Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Biochemical, physiological, and growth evaluation of different chickpea genotypes under varying salinity regimes

Avaliação bioquímica, fisiológica e de crescimento de diferentes genótipos de grão-de-bico sob vários regimes de salinidade

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Abstract

Biochemical and physiological parameters, growth, and yield of field crops especially salt sensitive crops like chickpea are affected adversely by salinity in arid to semi-arid regions. To investigate the effect of different salinity levels on growth, biochemical and physiological parameters of chickpea genotypes, a pot experiment following CRD, two factor factorial design, was conducted in the glasshouse at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan. Ten (10) kg of soil was filled in each pot and salinity levels were maintained @ S_0= 0 mM NaCl, S_1= 50 mM NaCl, S_2= 100 mM NaCl and S_3= 150 mM by applying NaCl and 5 genotypes of chickpea (KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, and CM-98) were used. At crop maturity, growth parameters, physiological, biochemical, and ionic parameters were measured using standard analysis procedures. Salinity reduced the growth and yield of all genotypes, but the rate of decrease was different among the genotypes tested. From the results, a decrease in K concentration, K/Na ratio, transpiration rate, stomatal conductance, N, and P was observed in all genotypes with the increase in salinity. An increase in salinity level increased the proline content (35.45%), crude protein (42%), H_2O_2 (19%), lipid peroxidation (62%), carbohydrates (23.22%), and Na^+ concentration (137%). The highest level of salinity, 150 mM NaCl has exhibited the highest salinity stress in all parameters. Genotype KK-2 and Bhakkar-11 showed a lower rate of relative decrease in yield (4.5 and 12%), K^+/Na^+ ratio (23.34 and 11.47%), and K^+ concentration (7.9 and 11%), respectively, and the lowest relative increase in Na^+ accumulation (20.3 and 0.48%), @ 50 mM salinity compared to control. Genotype KK-2 and Bhakkar-11 proved better @ 50mM salinity. The findings suggest that the critical level of the salinity must be kept in mind and the salt-tolerant genotypes should be cultivated in salt affected soils.

Keywords: salinity stress, climate change, yield reduction, salt tolerant genotypes.

Resumo

Parâmetros bioquímicos e fisiológicos, crescimento e rendimento de culturas de campo, especialmente culturas sensíveis ao sal, como grão-de-bico, são afetados negativamente pela salinidade em regiões áridas e semiáridas. Para investigar o efeito de diferentes níveis de salinidade no crescimento, parâmetros bioquímicos e fisiológicos de genótipos de grão-de-bico, um experimento em pote seguindo CRD, delineamento fatorial de dois fatores, foi conduzido na estufa do Instituto de Biotecnologia e Engenharia Genética, Universidade de Agricultura, Peshawar, Paquistão. Dez kg de solo foram preenchidos em cada vaso e os níveis de salinidade foram mantidos @ S0 = 0 mM NaCl, S1 = 50 mM NaCl, S2 = 100 mM NaCl e S3 = 150 mM aplicando NaCl e 5 genótipos de grão-de-bico (KK-2, Bhakkar-2011, Bittle-98, Punjab-2008 e CM-98). Na maturidade da cultura, parâmetros de crescimento, parâmetros fisiológicos, bioquímicos e iônicos foram medidos usando procedimentos de análise padrão. A salinidade reduziu o crescimento e a produtividade de todos os genótipos, mas a taxa de decréscimo foi diferente entre os genótipos testados. A partir dos resultados, observou-se diminuição da concentração de K, razão K/Na, taxa de transpiração, condutância estomática, N e P em todos os genótipos com o aumento da salinidade. Um aumento no nível de salinidade aumentou o teor de prolina (35,45%), proteína bruta (42%), H_2O_2 (19%), peroxidação lipídica (62%), carboi- dratos (23,22%) e concentração de Na+ (137%). O nível mais alto de salinidade, 150 mM NaCl, exibiu o maior estresse de salinidade em todos os parâmetros. Os genótipos KK-2 e Bhakkar-11 apresentaram menor taxa de diminuição relativa no rendimento (4,5 e 12%), razão K+/Na+ (23,34 e 11,47%) e concentração de K+ (7,9 e 11%), respectivamente, e menor aumento relativo no acúmulo de Na+ (20,3 e 0,48%), @ 50 mM de salinidade comparado ao controle. Os genótipos KK-2 e Bhakkar-11 se mostraram melhores @ 50mM de salinidade. Os resultados sugerem que o nível crítico de salinidade deve ser mantido em mente e os genótipos tolerantes ao sal devem ser cultivados em solos afetados pelo sal.

