SCREENING OF CHICKPEA GENOTYPES AGAINST SALINITY STRESS IN PETRI DISH ENVIRONMENT

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ABSTRACT: The leguminous crop chickpea (*Cicer arietinum* L.) has a great potential for nutrition, antioxidants, proteins, lipids, and carbs. Due to concerns with soil salinity/sodicity and climate change, the area used for chickpea cultivation is shrinking daily and the overall yield has decreased. Ten varieties of chickpea were subjected to salinity stress in a petri dish at four different NaCl concentrations: 0 mM, 50 mM, 100 mM, and 150 mM in a controlled condition experiment. The findings of the experiment revealed that Chattan and KK-1 do not survive under salinity stress. While Punjab-2008 and KK-2 demonstrated strong resilience to salinity stress. The results showed that at 0 mM of NaCl, KK-2 and Punjab-2008 had the highest germination rates (99%), followed by Bittle-98 and CM-98 (98%) The Chattan and KK-1 do not exhibit any germination at 150 mM NaCl. The data showed that Fakhr-e-thal had the smallest Radicle length (0.05 cm) at 150 mM NaCl, whereas Bhakkar-2011 at 50 mM NaCl had the largest Radicle length (4.90 cm). In comparison to 0 mM NaCl, the plumule length (cm) of the various chickpea genotypes showed that Bhakkar-2011 had the longest plumules (3.09 cm), Chattan had the shortest (0.80 cm), and KK-1 had no values at the greatest salinity levels. It is therefore concluded that increasing salinity badly affects the germination and growth in chickpea, but this stress can be mitigated by using salinity resilient genotypes.

Keywords: Salinity, Germination, Chickpea, Genotypes, Tolerance.

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INTRODUCTION

The production of chickpeas for human consumption is the second largest of all edible legumes worldwide. It is grown either on soil moisture that has been saved up after the rainy season or rice harvesting (South Asia, eastern Africa, and north-eastern Australia), or while it is raining (Canada and Mediterranean regions) (Berger *et al*., 2004). It is predicted that abiotic stresses are responsible for a 6.4-million-ton annual reduction in chickpea yield, out of a total annual production of 12 million tones (Gujaria, 2012).

Most of the factors contributing to lower crop production around the world are abiotic stresses. During the growth phases of crop plants, they are subjected to a variety of environmental variables that slow down their morphological growth. Because of the negative effects of salinity on germination, growth, and vigor, and ultimately crop yield, agricultural production suffers as a direct result of increased salinity (Shrivastava and Kumar, 2015). During the germination periods, the bulk of salinity inhibition signs will present themselves. Certain crop plants can tolerate the effects of abiotic factors because they have a diverse set of defense mechanisms at this stage. How plants respond to salinity is influenced both by the chemical make-up of the soil and the amount of salt that naturally occurs in the soil. The reactions of plants in salt-stressed conditions are substantially impacted by the genotypes of the plants as well as the developmental phases the plants are in at the time (Kaur *et al*., 2022). Chickpeas, like other pulse crops, are sensitive to salt, and salts containing chlorides have a considerable detrimental impact on the amount of chickpeas that may be produced (Yadav *et al*., 2019). Chickpeas are particularly susceptible to the damaging effects of salinity during the germination and early growth stages of the plant (Ceritoglu *et al*., 2020). In soils that have been damaged by salt, most plant species are unable to maintain their normal functionality in terms of growth and development because of changes in osmotic potential, a decrease in soil moisture, and the accumulation of a high level of sodium concentration in the soil solution (Lavrenko *et al*., 2019). This renders them unable to grow and develop normally, therefore, a reduction in the amount of water and nutrients was observed that were available to the plant in its soil (Rahneshan *et al*., 2018; Panuccio *et al*., 2014). The physiological dryness that results from salinity and the inability of plants to get critical nutrients are both caused by salinity (Shabala and Munns, 2017). The effects of salinity stress can be seen throughout every stage of the chickpea crop's development. A few examples of effects

that can be either quantitative or qualitative include seed germination (Ibrahim, 2016), seedling establishment (Yue *et al*., 2019), biochemical and physiological parameters (Noohpisheh *et al*., 2021), osmotic regulation (Zhang *et al*., 2010), reactive oxygen species level (Hassanuzzaman *et al*., 2021), antioxidants level (Garcia-Caparros *et al*., 2019), gene expression and regulation (Hussain *et al*., 2021), nutrient uptake (Heidari and Jamshid, 2010), plant growth (Safdar *et al*., 2019), yield, and yield components (Shahzad *et al*., 2019).

