic potential, delaying the mobilization of germination-related enzymes, limiting cell division and elongation process of seedling growth (Marques *et al*., 2011). Similar results were obtained by Shanko *et al*. (2017), Pimenta *et al*. (2021), Nejadhabibvash and Rezaee (2021), Nguyen *et al*. (2022).

Relative water content (%) and relative stress injury (%)

The chickpea genotypes exhibited a similar declining pattern in the relative water content % (RWC) with the augmentation of salt stress. RWC in coleoptile varied from 48.97 to 77.11% after 15 days of sowing and from 45.39 to 74.91% after 30 days of sowing at 10 dS m-1 of salt stress (Fig 4A). Among the chickpea genotypes, H 03-56 (26.16), HC 5 (23.76), HC 3 (26.58), H 12-63 (17.54), H 13-01 (17.50), H 14-21 (11.51), H 14-14 (20.80), H 14-11 (18.02), H 14-04 (23.15), H 14-01 (17.25), H 15-27(20.00), ICCV 4958 (24.93), H 15-25 (20.45) and H 15-23 (16.17) had comparatively more RWC along with a smaller reduction in RWC at 10 dS m-1 as compared to control value at 30 DAS (Fig 4B).

While practically all chickpea genotypes, with the exception of ICCV 92944 (33.59) and H 14-21 (38.80), had higher RWC content at 10 dS m^{-1} salt stress after 30 DAS than the control treatment (Fig 4A). Reversibly, relative stress injury (RSI %) in chickpea genotypes increased with the increment of salt stress *i.e.* 0 to 10 dS m^{-1} at both 15 and 30 DAS (Fig 4B). At 30 DAS, RSI in the coleoptiles of chickpea genotypes HC 1 (83.40), H 14-21 (76.74), H 14-14 (75.96), ICCV 4958 (76.30), and H 15-25 (81.28) (Fig 4B), and in radicles of chickpea genotypes H 03-56 (85.16), HC 5 (85.05), HC 3 (82.95), H 12-55 (84.67), H 12-22 (77.89) and H 14-01 (83.35) had a comparatively lesser increment in RSI at 10 dS m^{-1} of salt stress as compared to control (Fig 4B).

Excess salts in the soil concentrated around the root zone, causing a change in cell metabolism that resulted in a decrease in RWC and an increase in RSI due to detrimental effects on physiological processes like plant water status, ion homeostasis, and photosynthesis (Babar *et al*., 2014). In chickpea genotypes, the RWC and RSI could be used as direct measures of salt stress resistance. Based on these physiological parameters, the salt stress tolerant

Table 1: List of 20 *Desi* chickpea genotypes for salt sensitivity appraisal at seedling stage.

Genotypes	Pedigree	Source
ICCV 92944	[(GW 5/7 × P 327) × ICCL 83149]	JNKVV, Jabalpur
H 12-64	Advanced lines from HAU	CCSHAU, Hisar
H 13-01	HC 5 × H 04-31	CCSHAU, Hisar
H 14-01	HC 1 × H 04-31	CCSHAU, Hisar
H 03-56	Advanced lines from HAU	CCSHAU, Hisar
H 12-63	(HC 5 × H 00-256) × ICC 4958	CCSHAU, Hisar
H 14-21	Advanced lines from HAU	CCSHAU, Hisar
H 15-27	Advanced lines from HAU	CCSHAU, Hisar
HC 5	H 89-78 × H89-84	CCSHAU, Hisar
H 12-55	Advanced lines from HAU	CCSHAU, Hisar
H 14-14	Advanced lines from HAU	CCSHAU, Hisar
ICCV 4958	Collected from Jabalpur, Madhya Pradesh, India	JNKVV, Jabalpur
HC 1	F 61 × L550	CCSHAU, Hisar
H 12-22	HC 1 × (HC 1 × ICCV 96030)	CCSHAU, Hisar
H 14-11	(HC 5 × PDG 84-16) × (HC 5 × H 91-36)	CCSHAU, Hisar
H 15-25	Advanced lines from HAU	CCSHAU, Hisar
HC 3	L 550 × E 100 Y (m)	CCSHAU, Hisar
H 13-02	Advanced lines from HAU	CCSHAU, Hisar
H 14-04	H 03-56 × H 04-31	CCSHAU, Hisar
H 15-23	Advanced lines from HAU	CCSHAU, Hisar

