# Role of primed chickpea genotypes under different saline and soil conditions

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***Abstract-*** The experiment was conducted at Screen House of University of Science and Technology Bannu. Two soil types (Sandy, clay soil) as growth media and in order to evaluate salinity impacts four saline levels (0, 50, 100, & 150) mM were used. Four chickpea genotypes named Bhakar-2011, Thall-2006, Noor-2009 and Noor-2013 were selected from Chickpea Research Station Ahmadwala, Karak. The above-mentioned chickpea genotypes were primed with $CaCl_2$ (50 mM) solution for 12 hours at 25 $^oC$, while dry seeds of same genotypes were used as control. The pots were irrigated with a half-strength Hoagland solution after every 3rd day. When the chickpea seedling reached to V3 stage in pots, salt stress was applied. In comparison of soil types, sandy soil had high shoot, root fresh weight, shoot, root dry weight, high shoot to root dry weight ratio, shoot $Na^+$ and $K^+$ concentration, shoot $Na^+/K^+$ ratio, root $Na^+/K^+$ ratio, proline and chlorophyll as compared to clay soil. Primed seeds had lengthy shoots, roots, maximum shoot fresh weight, root fresh weight and their dry weights, more $K^+$ in shoot and root, and chlorophyll. Sodium chloride at the rate of 150 mM had increased shoot to root dry weight ratio, shoot & root $Na^+$ and $K^+$ concentration, root & shoot $Na^+/K^+$ ratios, and increase in proline content. Chickpea Thall-2006 had high shoot fresh weight, shoot dry weight and root $Na^+$ concentration. It is concluded that chickpea genotype Thall-2006 showed better performance under high salt stress when primed with calcium chloride in sandy soil.

***Index Terms***- Calcium Chloride, NaCl stress, Sand, Clay, *Cicer arietinum*, Performance

## I. INTRODUCTION

Ecosystem is key element of biodiversity and its disturbance significantly influence biodiversity. Ecosystem is high complex system of biotic and abiotic components and it is especially true for the soil in ecosystems and this complexity play an important role in strengthening biodiversity. Soil disturbance and climatic variations are the greatest threats to biodiversity (Mooney et al., 2005; IPCC, 2007). It is evident that unhealthy soil promotes species endangerment (Brook et al., 2003). It is very important to assess soil impacts on biodiversity for effective conservation efforts of plant biodiversity. Plant and soil create such resources that develop biodiversity more ever soil provides essential materials (Kier et al., 2005). Soil influence many ecological processes related to life (Hamilton and Hamilton, 2006).

Salinity-adversely affects plant growth, development and yield (Ghassemi et al., 2009) reduction of grain yield in different genotypes of oat yield was recorded (Zhao et al., 2009). In barley, cotton and wheat root growth, biomass and grain number were decreased (Katerji et al., 2009). The most salinity sensitive part is tomato fruit (Reina et al., 2005). Salinity decrease plant height (Yetisir and Uygur, 2009) and leaf growth and cell division in grasses (Ben et al., 2008). Salt stress reduce germination percentage, shoot and root lengths, fresh weight and seedling vigor in plants (Ahmad et al., 2013). High level of soil salinity can cause plants to wilt even in presence of adequate soil moisture (Horneck et al., 2007). The dry weight of roots is also decreased (Abbas et al., 2011). Countries with high salinity level have higher number of threatened plant species. Salinity threat like slow loss of species is very silent that it is hardly understand. Among all the other factors responsible for poor growth of plant in our country salinity is of prime importance. In our country 25% of the irrigated land is affected by some level of salinity which makes 1.4 million hectares of all agricultural land unsuitable for agriculture. Pakistan is the 8$^{th}$ country in terms of area affected with salinity. Annual loss of crops due to salinity in Pakistan have been estimated between 1.5 to 5.5 billion rupees (MNFS&R, 2013). The performance of plants in saline growth medium is affected by salinity alleviating agent in addition to priming significantly (Afzal et al., 2008). Germplasm having salt tolerance contain higher value of proline, $K^+/Na^+$ ratio and chlorophyll content under saline condition (Khan et al., 2009). Number of seed, biomass and harvest index increased when seeds were primed with $CaCl_2$ with respect to other priming agents when compared with control (Farooq *et al.*, 2008). Resistance to abiotic stress is also positively affected by priming (Jisha et al., 2016). Hydro priming for water deficit stress and primed chickpea seeds promotes yield in comparison to non-primed seeds (Kaur et al., 2002). Legume seeds gave best production when seeds primed overnight (Musa et al., 1999). Priming of seeds with water develops seedling vigor, yield and plants establishment of chickpea, rice, and maize in India (Harris et al., 1999). The Calcium Chloride ($CaCl_2$) in combination with priming results high fresh weight and dry weight in comparison with non-primed genotypes (Afzal et al., 2008). Exogenous application of calcium alters complete physiology of salt defense process (Cha-um et al., 2012). When Calcium ($Ca^{++}$) fertilizers are applied to the crops of saline soil overcome the negative effects of salinity. This research was designed to overcome salt

stress in different soil conditions to improve growth and development of primed chickpea genotypes.