The selection of salt-tolerant genotypes is the most effective strategy for mitigating the detrimental effects of salinity on the germination stage of plant growth (Hasegawa *et al*., 2000). It is common practice to evaluate a plant's ability to withstand salinity by comparing its biomass under salt stress to its biomass under control conditions over a prolonged period (Singh *et al*., 2021). Because of this, cultivating crops on ground that has been impacted by salt is both time-consuming and costly (Turan *et al*., 2007). In countries like India, Pakistan, and Ethiopia, amongst others, the cultivation of leguminous crops is primarily done for human consumption. As a result, selecting cultivars that are resistant to salt is essential to ensuring the expanding population has access to sufficient food supplies. In the current work, molecular measurements of responses of various chickpea types to altering salt concentrations were done in petri dish conditions.

MATERIALS AND METHODS

The experiment was conducted at Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar Pakistan. The experiment was carried out in Petri Dishes and ten verities were selected for screening of salinity stress at three levels.

Selection and Collection of seeds: A total of 10 local varieties of chickpea were selected for the experiment in a petri dish environment. These varieties were collected from the Grain Research Station, Ahmadwala, Karak, Pakistan Agricultural Research Institute, Dera Ismail Khan, Pakistan, *Arid Zone* Research Centre, Bhakkar, Pakistan and Ayub Agriculture Research Institute, Faisalabad, Pakistan.

This experiment contained ten different chickpea varieties and four salinity stress levels in petri dishes environment. Closed Petri dishes were placed in a sterile polypropylene tray with sterile distilled water. Pre-germinated (radicle of 4 to 5 cm length) chickpea seeds were simultaneously after a preconditioning step, placed on the fiberglass filter paper in Petri dishes. This co-culture system was maintained in the growth room at 20 ± 3 °C, with artificial light, and a humidity level of at least 70%. Chickpea seeds were surface sterilized using Sodium Hypochlorite (3%) solution for 60 seconds at room temperature (Sauer and Burroughs, 1986). One hundred and twenty Petri dishes were washed and sterilized in an autoclave. The Petri dishes were placed at a uniform distance with sterile filter papers placed inside them. In order to prevent the impact of light on roots, dishes were covered with aluminum foil. Four levels of salinity i.e. 0 mM, 50 mM, 100 mM and 150 mM were used. These concentrations were sodium chloride (NaCl) per liter of water (de-ionized). The distilled water was added to Petri dishes used as the untreated check. In order to avoid evaporation, the petri dishes were covered with paraffin (Asgharipour and Rafiei, 2011; Muhammad and Hussain, 2010) and then kept in a growth chamber with a photoperiod of 12 hours and at a temperature of 25°C ± 1°C. The salt solution of the desired concentration was applied to each treatment at a regular interval of 2 days. In the next step, nine seeds from each variety were treated with 0 mM, 50 mM, 100 mM, and 150 mM concentrations of NaCl/liter of water (distilled).

Germination Rate: The germination percentage is the number of seeds germinated compared to the total number of seeds in a petri dish (09 in our case), to be multiplied by 100. The following formula was adopted as described by Desalegne (1996).

Germination rate (GR):$= \frac{NT3+NT6+NT9+NT12}{Total\ number\ of\ seeds\ germinated}$

Where: NTn=number of seeds germinated while N=days (3, 6, 9, 12)

Plumule and Radicle length (cm): Plumule length (cm) was measured by the stem and embryo length. The radicle length (cm) was measured from the point of first cotyledons' node to the tip of the lengthiest root.