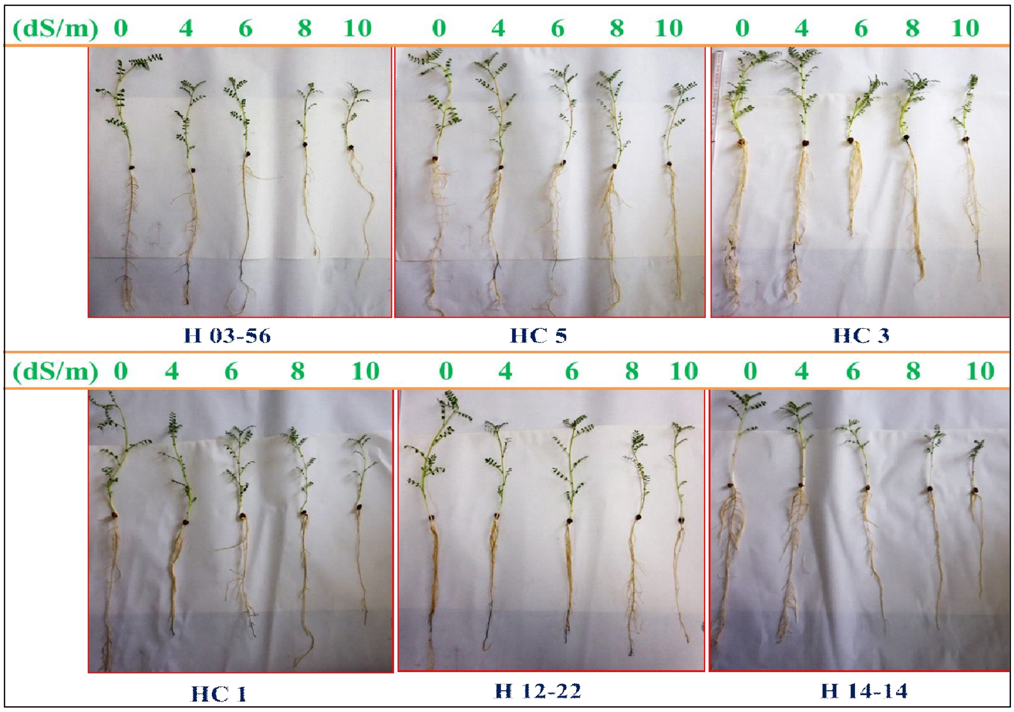


Fig 1: Seedling growth performance of chickpea genotypes under NaCl salt stress (0, 4, 6, 8 and 10 dS m^{-1}).