Palavras-chave: estresse salino, das alterações climáticas, redução de rendimento, genótipos tolerantes ao sal.

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Received: October 4, 2022 – Accepted: October 9, 2022

1. Introduction

Global climate change such as environmental pollution, scarcity of water, increasing human population, dwindling area available for cultivation, and rising salinization of soil and water characterize the beginning of the twenty-first century. Salinity is one of the most damaging factors in agriculture, causing significant losses in agricultural production, quality, and cultivated land area (Shahbaz and Ashraf, 2013). Most agricultural plants are very susceptible to salt as a result of long-term cultivation in more favorable circumstances (Hu et al., 2021). Soil salinity has affected about one-third of the irrigated arable land and levels are still rising. Salinity deteriorates the pattern of plant growth and development (Lazof and Bernstein, 1998). Saline soil contains sufficient neutral soluble salts and has an electric conductivity (EC) of the saturated soil extract (ECe) in the root zone that exceeds 4 dS m^{-1} (about 40 mM NaCl) at 25 °C. Most agricultural plants' yields are lowered at this ECe while many crops' yields are affected at lower ECe (Jamil et al., 2011). The average yields of all important crops are affected from 20 - 50% by drought and excessive soil salinity. These enormous losses are mostly attributable to drought and excessive soil salinity (Shrivastava and Kumar, 2015). Salinity affects the total farmed and irrigated agricultural fields globally (Saddiq et al., 2021). Salinized regions are growing at a 10% yearly rate due to a variety of factors such as irrigation with saline water, low precipitation, high surface evaporation, poor cultural practices, and weathering of native rocks. More than half of the arable land is estimated to be salinized by 2050 (Jamil et al., 2011; Hirich et al., 2014).

The overabundance of soluble salts in the soil induces osmotic stress, which causes ionic imbalance and particularly ion toxicity (Rauf and Tester, 2008), which can result in plant death (Rout and Shaw, 2001). The increase in crop salt tolerance is a very appealing strategy for dealing with the salinity problem. Hence, the investigation and selection of salt tolerant genotypes within a species over comparatively salt-sensitive ones using traditional selection and breeding approaches are the need of the hour (Singla and Garg, 2005).

Chickpea (*Cicer arietinum* L.) is the world's second most significant edible bean and an essential source of proteins in many nations (Gaur et al., 2012; Varshney et al., 2013). Furthermore, it is frequently utilized as feed and green manure. Chickpea seeds have a protein content of 12.6-29%, a fat content of 2.9-8.8%, and a carbohydrate content of 51-71% (Yadav et al., 2007). However, like many other leguminous crops, chickpea is extremely susceptible to salts (Ashraf and Waheed, 1993). Soil salinity is caused mostly by chloride and sulphate build-up in salty areas, which is a severe limitation in chickpea production in semi-arid arid areas. Although certain soils are naturally saline, secondary salinization is primarily caused by irrigation systems, and this is most dangerous to legume sustainability. Salinity has a wide range of impacts on chickpeas. Under the salty circumstance, seed germination is delayed as well as decreased, and vegetative plant development is restricted (Shaheenuzzamn, 2015). Therefore, tolerance to salinity is the only viable option. Because chickpeas are native to dry regions, they may be adapted to a variety of environmental stresses that consequently provides reasons to develop salt tolerant germplasm with little yield loss, and it may be an effective tool in alleviating the salinity problem to some extent (Rao et al., 2002). Resistance to salt stress is not based on a single feature, but rather on a complex set of genetic, morphological, and physiological properties. Salinity even in moist soils serves as a limiting factor to reduce the availability of water to growing tissue, resulting in what is known as "Physiological Drought." Salinity lowers agricultural production by reducing photosynthesis, respiration, and protein synthesis (Meloni et al., 2003).