## II. IDENTIFY, RESEARCH AND COLLECT IDEA

Experimental design and experimental Site

The experiment was conducted at Screen House of University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan. The complete randomized design (CRD) with three (3) factors was applied. The factor A consist of two soil types (Sandy and clayey), factor B include priming of seeds ( with and without $CaCl_2$), factor C consist of salinity levels (0, 50, 100, and 150) mM and factor D consists of chickpea genotypes i.e two kabuli (Bhakar-2011 and Thall-2006) and two desi types (Noor-2009 and Noor-2013). The above-mentioned chickpea genotypes were primed with $CaCl_2$ (50 mM) solution for 12 hours at 25 $^oC$, followed by surface drying, while dry seeds of same genotypes were used as control. The pots were irrigated with a half-strength Hoagland solution after every 3rd day. Standard pots size (380 $cm^3$) having capacity of 5 kg sterilized sandy and clayey soil. A thin cloth of muslin was placed at the bottom of each pot to prevent release of sand from the pot. Fifteen seeds of each chickpea genotypes were sown in pots at 5cm depth. At V3 stage of chickpea seedlings were salinized with 50, 100 and 150 mM NaCl with control. After completion of emergence thinning were done, remaining ten plants in each pot, irrigated half strength Hoagland solution after every 3-4 days. Physio-chemical properties of soil at depth of 0-15 cm at experimental site for sandy soil (pH7.7, EC 432.4µS/cm, Silt40%, Clay55 %, Bulk density 1.2, O.M 0.5%, WHC 42%, N 0.12%, P2 .75 mg/Kg, K68 mg/Kg and for clayey soil (pH7.4, EC517.4 µS/cm, silt34 %, Clay63 %, Bulk density1.5, O.M0.81 %, WHC46%, N1.11%, P3.1 mg/Kg, K75 mg/Kg)) . Daily Temperature ($^oC$), rainfall (mm), humidity (%) at experimental site of Bannu was obtained during the research experimental site (Table 1).

Data collection and procedure

The following observations were performed. Shoot length data was collected by taking five randomly selected plants were measured at maturity with scale ruler to determined averaged shoot length (Upadhyaya et al., 2002). Root length of the five randomly selected plants harvested for root length was determined with ruler and obtained mean length. Shoot fresh weight (g $plant^{-1}$) was determined by taking five plants were harvested from each treatment and then immediately weighed and the mean value of shoot weight was recorded. Root fresh weight (g $plant^{-1}$) was determined by taking roots and separated from the plants harvested for root weight in each treatment and recorded their mean weight. Shoot dry weight (g $plant^{-1}$) was determined by weighing the plants harvested for shoot fresh weight after drying in oven at 70 $^oC$, for 48 h (Avelar et. al., 2018). Root dry weight (g $plant^{-1}$) was calculated by taking the root samples from each experimental units and fresh root weight was kept in oven 70 $^oC$ for 48 h to obtain root dry weight (Avelar et. al., 2018). Root to shoot ratio was calculated by dividing root dry weight on shoot dry weigh. Shoot $Na^+$ concentration (mg $g^{-1}$ dry weight) was determined by followed the protocols of Benton et al. (1991). The concentration of $Na^+$ in shoot was recorded in dry shoots were ground for digestion. Taking Nitric acid ($HNO_3$) 10 ml was added to 0.5 g sample in concentrated form and keep all over the night for digestion in a conical flask. Concentrated perchloric acid ($HClO_4$) 4 ml was added to the above mentioned solution and given the temperature 200 $^oC$ resulting a colourless solution with white fumes after cooling and filtering than by adding 100 ml distilled water the volume was adjusted. The flame photometer was adjusting against standard solution to determine $Na^+$ content. Root $Na^+$ concentration (mg $g^{-1}$ dry weight) was determined according to Benton et al. (1991). The concentration of $Na^+$ in root was recorded, by drying roots and then ground it for digestion. Taking Nitric acid ($HNO_3$) 10 ml was added to 0.5 g sample in concentrated form and keep all over the night for digestion in a conical flask. Concentrated perchloric acid ($HClO_4$) 4 ml was added to the above mentioned solution and given the temperature 200 $^oC$ resulting a colorless solution with white fumes after cooling and filtering than by adding 100 ml distilled water the volume was adjusted. The flame photometer was adjusted against standard solution to determine Na+ content. Shoot $K^+$ concentration (mg $g^{-1}$ dry weight) was determined by followed Benton et al. (1991) protocols. Dry shoots were ground for digestion. Taking Nitric acid ($HNO_3$) 10 ml was added to 0.5 g sample in concentrated form and keep all over the night for digestion in a conical flask. Concentrated perchloric acid ($HClO_4$) 4 ml was added to the above mentioned solution and given the temperature 200 $C^o$ resulting a colorless solution with white fumes after cooling and filtering than by adding 100 ml distilled water the volume was adjusted. The flame photometer was adjusting against standard solution to determine $K^+$ content. Root $K^+$ concentration (mg $g^{-1}$ dry weight): Concentration of $K^+$ in root was recorded according to Benton et al. (1991). Dry roots were ground for digestion. Taking nitric acid ($HNO_3$) 10 ml was added to 0.5 g sample in concentrated form and keep all over the night for digestion in a conical flask. Concentrated perchloric acid ($HClO_4$) 4 ml was added to the above mentioned solution and given the temperature 200 $^oC$ resulting a colourless solution with white fumes after cooling and filtering than by adding 100 ml distilled water the volume was adjusted. The flame photometer was adjusting against standard solution to determine $K^+$ content. Shoot $Na^+/K^+$ ratio was determined by dividing the value of concentration of $Na^+$ by $K^+$. Root $Na^+/K^+$ ratio was determined by dividing the value of concentration of $Na^+$ by $K^+$. Proline content (µg $g^{-1}$ dry weight) of leaves in each sample was measured according to Bates et al. (1973) protocol. One-gram fresh samples of leaves in each treatment were ground with 40 ml of 3% sulphosalicylic acid. This homogeneous mixture was filter with 3 mm whatman filter paper and two ml of the filtrate was mixed with two ml of reagent (1.25 g Ninhydride, 20 ml Glacial acetic acid and 20 ml Orthophosphoric acid 6M and 2ml Acetic acid) in tubes of propylene. These tubes were incubated for 1 hour at 100 $^oC$ in water bath. After cooling four ml toluene was added to this mixture. The UV spectrometer (520 nm) was used to calculate proline concentration. Chlorophyll content (mg $cm^{-2}$) was measured at flowering stage in each pot of each treatment randomly with the help of AtLeaf chlorophyll meter (FT green LLC, 0.2 A/ +0.5 v, class B, made in USA) and the respective values were converted into mg $cm^{-2}$ by AtLeaf reference values.