Statistical Analysis: The experiment was performed under a complete randomized design (CRD) with a factorial arrangement. The difference between percentage and length was subjected to statistical analysis by using Statistix 8.1 to perform ANOVA (Analysis of Variance). The means significance ($P \leq .05$) for *F*-test were further subjected to mean separation using DMRT (Duncan's Multiple Range Test).

RESULTS

Germination Rate: The germination (%) data of chickpea varieties as affected by different drought levels are shown in Fig 1. The data showed that maximum germination rate was recorded in KK-2 and Punjab-2008 (99%) followed by Bittle-98 and CM-98 (98%) at 0 mM NaCl. It was noted that as the salinity levels increased, the germination rate (%) decreased accordingly. In the case of Chattan genotype, the highest germination rate (96.7%) was shown at 0 mM of NaCl, while the decline in germination rate was 21%, 87%, and 100% at 50 mM, 100 mM, and 150 mM of NaCl, respectively. In KK-1

genotype the highest germination rate (97%) was recorded in the control treatment (0 mM) among all salinity levels. The germination rate decline was 62%, 100%, 100% at 50 mM, 100 Mm, and 150 mM of NaCl, respectively, for the KK-1 genotype. The results showed that the highest germination rate (99%) for KK-2 was shown in the control treatment (0 mM) among all the salinity levels. At 50 mM of NaCl, the germination rate was 54% lower than the control treatment. At 100 mM of NaCl, it was 36% lower than control, while at 150 mM of NaCl, it was 68% lower than the control treatment where no salinity was applied. A similar trend was found in KK-3; where the germination rate was 97%, 75%, 45%, and 17% at 0, 50, 100, and 150 mM of NaCl, respectively. In Fakhr-e-thal, the highest germination rate was shown in the control treatment among all salinity levels. Simultaneously, the decline was 12%, %56, and 90% at 50, 100, and 150 mM of NaCl, respectively. The Indus genotype showed germination at all salinity levels but with a decreasing trend. The maximum decline was observed at 150 mM of NaCl (79%), followed by 100 mM (34%) and 50 mM (23%) as compared to the control treatment. The Bhakkar-2011 genotype also showed germination at all salinity levels but with a decreasing trend. The decline was maximum at 150 mM of NaCl (65%), followed by 100 mM (28%) and 50 mM (23%) as compared to the control treatment. The Bittle-98 genotype also showed germination at all salinity levels but with a decreasing trend. The decline was maximum at 150 mM of NaCl (83%), followed by 100 mM (79%) and 50 mM (29%) as compared to the control treatment. In CM-98, the highest germination rate was shown in the control treatment among all salinity levels. While, the decline was 87%, 63%, and 87% at 50, 100, and 150 mM of NaCl, respectively. Punjab-2008 genotype also showed germination at all salinity levels but with a decreasing trend. The decline was maximum at 150 mM of NaCl (51%), followed by 100 mM (45%) and 50 mM (44%) as compared to the control treatment. The germination trend was as KK-2=Punjab-2008 > Bittle-98 = CM-98 > KK-1 = KK-2 > Bhakkar-2011 > Indus > Chattan > Fakhar-e-thal. It is thus concluded that KK-2 and Punjab-2008 were the most tolerant varieties to NaCl in our appraisal. It is further concluded from our data that varieties KK-1 and Chattan emerged as the most susceptible varieties to salinity hence avoided to be planted on saline soils.