Tolerant chickpea genotypes showed improved proline content compared to salt-sensitive genotypes (Kaur et al., 2014). The salinity stress causes hyperosmotic stress and in severe cases causes oxidative stress in plants as well, which is responsible for the generation of reactive oxygen species (ROS) that are deleterious to plants (Ahmad et al., 2012; Azooz et al., 2011) by damaging biomolecules i.e., lipids, proteins, and nucleic acids (Tuteja et al., 2009). Proline serves as an osmolyte and scavenges singlet oxygen and free radicals like hydroxyl ions. It is also considered a redox potential regulator and protects macromolecules such as proteins, and DNA, and reduces enzyme denaturation caused by salts and heat, etc. (Kumar et al., 2010). Although chickpea is susceptible to salt, there is some evidence for salinity resistance in chickpea cultivars. The main objective of this study was to investigate the effect of salinity level on the growth, physiology, sodium and potassium content, and biochemical parameters in different chickpea genotypes as well as to identify threshold salinity levels for chickpea genotypes.

2. Materials and Methods

2.1. Pot experiment

A pot experiment was conducted in the glasshouse at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan. Ten (10) kg of soil was filled in each pot. Five genotypes of chickpeas (KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98) were used. For each genotype, four salinity levels, 0 mM NaCl, 50 mM NaCl, 100 mM NaCl, and 150 mM NaCl were applied. A completely Randomized Design (CRD) was used for the present study and each treatment combination was replicate thrice. Three (03) seeds from each genotype in each pot were maintained till maturity. At maturity, plants were harvested. Growth parameters viz., Root and shoot length, and yield was measured. Root: Shoot (R:S) was calculated by dividing root length (RL) by shoot length (SL).

2.2. Ion analyses

Mature leaves were dried in an oven, cut into small pieces, and weighed, followed by extraction in 5mL of 0.5M HNO_3 by shaking at room temperature for 48 hours (Munns, 2002). The concentrations of N and P were determined using UV Vis Spectrophotometer, while Na and K ions were determined by using a Flame photometer. K^+/Na^+ ratio was calculated by using the data of K^+ and Na^+ content analysis.

2.3. Physiological analyses

2.3.1. Proline content

Proline contents were extracted from 0.2 g of leaf tissues homogenized in 4 mL of an aqueous solution of sulfosalicylic acid by the method described by Bates et al. (1973) and Sairam and Tyagi (2004).

2.3.2. Lipid peroxidation

Lipid peroxidation was measured by observing malondialdehyde (MDA) following the method developed by Ohkawa et al. (1979).

2.3.3. Estimation of H_2O_2 content

The method developed by Bergmeyer and Bernt (1974) was used to determine the H_2O_2 content.

2.3.4. Protein concentration

The dye binding assay as developed by Bradford (1976) using Bovine Serum Albumin as a standard was used for quantification of protein concentration.

2.3.5. Reducing sugars

The presence of reducing sugars was conducted by adding fresh leaf samples into boiling water, followed by the addition of a minute amount of Benedict's reagent (Roberts, 1985), and then allowed to cool. During the next 4-10 minutes, the color of the solution was changed. The Spectrophotometer (UV Visible) was used for quantification.

2.3.6. Physiological parameters

Transpiration rate and stomatal conductance were determined by using Cyrus.

2.4. Statistical analysis

The experiment was laid out in CRD two-factor factorial. The applied treatment combination effects were estimated using a two-way analysis of variance (ANOVA) Steel et al. (1997). The least significant difference (LSD) test ($p<0.05$) was used for comparison among the treatment means. The correlation was computed by using XLSTAT software.

2.5. Quality assurance

Quality Assurance was maintained in all steps of analytical procedures. The reference standard sample was analyzed after five samples batch in each parameter. The chemicals used were analytical grades obtained from Sigma Aldrich along with a certificate of analysis, and these chemicals were chromatographically pure. The centrifuge tubes and laboratory consumables were dipped in 20% nitric acid (HNO_3) solution which was prepared with ultrapure water overnight and then flushed thoroughly with ultrapure water. Determination in Atomic Absorption Spectrophotometer, Flame photometer, and Spectrophotometer was carried out in three replicates, and the reported results were the average of three replicates along with the standard deviation. All determinations were performed at room temperature.