## I. WRITE DOWN YOUR STUDIES AND FINDINGS

*Shoot length*

Shoot length was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2). The seeds primed with $CaCl_2$ improved the shoot length in both the soils as compared to control while tallest plants were observed in sandy soil (32.3) as compared to clay soil (31.5). Shoot length gradually decreased when salt stress increasing from 50mM to 150mM in both the soil types. However, lengthy shoots were recorded in sandy soil as compared to clay soil. Chickpea variety Noor-2013 had longest shoot (29.8) followed by Bhakar-2011 (28.1), Thall-2006 (27.3) and Noor-2009 (24.9) in clay soil, while Noor-2009 showed maximum shoot length (32.9) followed by Noor-2013 (31.2), Thall-2006 (31.0) and Bhakar-2011 (29.5) in sandy soil. The interaction for priming × salinity had showed significant effect on shoot length in both the soil types.

*Root length*

Root length was significantly affected by priming, salinity levels for chickpea genotypes under clay and sandy soils (Table 2). The seeds primed with $CaCl_2$ promote root length in both the soils as compared to control. While longest roots were recorded in clay soil

(10.5) as compared to sandy soil (8.2). Root length was gradually decreased when salt stress increasing from 50mM to 150mM in both the soil types. However longest roots were recorded in clay soil as compared to sandy soil. Chickpea variety Thall-2006 lengthy roots (9.95) followed by Noor-2009 (9.37) Noor-2013 (9.27) and Bhakar-2011 (9.15) in clay soil while Noor-2013 show longest roots (8.2) followed by Noor-2009 (8.1), Bhakar-2011 (8.0) and Thall-2006 (7.8) in sandy soil. The interaction for priming × salinity has shown significant effect on root length in both the soil types.

*Shoot fresh weight*

Shoot fresh weight was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2). The seeds primed with $CaCl_2$ produced more fresh weight of shoot in both the soils as compared to control while maximum shoot fresh weight was recorded in sandy soil (1772.5) as compared to clay soil (487.6). Shoot fresh weight was gradually decreased when salt stress increasing from 50mM to 150mM in both the soil types however highest shoot fresh weight was recorded sandy soil as compared to clay soil. Chickpea variety Bhakar-2011 has highest fresh weight of shoot (417.0) followed by Noor-2013 (384.2) Thall-2006 (375.4), and Noor-2009(340.8) in clay soil while Thall-2006 showed maximum shoot fresh weight (1925.8) followed by Bhakar-2011 (1712.1), Noor-2013 (1670.4) and Noor-2009 (1603.8) in sandy soil. The interaction for priming × salinity has shown significant effect on shoot fresh weight in both the soil types.

*Root fresh weight*

Root fresh weight was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2). The seeds primed with $CaCl_2$ produce more root fresh weight in both the soils as compared to control while maximum root fresh weight was recorded in sandy soil (183.7) as compared to clay soil (101.4). Root fresh weight was gradually decreased when salt stress increasing from 50mM to 150mM in both the soil types however highest root fresh weight was recorded in clay soil as compared to sandy soil. Chickpea variety Bhakar-2011 has more root fresh weight (87.3) followed by Thall-2006(78.5), Noor-2013 (77.2) and Noor-2009 (72.8) in clay soil while Bhakar-2011 show maximum root fresh weight (182.5) followed by Noor-2009 (166.7), Noor-2013 (165.5) and Thall-2006 (165.4) in sandy soil. The interaction for priming × salinity has shown significant effect on root fresh weight in both the soil types.

*Shoot dry weight*

Shoot dry weight was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2). The seeds primed with $CaCl_2$ improved the shoot dry weight in sandy soil as compared to control while maximum shoot dry weight was recorded in sandy soil (220.2) as compared to clay soil (28.5). Shoot dry weight was gradually increased when salt stress increasing from 50mM to 150mM in clay soil while in sandy soil shoot dry weight decreased with increase in salinity. Highest shoot dry weight was recorded sandy soil as compared to clay soil. Chickpea variety Noor-2013 has highest shoot dry weight (29.7) followed by Noor-2009 (28.4) Thall-2006 (27.5) and Bhakar-2011 (27.4) in clay soil while Thall-2006 show maximum shoot dry weight (264.1) followed by Bhakar-2011 (220.2), Noor-2013 (195.3) and Noor-2009 (186.6) in sandy soil. The interaction for priming × salinity has shown significant effect on shoot dry weight in both the soil types.

*Root dry weight*

Root dry weight was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2). The seeds primed with $CaCl_2$ produce high dry weight of root in both the soils as compared to control while maximum root dry weight was recorded in sandy soil (21.6) as compared to clay soil (12.7). Root dry weight was gradually decreased when salt stress increasing from 50mM to 150mM in both the soil types however highest root dry weight was recorded in sandy soil as compared to clay soil. Chickpea variety Bhakar-2011 has more root dry weight (11.2) followed by Noor-2013(10.2), Thall-2006(9.5) and Noor-2009 (8.3) in clay soil while Bhakar-2011 has more root dry weight (20.9) followed by Noor-2013(19.5), Thall-2006(19.3) and Noor-2009 (18.4) in sandy soil. The interaction for priming × salinity has shown significant effect on root dry weight in both the soil types.

*Shoot to root dry weight ratio*

Shoot to root dry weight ratio was not significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 2 & 4). The seeds primed with $CaCl_2$ showed low ratio of shoot to root dry weight in both the soils as compared to control. While maximum shoot to root dry weight ratio was recorded in sandy soil (13.6) as compared to clay soil (7.4). Shoot to root dry weight ratio was gradually increased when salt stress increasing from 50mM to 150mM in clay soil while decreased in sandy soil. Highest shoot to root dry weight ratio was recorded sandy soil as compared to clay soil. Chickpea varieties had significant effect on shoot to root dry weight ratio (Table. 3). Chickpea variety Noor-2009 has more shoot to root dry weight ratio (8.4) followed by Thall-2006 (7.2), Noor-2013 (7.1) and Bhakar-2011 (6.0) in clay soil while Thall-2006 show

maximum shoot to root dry weight ratio (13.9) followed by Noor-2013 (13.8), Bhakar-2011(11.7) and Noor-2009 (10.8) in sandy soil. The interaction for priming × salinity has shown non-significant effect on shoot to root dry weight ratio in both the soil types.