Radicle length: Four levels of salinity (Level 1(S_0):0 mM NaCl; Level 2 (S_2): 50 mM NaCl; Level 3 (S_3): 100 mM NaCl; Level 4 (S_4): 150 mM NaCl) were used to determine the effect of salinity on radicle length. The data depicted that the highest Radicle length was determined in Bhakkar-2011 L2 level of salinity followed by L1 level and L3 level, respectively. In comparison, the lowest Radicle length was recorded in Chattan @ at Levels 3 and 4 of salinity. The data showed that the treatment performance varies with salinity levels as well as influenced with the chickpea genotype (Table 2). The Chattan genotype showed a decline in radicle length as the salinity levels increased; the control treatment where 0 mM of NaCl was maintained showed the highest radicle length (2.23 cm) compared to the other salinity levels. The decline in radicle length with salinity levels was 129% at Level 2, 478% at Level 3 and 1116% at Level 4. The genotype KK-1 showed the same trend concerning salinity. The control treatment showed maximum radicle length (2.87 cm) compared to the other salinity levels. The decline in radicle length was 104%, 716%, 2149% at Level 2, Level 3 and Level 4 of NaCl, respectively, for the KK-1 genotype. Likewise, KK-2 genotype depicted the highest radicle length (3.80 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 134%., 155%, and 334% at Level 2, Level 3 and Level 4 of NaCl, respectively, for the genotype concerned. Whereas KK-3 genotype also portrayed the highest radicle length (3.60 cm) in the control treatment (0 mM) among all salinity levels. Its decline in radicle length was to the extent of 135%, 228%, and 899% at 50 mM, 100 mM, and 150 mM of NaCl, respectively. The Fakhr-e-Thal genotype portrayed the highest radicle length (3.57 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 136%, 182%, and 7133% at 50 mM, 100 mM, and 150 mM of NaCl, respectively, for the genotype under study. The Indus genotype witnessed the highest radicle length (3.43 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 118%, 366%, 763% at Level 2, Level 3 and Level 4 of NaCl, respectively, for the Indus genotype. The Bhakkar-2011 genotype also exhibited the highest radicle length (4.83 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 93%, 95%, and 188% at at Level 2, Level 3 and Level 4 of NaCl, respectively, as evaluated for the Bhakkar-2011 genotype (Table 4.1.).

The Bittle-98 genotype designated the highest radicle length (4.20 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 105%, 95% and 167% at at Level 2, Level 3 and Level 4 of NaCl, respectively for Bittle-98 genotype. The CM-98 genotype designated the highest radicle length (4.23 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 104%, 106% and 395% at at Level 2, Level 3 and Level 4 of NaCl, respectively for CM-98 genotype. The radicle length as evaluated in Punjab-2008 genotype showed the highest radicle length (4.63 cm) in the control treatment (0 mM) among all salinity levels. The decline in radicle length was 102%, 107%, and 661% at at Level 2, Level 3 and Level 4 of NaCl, respectively for the CM-98 genotype (Table 4.1.).

The data in Table 4.1further depicts that based on the tolerance at the highest rates of application (S_3), varieties KK-2 (1.13), Brittal-98 (2.47), and Bhakkar-2011 (2.53) gained the longest radicle length at the highest rate of NaCl application i.e., 150 mM (S_3) (Table 2), which are suitable for cultivation in the saline canal irrigated areas including the tube well-irrigated soils.

Plumule Length (cm): The plumule length (cm) of chickpea genotypes against 0 mM NaCl as exhibited in Fig 2(a) showed the longest plumule length (3.09 cm) as measured in Bhakkar-2011 and the lowest (0.80 cm) in Chattan. The plumule length at 0 mM NaCl trend from the highest to the lowest was as Bhakkar-2011 > Punjab-2008 > CM-98 > Bittle-98 > KK-2 > Fakhr-e-Thal > KK-3 > Indus > KK-1 > Chattan. The plumule length at 50 mM NaCl trend in decreasing order was as Bhakkar-2011 > Punjab-2008 > CM-98 > Bittle-98 > KK-2 > KK-3 > Fakhr-e-Thal > Indus > Chattan > KK-1 (Fig 2b). In case of 100 mM of NaCl, it was observed that the maximum plumule length (3.40 cm) was recorded in Bhakkar-2011 and the lowest in KK-1. The trend was as Bhakkar-2011 > Punjab-2008 > CM-98 > Bittle-98 > KK-2 > Indus > KK-3 > Fakhr-e-Thal > Chattan > KK-1 (Fig 2c). In case of highest level of salinity (150 mM NaCl), the high plumule length (1.67 cm) was shown in Bhakkar-2011 while Chattan and KK-1 showed no plumule length indicating their highest sensitivity to salinity. The trend was as follows Bhakkar-2011 > Punjab-2008 > Bittle-98 > CM-98 > KK-2 > Indus > KK-3 > Fakhr-e-Thal > Chattan > KK-1 (Fig 2d).