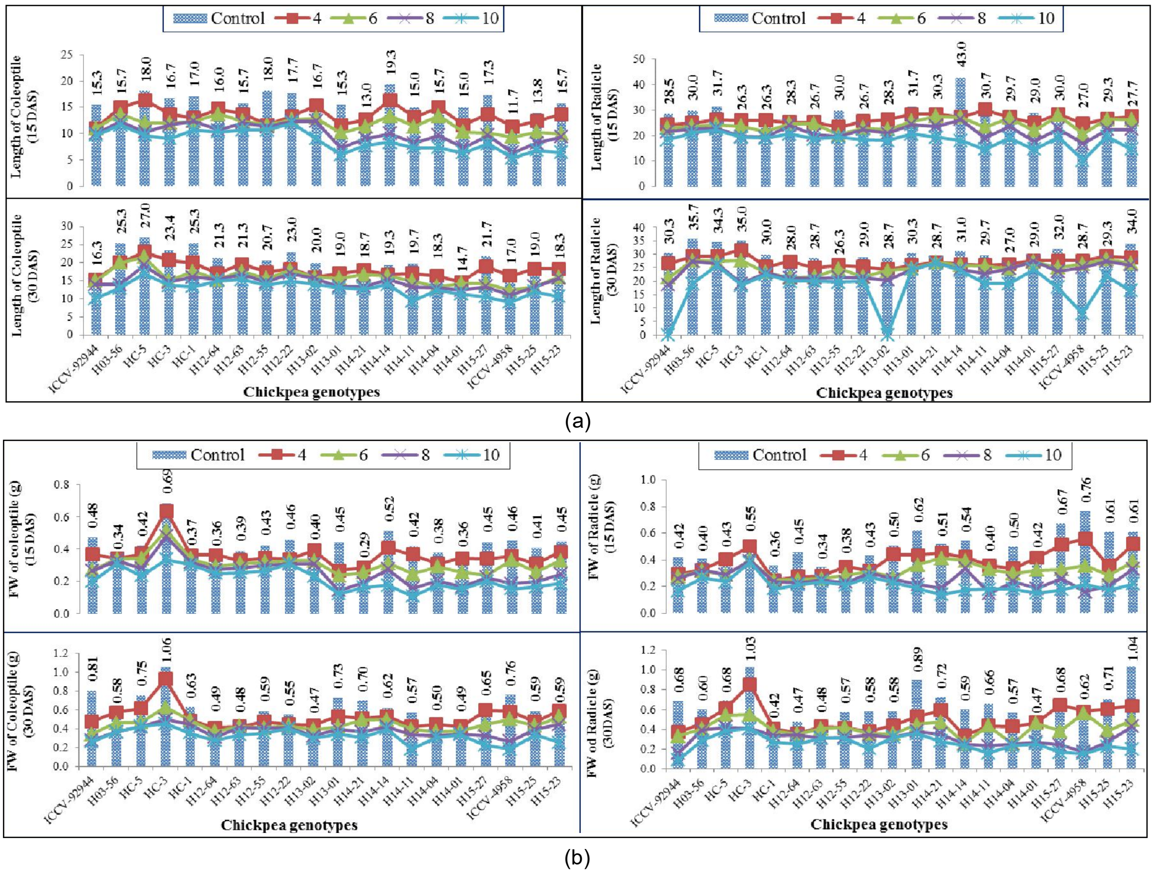


Fig 2: (A) Length (cm) and (B) Fresh weight (FW) of coleoptile and radicle among chickpea genotypes at 15 and 30 DAS under salt stress (0, 4, 6, 8 and 10 dS m^{-1}).