*$Na^+$ concentration of shoot*

$Na^+$ concentration of shoot was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ have less amount of shoot $Na^+$ concentration in both the soils. $Na^+$ concentration of shoot was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest $Na^+$ concentration of shoot was recorded in sandy soil as compared to clay soil. Chickpea variety Noor-2009 has more $Na^+$ concentration of shoot (29.3) followed by Noor-2013 (29.0), Thall-2006(28.6) and Bhakar-2011(28.0) in clay soil while Noor-2013 show maximum $Na^+$ concentration of shoot (28.7) followed by Bhakar-2011(28.6), Thall-2006(28.3) and Noor-2009 (28.1) in sandy soil. The interaction for priming × salinity has shown significant effect on $Na^+$ concentration of shoot in clay soil while for sandy soil types it was non-significant.

*$K^+$ concentration of shoot*

$K^+$ concentration of shoot was significantly affected by priming, salinity levels for chickpea genotypes under clay and sandy soils (Table 3). The seeds primed with $CaCl_2$ has more amount of shoot $K^+$ concentration in both the soils as compared to control while maximum $K^+$ concentration of shoot was recorded in clay soil (30.6) as compared to sandy soil (27.5). $K^+$ concentration of shoot was gradually decreased when salt stress increasing from 50mM to 150mM in clay soil however in sandy soil it was increased. Highest $K^+$ concentration of shoot was recorded in clay soil as compared to sandy soil. Chickpea variety Noor-2013 has more $K^+$ concentration of shoot (29.6) followed by Thall-2006(29.4), Bhakar-2011(29.2) and Noor-2009 (29.0) in clay soil while Noor-2013 show maximum $K^+$ concentration of shoot (27.7) followed by Bhakar-2011(25.4), Thall-2006(25.3) and Noor-2009 (24.4) in sandy soil. The interaction for priming × salinity has shown significant effect on $K^+$ concentration of shoot in both types of soils.

*$Na^+$ concentration of root*

$Na^+$ concentration of root was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ has low concentration of root $Na^+$ in both the soils as compared to control while maximum $Na^+$ concentration of root was recorded in sandy soil (32.2) as compared to clay soil (21.3). $Na^+$ concentration of root was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest $Na^+$ concentration of root was recorded sandy soil as compared to clay soil. Chickpea variety Noor-2009 has more $Na^+$ concentration of root (21.3) followed by Noor-2013 (20.7), Thall-2006 (20.3) and Bhakar-2011 (19.7) in clay soil while Noor-2013 and Noor-2009 show maximum $Na^+$ concentration of root (32.3) followed by Bhakar-2011 (32.2) and Thall-2006 (31.6) in sandy soil. The interaction for priming × salinity has shown significant effect on $Na^+$ concentration of root in both the soil types.

*$K^+$ concentration of root*

$K^+$ concentration of root was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ have high concentration of root $K^+$ in both the soils as compared to control while maximum $K^+$ concentration of root was recorded in sandy soil (26.2) as compared to clay soil (12.3). $K^+$ concentration of root was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest $K^+$ concentration of root was recorded sandy soil as compared to clay soil. Chickpea variety Bhakar-2011 has more $K^+$ concentration of root (12.5) followed by Thall-2006 (12.3), Noor-2013 (12.0) and Noor-2009 (11.6) in clay soil while Noor-2013 show maximum $K^+$ concentration of root (26.4) followed by Bhakar-2011 (26.1), Thall-2006 (24.7) and Noor-2009 (24.5) in sandy soil. The interaction for priming × salinity has shown non-significant effect on $K^+$ concentration of root in both the soil types.

*Shoot $Na^+/K^+$ ratio*

Shoot $Na^+/K^+$ ratio was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ has low ratio of shoot $Na^+/K^+$ in both the soils as compared to control while maximum shoot $Na^+/K^+$ ratio was recorded in sandy soil (1.2) as compared to clay soil (1.0). Shoot $Na^+/K^+$ ratio was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest shoot $Na^+/K^+$ ratio was recorded sandy type soil as compared to clay soil. Chickpea genotypes Noor-2009 and Noor-2013 has more shoot $Na^+/K^+$ ratio (1.0) followed by Thall-2006 and Bhakar-2011 (0.9) in clay soil while Noor-2009 show maximum shoot $Na^+/K^+$ ratio (1.3) followed by Bhakar-2011, Thall-2006 (1.2) and Noor-2013 (1.1) in sandy soil. The interaction for priming × salinity has shown non-significant effect on shoot $Na^+/K^+$ ratio in both the soil types.

*Root $Na^+/K^+$ ratio*

Root $Na^+/K^{++}$ ratio was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ has low ratio of root $Na^+/K^+$ in both the soils as compared to control while maximum root $Na^+/K^+$ ratio was recorded in clay soil (1.9) as compared to clay soil (1.3). Root $Na^+/K^+$ ratio was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest root $Na^+/K^+$ ratio was recorded clay soil as compared to sandy soil. Chickpea variety Noor-2013 has more root $Na^+/K^+$ ratio (1.9) followed by Bhakar-2011, Thall-2006 and Noor-2009 (1.7) in clay soil while Thall-2006 and Noor-2009 show maximum root $Na^+/K^+$ ratio (1.3) followed by Bhakar-2011 and Noor-2013 (1.2) in sandy soil. The interaction for priming × salinity has shown non-significant effect on root $Na^+/K^+$ ratio in both the soil types.