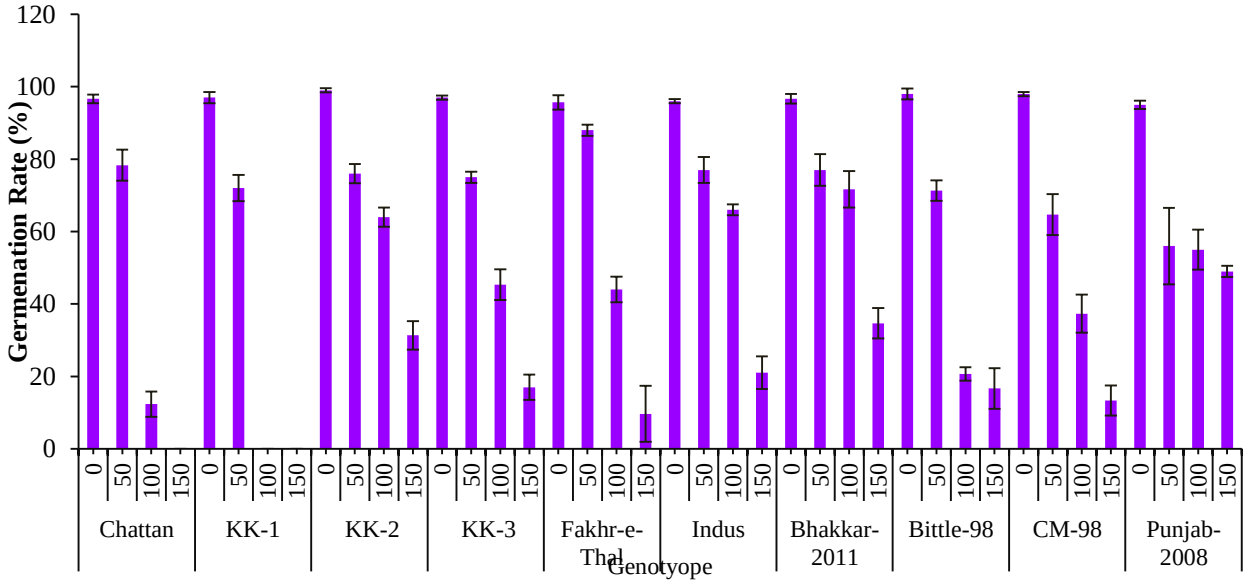

Fig 1. The germination rate of Chickpea genotypes at different salinity levels

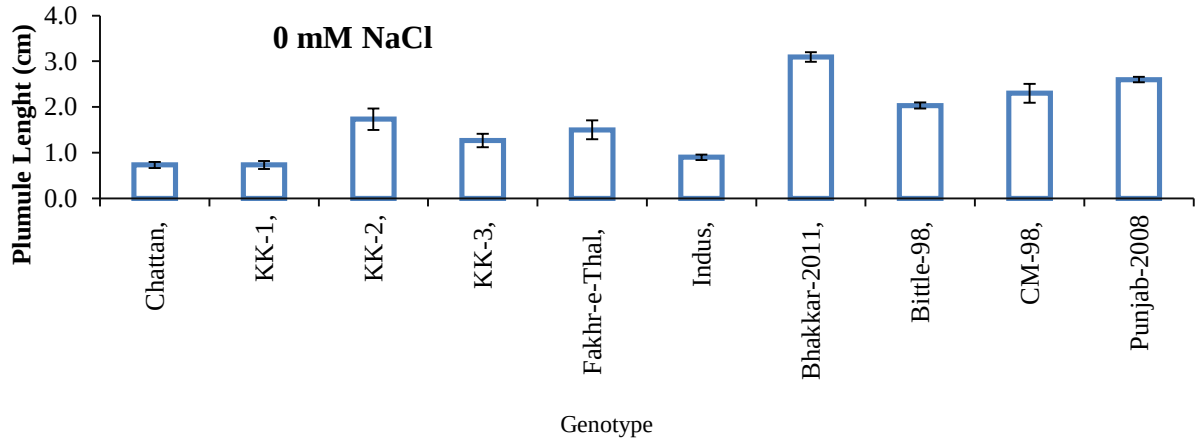

Fig 2(a). The effect of Salinity (0 mM NaCl) on the growth of plumule length of chickpea under petri dish condition