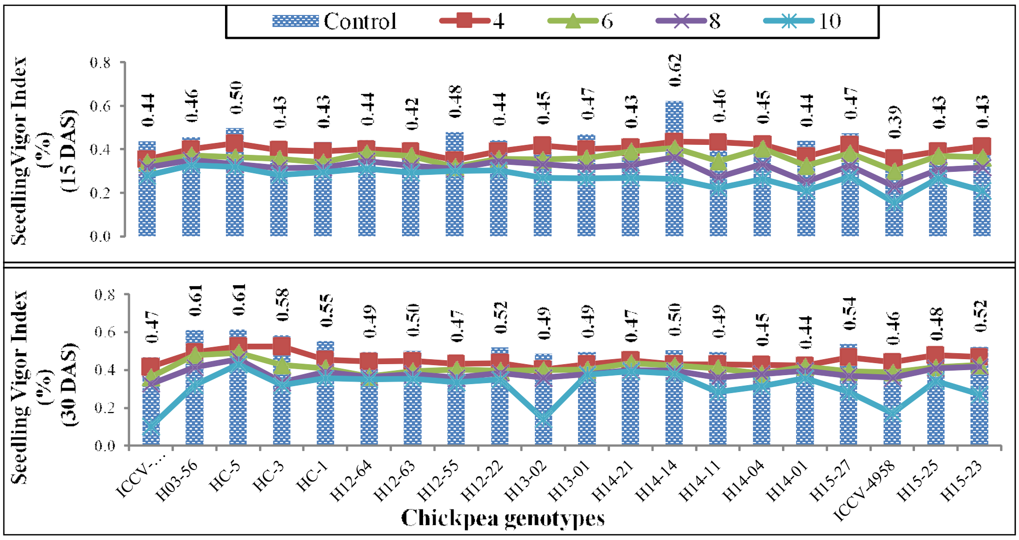
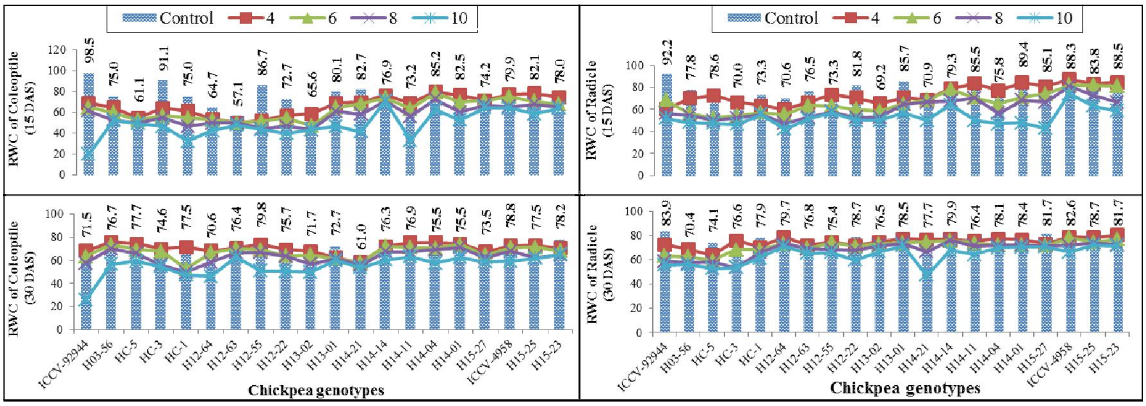
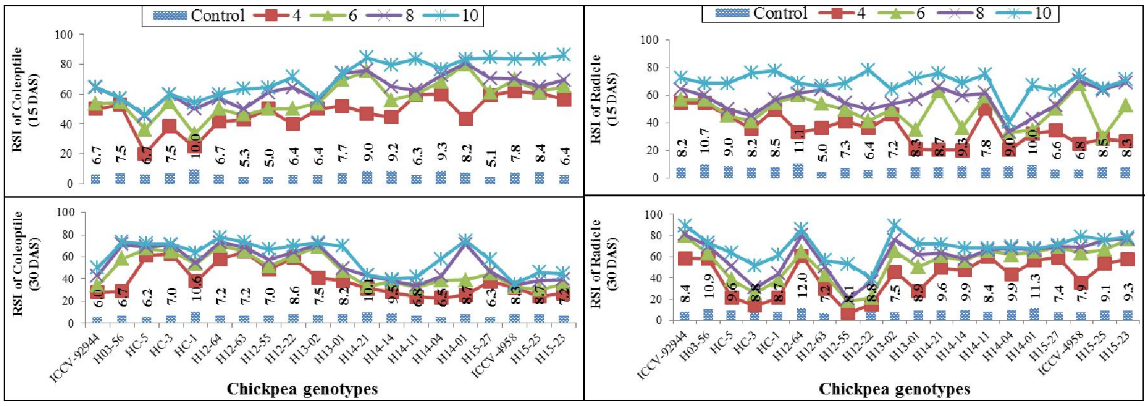


Fig 3: Seedling vigor index (%) of various chickpea genotypes at 15 and 30 DAS under salt stress (0, 4, 6, 8 and 10 dS m^{-1}).



(a)



(b)

Fig 4: (A) Relative water content % (RWC %) and (B) relative stress injury % (RSI %) of coleoptile and radicle among chickpea genotypes at 15 and 30 DAS under salt stress (0, 4, 6, 8 and 10 dS m^{-1}).

genotypes *viz*., H 03-56, HC 5, HC 3, HC 1, H 12-55, H 12-22, H 13-01, H 14-21, H 14-14, H 14-01, ICCV 4958 and H 15-25 had been screened and identified. These results are also in concomitant with Dudhe and Kumar (2016), Gaurav *et al*. (2016), Nasiri *et al*. (2021) and Kaur *et al*. (2021).

CONCLUSION

Salt stress conditions cause a delay in seed germination which led to greater exposure of seeds to pathogenic action, insect-pest attack and substantial loss in seedling vigor. It has the potential to hinder cell division and elongation, as well as limit water availability and photosynthesis, resulting in a reduction of plant growth. Seedling biomass production, growth, and development processes are all sensitive to salt stress, hence these could be employed as good selection criteria for determining salt stress tolerance in chickpea genotypes. Based on seedling growth and physiological performance under laboratory conditions, the chickpea genotypes HC 5, HC 3, HC 1, H 12-55, H 14-14, H 14-01 and H 15-25 performed relatively better than others at 10 dS m-1 salt stress and could be used as a source of tolerance in breeding programmes to develop salt-tolerant chickpea genotypes.

Conflict of interest

The authors declare that there is no conflict of interest.

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