*Leaf proline content*

Leaf proline content was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3 & 5). The seeds primed with $CaCl_2$ low concentration of leaf proline in both the soils as compared to control while maximum leaf proline content were recorded in clay soil (0.7) as compared to sandy soil (0.6). Leaf proline content was gradually increased when salt stress increasing from 50mM to 150mM in both the soil types however highest leaf proline content was recorded clay soil as compared to sandy soil.

Chickpea variety Bhakar-2011, Thall-2006 and Noor-2013 has more leaf proline content (0.7) followed by Noor-2009 (0.6) in clay soil while in sandy soil all genotypes have same value (0.5) of leaf proline. The interaction for priming × salinity has shown significant effect on leaf proline content in both the soil types.

*Chlorophyll content*

Chlorophyll content was significantly affected by priming, salinity levels for chickpea genotypes under clayey and sandy soils (Table 3). The seeds primed with $CaCl_2$ improved the chlorophyll content in clay soil but declined in sandy soil as compared to control. Maximum chlorophyll content was recorded in clay soil (78.7) as compared to sandy soil (75.3). Chlorophyll content was gradually decreased when salt stress increasing from 50mM to 150mM in clay soil however in sandy soil it was increased. Highest chlorophyll content was recorded clay soil as compared to sandy soil. Chickpea variety Noor-2009 has more chlorophyll content (77.5) followed by Bhakar-2011 (77.3), Thall-2006 (77.1) and Noor-2013 (75.7) in clay soil while Thall-2006 show maximum chlorophyll content (76.6) followed by Bhakar-2011, Noor-2009 (76.0) and Noor-2013 (73.2) in sandy soil. The interaction for priming × salinity has shown significant affect on chlorophyll content in sandy soil but non-significant in clay soil type.

## IV. DISCUSSION

Salinity has been shown to decrease in shoot length and root length gradually while priming enhanced length as compared to control in both types of soils. The reduction in root and shoot development with increased salinity due to toxic effects of the $Na^+$ and $Cl^-$ used and disturbed uptake of nutrient. Salt stress inhibited the root and shoot length but root length was more affected than shoot length. The shoot and root length is the most important factors for salt stress because it directly receives salts from roots and roots receive salts from soil therefore shoot and root length provides an important clue to the response of plants to salinity (Jamil and Rha, 2004). The salt ions may cause inhibition of plant growth as these ions have inhibitory effect. Khan et al. (2017) also approved that seed primed with GA promotes shoot length in chickpea. Similar results were achieved by Anwar et al. (2021) who determined that priming of gram seed considerably improved plant height under saline stress. Saline soil might inhibit cell division, cell elongation as mentioned by Radi et al. (2013). Pre sowing seed treatment was effective in subsiding the negative effects of salinity on plant height of chickpea. Basra et al. (2002) described that shoot length was encouraging in wheat by pre sowing seed treatment. Basra et al., (2005) also found that priming proved to be more effective than control for plant height. The findings of the present study are in accord with findings of Jamil et al. (2007) who reported that the root growth was more adversely affected compared to shoot growth by salinity. The roots of seedling were more sensitive than the shoots (Nakamura 2021). Actually, a higher decrease of shoot length as compared to root in salt stressed plants is a common evidence in many species (Munns, 2002), and obtained similar results in three Brassica species including *Brassica oleracea* and *Brassica napus* (Jamil et al., 2005). Similarly dry weight basis found that the shoot/root ratio was increased during priming (Arif et al., 2019). Similarly, Basra et al., (2002) also demonstrated that shoot length was increased in wheat seeds subjected to priming. On the other hand, Jamil et al., (2006) observed an increase of shoot/root ratio in two Brassica species, including Brassica oleracea. Kaya and Ipek (2003) observed a similar result in safflower.

The fresh and dry biomass for shoot and root was significantly influenced by salinity and pre sowing seed treatment in both types of soils. The seed priming of chickpea increased shoot, root biomass, number of seeds, 1000-seed weight and or number of pods per plant (kaur et al. 2002). The fresh and dry biomass of roots and shoots of primed plants were higher as compared to the biomass of control one in saline soil (Sarwar et al., 2006). These results confirm the findings of Basra et al., (2005) who reported that seed priming increased root and shoot fresh weights. Grain yield, fresh and dry shoot biomass of spring wheat improved by different priming treatments (Iqbal et al., 2006). Significant effects of priming on broad bean seeds (*Vicia faba*) were observed for different growth parameters (Sallam, 1999). The harvest index was not significantly affected by salinity and priming in both types of saline soils.

Significant results of $Na^+$ and $K^+$ concentration of shoot and root was observed in chickpea genotypes affected by salinity in both types of soils. Priming promotes $K^+$ while $Na^+$ concentration is demoted. The $Na^+$ concentration gradually increases with increase in salt level which leads to Na+ toxicity ultimately. The increase in salinity levels negatively influence $K^+$ concentration and there is direct competition between $Na^+$ and $K^+$ for root transporters as the physiochemical properties of both ions are same. Therefore, $K^+$ uptake by the roots decreased due to inhibitory effect $Na^+$ high concentration (Ghars et al., 2008). The most important ion for plant growth is potassium $K^+$ which is required as osmotic regulator and enzyme cofactor (Kamel and El-Tayeb, 2004). Competition was found at transport and catalytic sites due to the similarities between $Na^+$ and $K^+$ which results in binding of $K^+$ and maintain a high cytosolic $K^+$:$Na^+$ ratio which is helps in salt tolerance (Mian et al., 2011; Huq and Larher, 1985). It is observed that the $Na^+$:$K^+$ ratio increased with the salinity which means that accumulation of $Na^+$ than $K^+$ under salt stress which is expected for a salt sensitive crop like chickpea (Hirich et al, 2014). The results obtained in this research support the observations of Amini and Ehsanpour (2005) and Saleh (2011) who stated that $Na^+$: $K^+$ increased parallel to salinity level in tomato and cotton vegetative plant parts. In halophytes the $Na^+$:$K^+$ ratio independent of external salt level because these crops accumulate $K^+$ rather than $Na^+$ in order to avoid toxic effects of salinity (Samiullah and Bano, 2011). The low ratio of $K^+/Na^+$ interrupt biosynthesis of cell protein while competition build up between $Na^+$ and $K^+$ for binding sites of cellular function, but $Na^+$ fails in enzyme activation like $K^+$ ions (Tester and Davenport 2003).