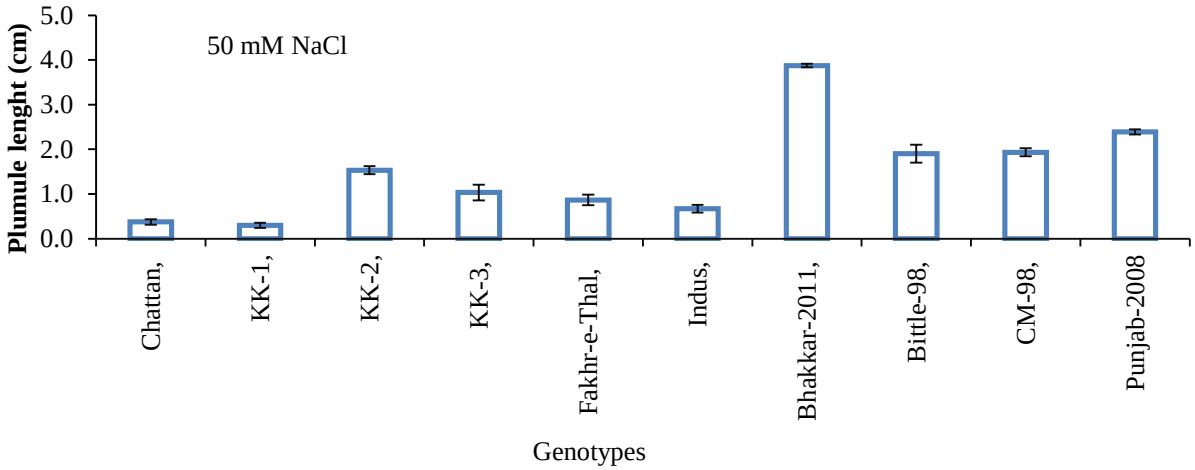

Fig 2(b). The effect of Salinity (50 mM NaCl) on the growth of plumule length of chickpea under petri dish condition

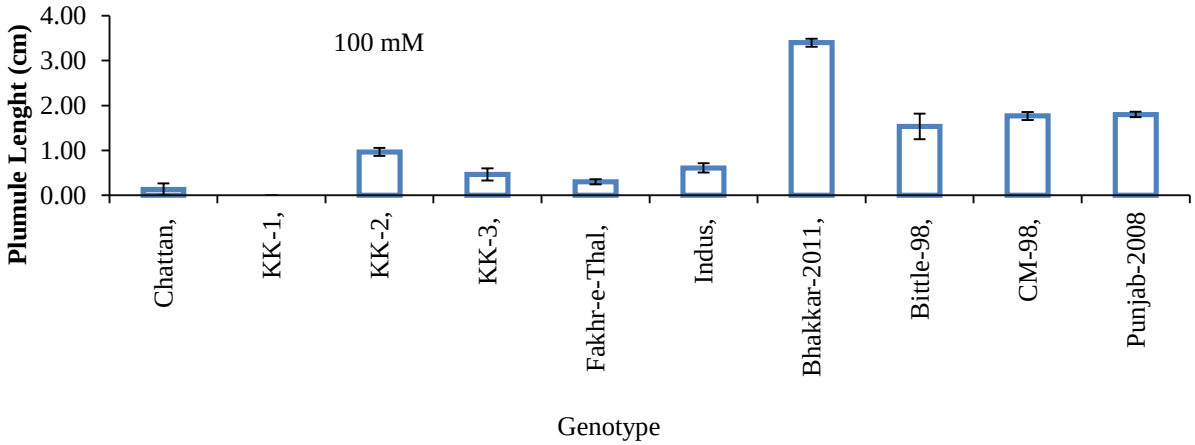

Fig 2(c). The effect of Salinity (100 mM NaCl) on the growth of plumule length of chickpea under petri dish condition.