3. Results

3.1. Growth responses to salinity

3.1.1. Shoot Length (SL)

The SL of chickpea genotypes was affected significantly by salinity levels and genotypes as shown in Figure 1a. The data depicted that the maximum SL (67.4 cm) was exhibited in the variety Punjab-2008 in the control treatment (S_0). The data also revealed that the lowest SL was observed in the Bittle-98 genotype at S_3 (150 mM). In all genotypes, the highest SL was recorded in the control treatment and the length dwindled as the salinity levels increased. The trend for SL (cm) was as Punjab-2008> > CM-98 > Bhakkar-2011> KK-2 > Bittle-98.

3.1.2. Root Length (RL)

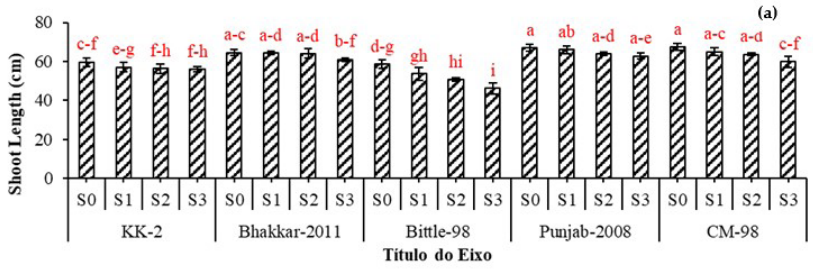
The data for RL variation was significantly affected by different salinity stress levels, similarly, a significant effect was observed among genotypes as shown in Figure 1b. The data depicted that the highest RL was possessed by Punjab-2008 at S_0 (0 mM) i.e., 24.9 cm. The data revealed that the minimum RL (10.73 cm) was shown by Bittle-98 at S_3 (150 mM). The trend for RL (cm) was as Punjab-2008 > Bhakkar-2011 > CM-98 > KK-2 > Bittle-98 at 0 mM (S_0). This trend was changed at the highest salt level (S_3) as Bhakkar-2011 > KK-2 > Punjab-2008 > CM-98 > Bittle-98.

3.1.3. Seed yield (g pot^{-1})

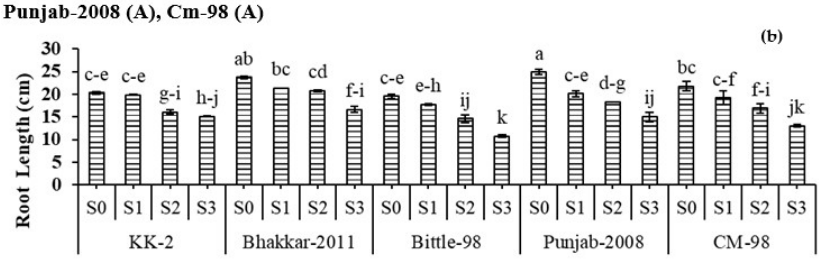
The data regarding grain yield are presented in Figure 2a and it was affected significantly by the different levels of salt application and a differential significant effect was observed among the genotypes ($p \leq 0.05$) studied. The data revealed that the highest grain yield was found in Bhakkar-2011 at S0 (19.8 g pot^{-1}), while the lowest grain yield was found in CM-98 at the highest rate of salinity S_3 (1.83 g pot^{-1}). The trend for grain yield (g pot^{-1}) was Bhakkar-2011 > Punjab-2008 > KK-2 > Bittle-98 > CM-98. Salinity decreased the yield of all genotypes. The lowest percent decrease was recorded in KK-2 (4.5%) and Bhakkar-2011 (12%) at a salinity of 50 mM compared to the respective control.