The effect of priming and salinity was significantly influenced chlorophyll concentration in both types of saline soils. The present study revealed that high salinity stress has a negative influence on the growth and yield of chickpea due to decrease chlorophyll. It shows that salinity limits photosynthesis in chickpea. The suppressive effect of salinity on chlorophyll content may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the degradation of chlorophyll pigments as well as damaging the photosynthetic apparatus (Radi et al., 2013). Plants having salt tolerance show little increase or no change in chlorophyll levels in salt stress

while salt sensitive plants show clear reduction in chlorophyll value (Stepien and Johonson, 2009; Ashraf and Harris, 2013). Reduction in plant pigments due to salinity is considered because of slow synthesis or fast breakdown of the pigments in cells (Ashraf, 2005). The Cl− accumulation contribute to lowering of leaf chlorophyll in Faba bean (Tavakkoli et al., 2010). The pre sowing melatonin application positively affected the photosynthetic pigments of plants (Dawood, and El-Awadi. 2015). The result of this research are in line with XD et al. (2010) who reported that melatonin treatment before sowing played an important role in preservation of chlorophyll and photosynthesis. In addition, Arnao and Hernández-Ruiz (2009) cited that melatonin treatments delayed senescence in barley and control chlorophyll degradation. The pretreatment of *Malus hupehensis* Rehd with different chemicals under high salinity conditions significantly improved plant growth and photosynthetic capacity (Li et al. 2012). Zhang *et al.* (2013) stated that priming with melatonin significantly prevent chlorophyll degradation in cucumber seedlings. The exogenous application of chemicals molecule significantly reduced chlorophyll degradation and increased the photosynthetic efficiency of many plants under salinity (Tan et al., 2012; Wang et al., 2013).

The effect of priming and salinity was significantly influenced concentration of proline in both types of saline soils. The present study revealed that high salinity stress has results more and more proline in leaves and stem of chickpea genotypes which show severity of stress. It shows that proline overcome the effects of salinity on the other hand priming discourage proline production which means that priming neutralized adverse effects of salinity on chickpea genotypes. It is reported that osmoregulation adjustment and anti-oxidative activity by proline provide protection against salt stress (Matysik et al., 2002). Previous examinations proved the same results like this study that proline accumulation in leguminous plants occur due to salt stress (El Moukhtari et al., 2020). The several plants showed accumulation of proline in when exposed to high soil salinity (Heidari et al., 2011). Similarly proline act as a mediator of osmotic adjustment thus stabilizingg the effect of salt accumulated plant cells. Salinity encourage proline accumulation in chickpea (Eyidogan and Öz, 2007). New amino acids produced by plants in response to salt stress that help them to overcome negative impacts of salinity (Goudarzi and Pakniyat, 2009). Among those amino acids, one important amino acid proline is very important to accumulates in large quantities in response to salinity to protect the cell (Shafi et al., 2011). The proline contents increased parallel with the increase in salinity (Rasool et al. 2012). Moreover, concentration of proline has been reported to rise under NaCl stress in peas (Ahmad et al., 2013), lentil (Misra and Saxena, 2009), tomato (Ali et al., 2011; Amini and Ehsanpour, 2005), pepper (Chookhampaeng, 2011), sugar beet (Farkhondeh et al., 2012), rice and maize (Turan et al., 2009).

*B. Use of Simulation software*

Data was collected on different parameters using statistical software (Statistics 8.1) for analyses. This analysis was carried out for obtaining means and applying least significant difference tests.

## II. GET PEER REVIEWED

Senior Professor Reviewed the paper and found satisfactory for submission for publication in any Journal.

## III. CONCLUSION

In comparison of soil types, sandy soil had high shoot & root fresh weight, shoot & root dry weight, shoot to root dry weight ratio, increased shoot $Na^+$ and $K^+$ concentration, shoot $Na^+/K^+$ ratio, root $Na^+/K^+$ ratio, proline and chlorophyll as compared to clay soil. Among different salinity levels high salt stress (150 mM) reduced shoot and roots length, shoot & root fresh and dry weight and chlorophyll content. Primed seeds had increased shoot and root length, shoot & root fresh and dry weight, increased shoot and root, $K^+$ concentration, and chlorophyll content. Among different chickpea genotypes, Thall-2006 had maximum root length, shoot fresh weight, shoot dry weight, and root $Na^+$ concentration. It is recommended that priming with $CaCl_2$ promoted tolerance in Thall-2006 chickpea under the saline condition in sandy soil.

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**Table 1. Average maximum, minimum, temperature (ºC), rainfall (mm), humidity (%) and solar radiation ($MJm^{-2}day^{-1}$) of experimental site.**

| Months | maxTemp | Min Temp | Rain fall | Solar Radiation | Humidity |
|---|---|---|---|---|---|
| October | 31.2 | 17.7 | 00.6 | 05.4 | 63 |
| November | 24.3 | 08.4 | 00.9 | 04.8 | 67 |
| December | 20.5 | 05.0 | 00.0 | 04.3 | 80 |
| January | 19.5 | 04.3 | 01.1 | 04.1 | 84 |
| February | 21.3 | 09.0 | 02.5 | 04.7 | 70 |
| March | 23.8 | 11.5 | 03.9 | 05.3 | 69 |
| April | 30.4 | 17.5 | 03.8 | 07.0 | 57 |
| May | 35.4 | 21.7 | 01.3 | 08.0 | 52.6 |