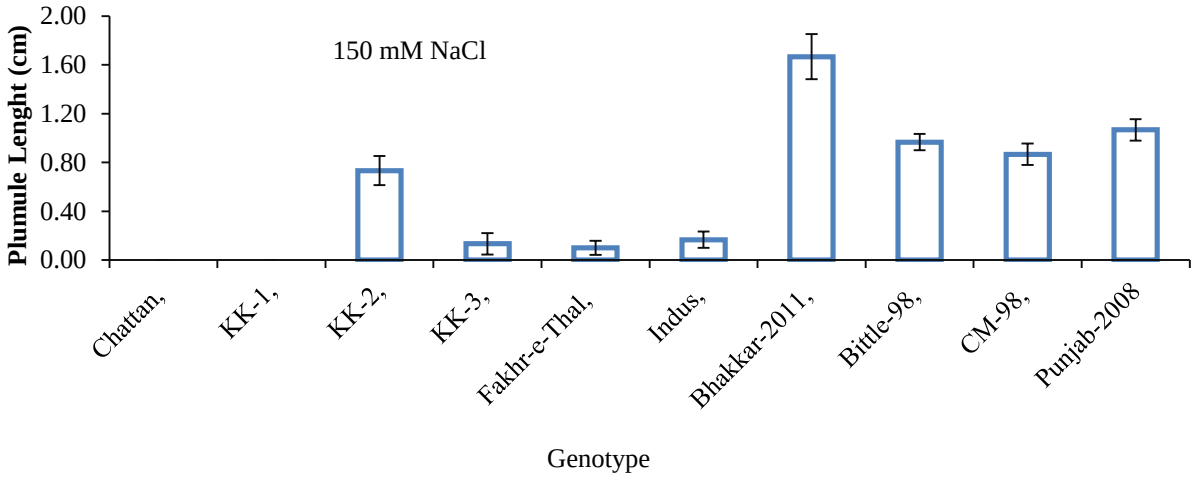

Fig 2(d). The effect of Salinity (150 mM NaCl) on the growth of plumule length of chickpea under petri dish condition

Table 1 Details of collected chickpea varieties.

Sr. No.	Variety Name
01	KK-1
02	KK-2
03	KK-3
04	CM-98
05	Punjab-2008
06	Indus-2016
07	Bittle-98
08	Bhakkar-2011
09	Chattan
10	Fakhr-e- Thal

Table 2. Radicle Length (cm) of chickpea genotypes against salinity stress levels.

Genotype	Salinity Level	Radicle Length (cm)	Genotype	Salinity Level	Radicle Length (cm)
Chattan	S_0	2.23 ± 0.24 g-j	Indus	S_0	3.43 ± 0.15 d-f
	S_1	1.70 ± 0.35 i-l		S_1	2.83 ± 0.07 e-g
	S_2	0.47 ± 0.42 m-o		S_2	0.93 ± 0.17 ln
	S_3	0.20 ± 0.20 n-o		S_3	0.45 ± 0.09 m-o
KK-1	S_0	2.87 ± 0.15 e-g	Bhakkar-2011	S_0	4.83 ± 0.07 a
	S_1	2.67 ± 0.19 f-h		S_1	4.90 ± 0.25 a
	S_2	0.40 ± 0.21 m-o		S_2	4.80 ± 0.25 a
	S_3	0.13 ± 0.13 no		S_3	2.53 ± 0.72 g-i
KK-2	S_0	3.80 ± 0.17 b-d	Bittle-98	S_0	4.20 ± 0.15 a-d
	S_1	2.77 ± 0.15 e-h		S_1	3.83 ± 0.03 b-d
	S_2	2.40 ± 0.25 g-j		S_2	4.20 ± 0.17 a-d
	S_3	1.13 ± 0.35 k-m		S_3	2.47 ± 0.67 g-i
KK-3	S_0	3.60 ± 0.15 c-e	CM-98	S_0	4.23 ± 0.24 a-d
	S_1	2.60 ± 0.25 f-h		S_1	3.90 ± 0.25 b-d
	S_2	1.57 ± 0.29 j-l		S_2	3.83 ± 0.43 b-d
	S_3	0.40 ± 0.30 m-o		S_3	1.07 ± 0.12 lm
Fakhr-e-Thal	S_0	3.57 ± 0.24 c-e	Punjab-2008	S_0	4.63 ± 0.09 ab
	S_1	2.57 ± 0.19 gh		S_1	4.33 ± 0.38 a-c
	S_2	1.93 ± 0.09 h-k		S_2	4.13 ± 0.80 a-d
	S_3	0.05 ± 0.05 o		S_3	0.70 ± 0.31 m-o