3.1.4. Root shoot ratio

Root to shoot ratio is calculated using SL and RL and a statistically non-significant effect of salinity was recorded among the genotypes ($p >0.05$). Decreased root shoot ratio was observed in increasing levels of salinity. The values ranged from 0.22 to 0.37 (Figure 2b). The highest values were recorded in Punjab-2008 at S_0 (0 mM), while the lowest values were found in CM-98 at the highest level of salinity stress i.e., S_3 (150 mM). Under salinity stress, KK-2 and Bhakkar-2011 proved tolerant due to their lowest decrease in values compared to their control, while other genotypes showed more decrease compared to these genotypes. The highest values were found in the S_0 level of salinity as compared to all other salinity levels (0 mM NaCl, 50 mM NaCl, 100 mM NaCl) studied.

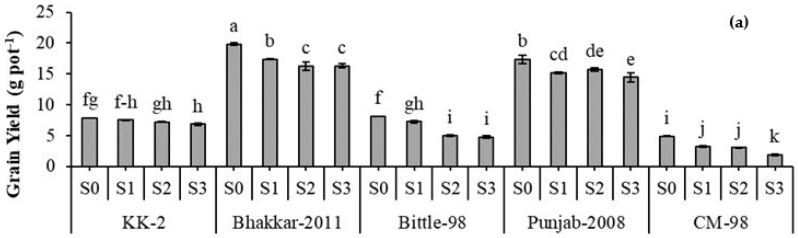


The level of significance for varities are: kk-2 (B), Bhakkar -2011 (A), Bittle-98 (C) , Punjab-2008 (A), Cm-98 (A)



The level of significance for varities are: KK-2 (B), Bhakkar -2011 (A), Bittle-98 (C) , Punjab-2008 (A), CM-98 (B)

Figure 1. Effect of salinity stress on SL (a) and RL (b) of chickpea genotypes: salinity levels=S0: 0 mM NaCl, S1: 50 mM NaCl, S2:100 mM NaCl, S3: 150 mM NaCl. Genotypes= KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98. Data labels represnts the level of significance for multiple comparison between all combination of treatments @ 0.05 probability level. Error bar shows standard error.



The level of significance for varities are: KK-2 (C), Bhakkar -2011 (A), Bittle-98 (D) , Punjab-2008 (B), Cm-98 (E)

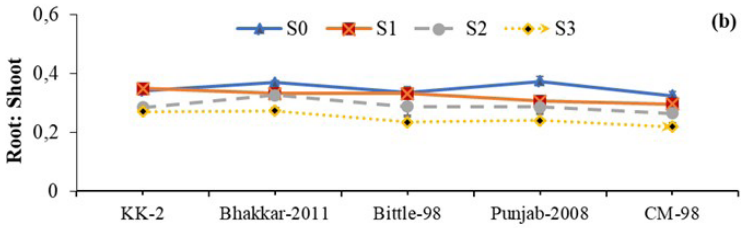


Figure 2. Effect of salinity stress yield (a), and R:S (b) of chickpea genotypes: salinity levels=S0: 0 mM NaCl, S1: 50 mM NaCl, S2:100 mM NaCl, S3: 150 mM NaCl. Genotypes= KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98. Data labels represnts the level of significance for multiple comparison between all combination of treatments @ 0.05 probability level. Error bar shows standard error.

3.2. Biochemical responses

3.2.1. Proline contents

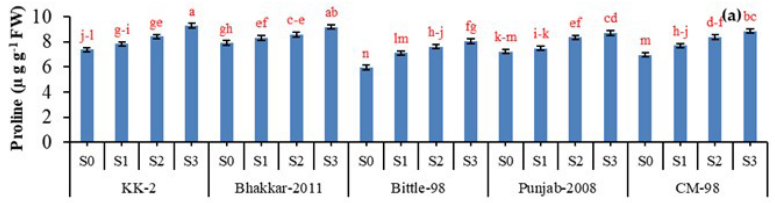
The Proline contents produced in chickpea genotypes were significantly affected ($p \leq 0.05$) by different salinity levels as well as by the genotypes shown in Figure 3a. The proline contents ranged from 5.96 to 9.28 µg g^{-1} FW. The highest proline contents (µg g^{-1} FW) were recorded in KK-2 genotype at S_3 (150 mM), while the lowest proline contents were found in Bittle-98 at the lowest level of salinity stress, i.e., S_0 (0 mM). The highest proline contents were found in the S_3 level of salinity as compared to all other salinity levels (0 mM NaCl, 50 mM NaCl, 100 mM NaCl, 150 mM NaCl). It was recorded that the proline contents were higher where salinity level was higher as compared to the control treatment in all genotypes.