**Table 2. Root length, shoot length, shoot fresh weight, root fresh weight, shoot, dry weight, root dry weight, shoot/root dry biomass ratio as affected by priming, salinity levels, soil types and chickpea genotypes.**

| Soil Types | Shoot length | Root Length | Shoot Fresh Weight | Root Fresh Weight | Shoot dry Weight | Root dry weight | Shoot & root dry biomass ratio |
|---|---|---|---|---|---|---|---|
| So1= Clay | 31.14 a | 9.24 a | 1728 a | 78.91 a | 216.53 | 19.52 | 17.274 |
| So2=Sandy | 27.52 b | 9.02 b | 379.3 b | 19.52 b | 56.30 | 9.81 | 12.542 |
| **LSD** | **1.8582** | **0.3548** | **180.97** | **7.9408** | **NS** | **NS** | **NS** |
| **Priming** | | | | | | | |
| P1= 0 | 30.71 a | 9.4583 a | 1130.1 a | 61.48 a | 149.79 | 17.12 | 20.049 |
| P2=Primed | 27.95 b | 7.80 b | 977.3 b | 36.96 b | 123.04 | 12.22 | 9.767 |
| **LSD** | **1.8582** | **0.7020** | **180.97** | **7.9408** | **NS** | **NS** | **NS** |
| **Salinity** | | | | | | | |
| S1= 0 mM | 35.771 a | 10.13 a | 1344.0 a | 67.77 a | 187.63 a | 20.56 a | 26.658 |
| S3= 50 mM | 28.98 b | 8.52 b | 1196.6 a | 54.70 b | 166.10 a | 14.27 b | 11.688 |
| S2= 100 mM | 27.29 bc | 8.27 b | 886.9 b | 40.77 c | 103.69 b | 13.52 b | 11.374 |
| S4= 150 mM | 25.27 c | 7.60 b | 787.3 b | 33.63 c | 88.25 b | 10.3 c | 9.911 |
| **LSD** | **0.9928** | **0.9928** | **255.94** | **11.230** | **33.522** | **1.9653** | **NS** |
| **Genotypes** | | | | | | | |
| V1=Bhakar-2011 | 30.50 | 8.83 | 1150.6 | 54.08 | 159.83 a | 16.06 a | 29.530 |
| V2=Thall-2006 | 29.10 | 8.58 | 1064.5 | 48.92 | 138.73 ab | 14.83 ab | 10.762 |
| V3=Noor-2009 | 28.91 | 8.56 | 1027.3 | 48.312 | 125.08 b | 14.41 ab | 10.516 |
| V4= Noor-2013 | 28.79 | 8.54 | 972.3 | 45.56 | 122,02 b | 13.35 b | 8.823 |
| **LSD** | **NS** | **NS** | **NS** | **NS** | **33.522** | **1.9653** | **NS** |

Means of the same category followed by different letters are significantly different at 5 % level of probability using LSD test.

**Table 3. Shoot $Na^+$ conc. root $Na^+$ conc. shoot $K^+$, root $K^+$ conc., shoot $Na^+/K^+$ ratio, root $Na^+/K^+$ ratio, chlorophyll and proline as affected by priming, salinity levels, chickpea genotypes under clay and sandy soil types.**

| Soil Types | Shoot $Na^+$ Cont. | Root $Na^+$ Cont. | Shoot $K^+$ Cont. | Root $K^+$ Cont. | Shoot $Na^+/K^{++}$ Ratio | Root $Na^+/K^{++}$ Ratio | Chlorophyll | Proline |
|---|---|---|---|---|---|---|---|---|
| So1= Clay | 28.69 | 32.13 a | 29.92 | 25.42 a | 1.1943 a | 1.7442 a | 76.91 a | 0.674 a |
| So2=Sandy | 28.40 | 20.45 b | 25.78 | 12.10 b | 0.9656 b | 1.2528 b | 75.43 b | 0.533 b |
| **LSD** | **NS** | **0.6956** | **NS** | **NS** | **0.0650** | **0.0968** | **0.9685** | **0.0205** |
| **Priming** | | | | | | | | |
| P1= 0 | 28.59 | 27.00 | 29.07 | 19.25 | 1.1153 a | 1.5774 a | 76.99 a | 0.6438 a |
| P2=Primed | 28.50 | 25.58 | 25.92 | 18.28 | 1.0447 b | 1.4196 b | 75.34 b | 0.5629 b |
| **LSD** | **NS** | **NS** | **NS** | **NS** | **0.0650** | **0.0968** | **0.9685** | **0.0205** |
| **Salinity** | | | | | | | | |
| S1= 0 mM | 32.69 a | 29.52 a | 29.35 a | 20.47 a | 1.2090 a | 1.8310 a | 77.15 a | 0.7996 a |
| S3= 50 mM | 30.83 b | 28.16 b | 27.33 b | 18.20 b | 1.1654 a | 1.4950 a | 76.21 ab | 0.6683 b |
| S2= 100 mM | 26.77 c | 26.65 c | 27.12 b | 18.60 b | 1.0946 a | 1.4908 b | 76.19 ab | 0.5464 c |
| S4= 150 mM | 23.90 d | 20.85 d | 26.19 b | 17.00 c | 0.8509 b | 1.1771 c | 75.13 b | 0.3987 d |
| **LSD** | **1.1214** | **0.8423** | **1.2183** | **1.044** | **0.1205** | **0.1795** | **1.3697** | **0.0291** |
| **Genotypes** | | | | | | | | |
| V1=Bhakar-2011 | 28.81 | 26.77 | 28.66 a | 19.29 a | 1.1577 | 1.5840 | 76.85 a | 0.6198 |
| V2=Thall-2006 | 28.69 | 26.50 | 27.35 b | 19.20 a | 1.0699 | 1.4979 | 76.77 a | 0.6015 |
| V3=Noor-2009 | 28.47 | 25.97 | 27.29 b | 18.50 ab | 1.0517 | 1.4577 | 76.60 a | 0.5985 |
| V4= Noor-2013 | 28.27 | 25.94 | 26.69 b | 18.02 b | 1.0406 | 1.4544 | 74.44 b | 0.5935 |
| **LSD** | **NS** | **NS** | **1.2183** | **1.044** | **LSD** | **NS** | **1.3697** | **NS** |

Means of the same category followed by different letters are significantly different at 5 % level of probability using LSD test.