Values are the means of replicates ± SE, Values sharing the same letter (s) are non-significant from each other

DISCUSSION

According to Rana *et al.* (2015), the first germplasm collections are based primarily on morphological and agronomic traits and are extremely important in breeding activities. Phenotypic exposure of common beans to the changing environment establishes the adaptation to the local environmental conditions (De Ron *et al.*, 2019). Furthermore, because only a small number of plants can produce enough seeds in a single year, local legumes tend to grow increasingly uniform (Ebert, 2014). The genotypes of chickpeas were gathered from the Gram Research Station in Ahmadwala, Karak, Arid Zone Research Institute Bhakkar, Pakistan Agriculture Research Council, Arid Zone Research Center Dera Ismail Khan, and the Ayub Agriculture Research Institute Faisalabad. Statistical tools were used to examine the findings and create a database for next research. It is believed that diversity or variation is a universal tool of existence and a supportive feature of nature. It makes it possible for life to survive in the face of adverse environmental shifts and climate change. Plants may adapt to and survive in a changing climate thanks to genetic variation. All 10 local genotypes were genetically and morphologically varied in the current investigation. The findings revealed that the radicle length reduced as saline levels rose (Table 2). Wu *et al.* (2011) also noted that the increase in salt caused a decrease in radicle lengths. High salt concentration and the osmotic effect are detrimental to seed growth and germination as well as radicle emergence (Ren *et al.*, 2020).

A salt content of 0 mM produced the maximum germination rate (Fig 1). The outcomes of this experiment concur with those of earlier research by Khodarahmpour *et al*. (2012). These experimental findings support Akbarimoghaddam *et al*. (2011) finding that a decrease in germination % was brought on by the osmotic effect of salt concentration present in the growing medium (soil/water). Raising the salt content in the growing medium (in our case, a petri dish) has a detrimental effect on the length of the radicle and plumule because salinity hinders their growth. Unexpectedly increasing the salt content of the growth medium can cause cell division to be disrupted and water absorption to be inhibited by reducing the osmotic potential (Baranova and Gulevich, 2021). According to the study, salt has a deleterious impact on the plumule and radicle's length. Due to ionic toxicity and disruptions in food intake, the salinity stress shortens plumule and radicle length (Shakri *et al*., 2022). Due to osmotic effects on water absorption, salt stress also reduces the manufacturing of enzymes and plant growth hormones. When crop seed is exposed to salt stress in the soil, the seed cell membranes are damaged because the permeability of the membranes increases, Ca2+ is replaced with Na+, and K+ is allowed to leak out (Chen *et al*., 2020). As a result, one of the most important indicators of how a crop plant will react to salinity stress is the length of the radicle. The results of our investigation showed that Bhakkar-2011 at a salinity level of 50 mM NaCl had the longest radicles, followed by levels of 0 mM and 100 mM NaCl. The lowest radicle length was demonstrated in Chattan at salinities of 150 mM NaCl and 100 mM NaCl, respectively. The outcomes demonstrated that salinity levels affect the treatment's efficacy. With an increase in salt content, the length of the radicle and plumule reduced. These results are consistent with several past results (Abbas *et al*., 2013).

Conclusion: The key findings from the present study are that chickpea genotypes selected for tolerance and sensitivity to the salinity stresses have shown that some varieties have found to be extremely resilient to these stresses while some were extremely vulnerable. At very initial stage the plant is extremely vulnerable to stresses due to absence of proper development of defense mechanisms. Therefore, the genotypes that resist at that stage will suggestively be defensive in the field. These are therefore be recommended to further tested on mass scale to be proposed for saline soils.

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