3.2.2. Lipid peroxidation

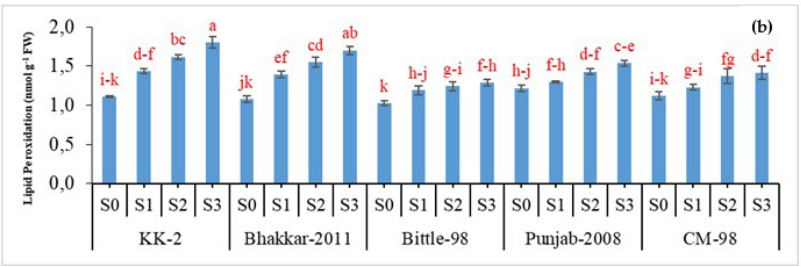
Lipid Peroxidation was significantly affected ($p \leq 0.05$) by salinity levels and the genotypic effect (Figure 3b). The Lipid Peroxidation ranged from 1.02 to 1.82 mmol g^{-1} FW. The highest lipid peroxidation (62%) was produced in the KK-2 genotype at S_3 (150 mM) compared to the control. At the same time, the lowest Lipid peroxidation occurred in Bittle-98 in the control treatment (0 mM) compared to higher levels of salinity. Whereas the highest Lipid peroxidation was found in the S_3 level of salinity as compared to all salinity levels. It was obvious that the Lipid Peroxidation was more produced where salinity level was higher as compared to control treatment in all genotypes.

3.2.3. Hydrogen peroxide (H_2O_2)

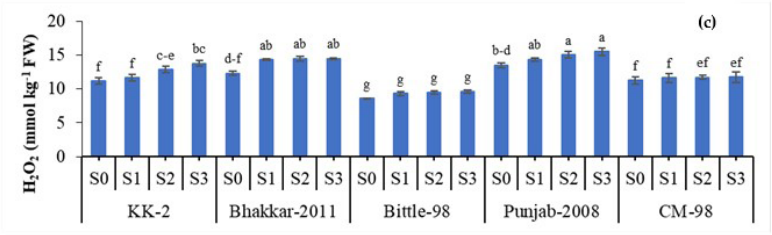
H_2O_2 was significantly ($p \leq 0.05$) affected statistically by salinity levels as well as by the genotypes (Figure 3c). The H_2O_2 contents ranged from 8.59 to 15.47 mmol kg^{-1} FW. The highest H_2O_2 was produced in the Punjab-2008 genotype at S_3 (150 mM), while the lowest H_2O_2 occurred in Bittle-98 in the control treatment (0 mM). The higher H_2O_2 was generated in S_3 and S_2 levels of salinity as



The level of significance for varities are: kk-2 (B), Bhakkar -2011 (A), Bittle-98 (D) , Punjab-2008 (C), Cm-98 (C)



The level of significance for varities are: kk-2 (A), Bhakkar -2011 (AB), Bittle-98 (D) , Punjab-2008 (B), CM-98 (C).



The level of significance for varities are: kk-2 (C), Bhakkar -2011 (B), Bittle-98 (E) , Punjab-2008 (A), CM-98 (D)

Figure 3. Effect of salinity stress on proline content (a), lipid peroxidation (b) and H_2O_2 (c) of chickpea genotypes: salinity levels=S0: 0 mM NaCl, S1: 50 mM NaCl, S2:100 mM NaCl, S3: 150 mM NaCl. Genotypes= KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98. Data labels represnts the level of significance for multiple comparison between all combination of treatments @ 0.05 probability level. Error bar shows standard error.