**Table 4. Analysis of variance for Root length, shoot length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, shoot/root dry biomass ratio as affected by priming, salinity levels, soil types and chickpea genotypes.**

| Source | DF | Shoot | Root | Shoot fresh | Root fresh | Shoot dry | Root dry | Shoot $Na^+$ | Root $Na^+$ |
|---|---|---|---|---|---|---|---|---|---|

| | | length | length | wt | wt | weight | weight | conc | conc |
|---|---|---|---|---|---|---|---|---|---|
| Soil Types (SO) | 1 | 627.13 | 71.297 | 8.731 E+07 | 169338 | 1232323 | 4524.08 | 3.797 | 6545.01 |
| Priming(P) | 1 | 365.76 | 131.672 | 1120421 | 28861 | 34347 | 1150.52 | 0.422 | 94.92 |
| Salinity (SA) | 3 | 995.82 | 54.852 | 3255608 | 11022 | 110318 | 883.03 | 754.547 | 698.16 |
| Genotypes (V) | 3 | 30.09 | 0.894 | 269380 | 607 | 14230 | 60.18 | 2.936 | 7.94 |
| SO*P | 1 | 1276.17 | 73.755 | 195522 | 20049 | 87 | 31.69 | 27.755 | 3.26 |
| SO*SA | 3 | 299.87 | 8.005 | 2596733 | 6723 | 92192 | 167.06 | 588.311 | 235.19 |
| SO*V | 3 | 117.10 | 0.547 | 219275 | 305 | 14803 | 1.35 | 6.144 | 5.57 |
| P*SA | 3 | 508.85 | 85.019 | 2222648 | 6009 | 48990 | 458.08 | 31.047 | 10.99 |
| P*V | 3 | 80.73 | 5.811 | 959245 | 85 | 17196 | 117.59 | 8.769 | 4.82 |
| SA*V | 9 | 58.26 | 6.343 | 562437 | 302 | 6711 | 102.75 | 3.616 | 6.30 |
| SO*P*SA | 3 | 109.30 | 7.880 | 1266493 | 4502 | 42214 | 384.85 | 13.436 | 27.77 |
| SO*P*V | 3 | 11.03 | 9.394 | 1043155 | 422 | 20713 | 221.01 | 2.658 | 1.49 |
| P*SA*V | 9 | 72.62 | 7.158 | 361994 | 339 | 5564 | 34.35 | 3.542 | 4.91 |
| SO*SA*V | 9 | 32.44 | 5.181 | 555080 | 562 | 7342 | 133.26 | 6.917 | 3.00 |
| SO*P*SA*V | 9 | 32.44 | 5.482 | 473307 | 618 | 7138 | 64.34 | 5.190 | 4.51 |
| Error | 128 | 42.33 | 6.042 | 401536 | 773 | 6889 | 3030.7 | 7.708 | 4.35 |
| Total | 191 | | | | | | | | |
| Co-efficient variance: | | 25 | 28.4 | 46.28 | 56.49 | 60.84 | 449.21 | 9.28 | 7.93 |

**Table 5. Analysis of variance for shoot $Na^+$ conc. root $Na^+$ conc. shoot $K^+$, root $K^+$ conc., shoot $Na^+/K^+$ ratio, root $Na^+/K^+$ ratio, chlorophyll and proline as affected by priming, salinity levels, chickpea genotypes under clay and sandy soil types.**

| Source | DF | Shoot $K^+$ conc | Root $K^+$ conc | Shoot $Na^+/K^+$ | Root $Na^+/K^+$ | Proline | Chlorophyll |
|---|---|---|---|---|---|---|---|
| Soil Types (SO) | 1 | 616.333 | 8520.01 | 2.47191 | 11.5886 | 0.96050 | 105.021 |
| Priming(P) | 1 | 475.021 | 45.05 | 0.23539 | 1.1954 | 0.31363 | 130.021 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Salinity (SA) | 3 | 85.264 | 98.01 | 1.19669 | 3.4234 | 1.40487 | 32.736 |
| Genotypes (V) | 3 | 33.375 | 16.60 | 0.13509 | 0.1746 | 0.00629 | 64.306 |
| SO*P | 1 | 11.021 | 19.38 | 0.02338 | 0.2415 | 0.00500 | 168.75 |
| SO*SA | 3 | 87.597 | 69.05 | 0.96753 | 3.6496 | 0.20972 | 45.146 |
| SO*V | 3 | 16.069 | 9.51 | 0.02404 | 0.0747 | 0.00865 | 8.188 |
| P*SA | 3 | 130.59 | 1.17 | 0.03188 | 0.0253 | 0.08214 | 8.035 |
| P*V | 3 | 17.507 | 2.38 | 0.11077 | 0.0153 | 0.00695 | 6.243 |
| SA*V | 9 | 5.620 | 3.95 | 0.04458 | 0.0681 | 0.00657 | 9.134 |
| SO*P*SA | 3 | 76.646 | 13.81 | 0.06361 | 0.1040 | 0.07349 | 56.319 |
| SO*P*V | 3 | 22.201 | 6.38 | 0.05012 | 0.0298 | 0.00130 | 0.389 |
| P*SA*V | 9 | 15.169 | 7.80 | 0.05236 | 0.0369 | 0.00282 | 7.016 |
| SO*SA*V | 9 | 10.463 | 6.32 | 0.02811 | 0.0786 | 0.01110 | 11.220 |
| SO*P*SA*V | 9 | 8.882 | 5.26 | 0.01373 | 0.0292 | 0.00637 | 6.718 |
| Error | 128 | 9.099 | 6.68 | 0.05359 | 0.1172 | 0.00517 | |
| Total | 191 | | | | | | |
| Co-efficient variance: | | 10.97 | 13.78 | 20.24 | 22.84 | 11.92 | 4.45 |

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