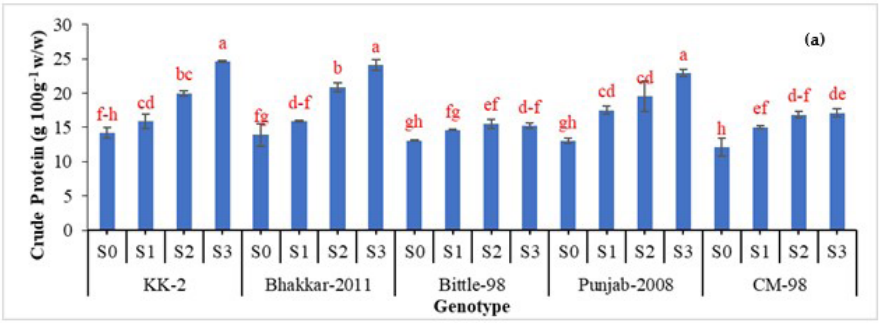
compared to all other salinity levels. The trend for H_2O_2 production was as Punjab-2008 > Bhakkar-2011 > KK-2 > CM-98 > Bittle-98. KK-2 and CM-98 showed the lowest percent increase (1.1 and 3.4%, respectively) at 50mM salinity compared to control and all other salinity levels

3.2.4. Crude protein contents

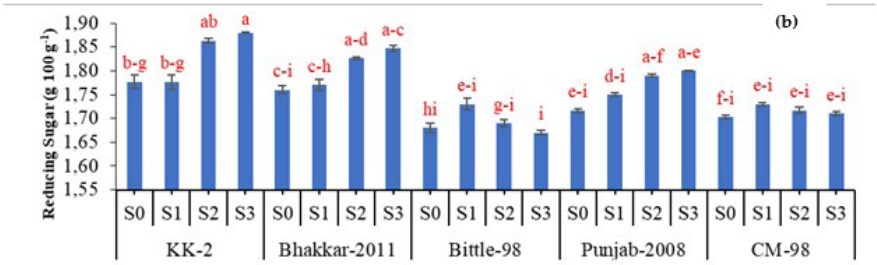
The effect of salinity levels and genotypes was significant on crude protein contents ($p \leq 0.05$) Figure 4a. The crude protein contents ranged from 12.12 to 24.65 g 100 g^{-1}. The highest crude protein contents were produced in the KK-2 genotype at S_3, while the lowest crude protein contents occurred in CM-98 at S_0. The trend for crude protein contents were as KK-2 > Bhakkar-2011 > Punjab-2008 > Bittle-98 > CM-98.

3.2.5. Reducing sugar contents

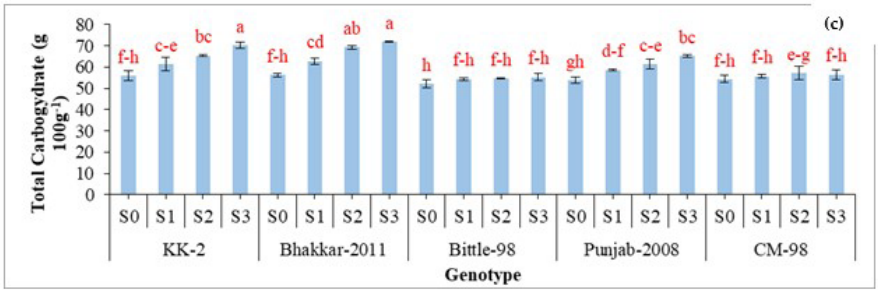
The reducing sugar contents were affected significantly by salinity as well as by genotypes ($p \leq 0.05$) (Figure 4b). The reducing sugar contents ranged from 1.67 to 1.88 g 100 g^{-1}. The highest reducing sugar contents were produced in the KK-2 genotype at S_3 (150 mM), while the lowest



The level of significance for varities are: KK-2 (A), Bhakkar -2011 (AB), Bittle-98 (C) , Punjab-2008 (B), CM-98 (C)



Reducing sugar: The significant varities are KK-2 (A), Bhakkar -2011 (AB), Bittle-98 (C) , Punjab-2008 (B), CM-98 (C)



The level of significance for varities are: kk-2 (A), Bhakkar -2011 (A), Bittle-98 (C) , Punjab-2008 (B), Cm-98 (C)

Figure 4. Effect of salinity stress on crude protein content (a), Reducing sugars (b) and total carbohydrates (c) of chickpea genotypes: salinity levels=S0: 0 mM NaCl, S1: 50 mM NaCl, S2:100 mM NaCl, S3: 150 mM NaCl. Genotypes= KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98. Data labels represnts the level of significance for multiple comparison between all combination of treatments @ 0.05 probability level. Error bar shows standard error.

reducing sugar contents occurred in Bittle-98 at S_3 (150 mM). The trend for reducing sugar contents production was as KK-2 > Bhakkar-2011 > Punjab-2008 > CM-98 >Bittle-98. In the case of the KK-2 genotype, the lowest levels of salinity (0 mM and 50 mM) showed the lowest reducing sugar contents, while at higher levels of salinity (100 mM and 150 mM) higher content of reducing sugars was recorded. There was a meager difference among salinity levels, while the difference was more pronounced among the genotypes.

3.2.6. Total carbohydrate contents

The total carbohydrate contents are significantly affected ($p \leq 0.05$) by genotypes as well as by salinity levels (Figure 4c. The total carbohydrate contents ranged from 72.03 to 52.06 g 100 g^{-1}. The highest total carbohydrate contents were exhibited in Bhakkar-2011 at S_3, while the lowest carbohydrate contents were recorded in the genotype Bittle-98 S_0. In the case of the KK-2 genotype, the increase in total carbohydrate contents increased from 55.9 in control to 70.32 in S_3. In the case of the Bhakkar-2011 genotype, the increment in total carbohydrate contents from control (56.20) to (72.03) in S_3. In the case of the Bittle-98 genotype, the increment in total carbohydrate contents was non-significant as compared to the control treatment, while in the case of Punjab-2008, there was a significant difference among the applied salinity levels for total carbohydrate contents. In CM-98, the total carbohydrate contents were the lowest in the control treatment, while the maximum in the highest salinity level.

3.3. Physiological responses of chickpea genotypes under salinity stress

3.3.1. Stomatal Conductance (SC)

The stomatal conductance (SC) in chickpea genotype leaves is shown in Figure 5a. The statistically significant variation ($p \leq 0.05$) in SC rate among the chickpea genotypes was recorded as well as salinity effect was also significant ($p \leq 0.05$). The statistically significant variation in transpiration rate among the chickpea genotypes was recorded, while the salinity effect was also significant at $p \leq 0.05$. The SC ranged from 0.07 to 0.55 mol H_2O m^{-2} s^{-1} among the genotypes studied. The highest SC occurred in the KK-2 genotype at S_0 (150 mM), while the lowest SC was recorded in Bittle-98 at S_3. Variation existed among the salinity levels as in the case of the KK-2 genotype; the control treatment (0 mM) showed the highest (0.55 mol H_2O m^{-2} s^{-1}) values of stomatal conductance as compared to S_1, S_2, and S_3 levels of salinity. In the case of the Bhakkar-2011 genotype, a similar trend was found as the highest value (0.52 mol H_2O m^{-2} s^{-1}) was shown in the control treatment (0 mM), while in S_1, S_2, and S_3 the values of stomatal conductance were 0.44 mol H_2O m^{-2} s^{-1}, 0.36 mol H_2O m^{-2} s^{-1} and 0.29 mol H_2O m^{-2} s^{-1}, respectively. While, in the case of Bittle-98, there was a decreasing trend of stomatal conductance against salinity levels increment. In the case of Punjab-2008, at S_0, S_1, S_2, and S_3, the stomatal conductance was 0.50 mol H_2O m^{-2} s^{-1}, 0.35 mol H_2O m^{-2} s^{-1}, 0.32 mol H_2O m^{-2} s^{-1} and 0.27 mol H_2O m^{-2} s^{-1}, respectively. In the case of CM-98, the highest value was 0.44 mol H_2O m^{-2} s^{-1} in the control treatment (0 mM), while the lowest was 0.11 mol H_2O m^{-2} s^{-1} at S_3 (150 mM).

3.3.2. Transpiration rate

Statistically significant variation in transpiration rate among the chickpea genotypes was recorded, while the salinity effect was also significant at $p \leq 0.05$, Figure 5b. The transpiration rate ranged from 1.06 to 2.93 mol H_2O m^{-2} s^{-1}. The highest transpiration rate was recorded in KK-2 genotype at S_0 (0 mM), while the lowest transpiration rate occurred in Bittle-98 at S_3 (150 mM).

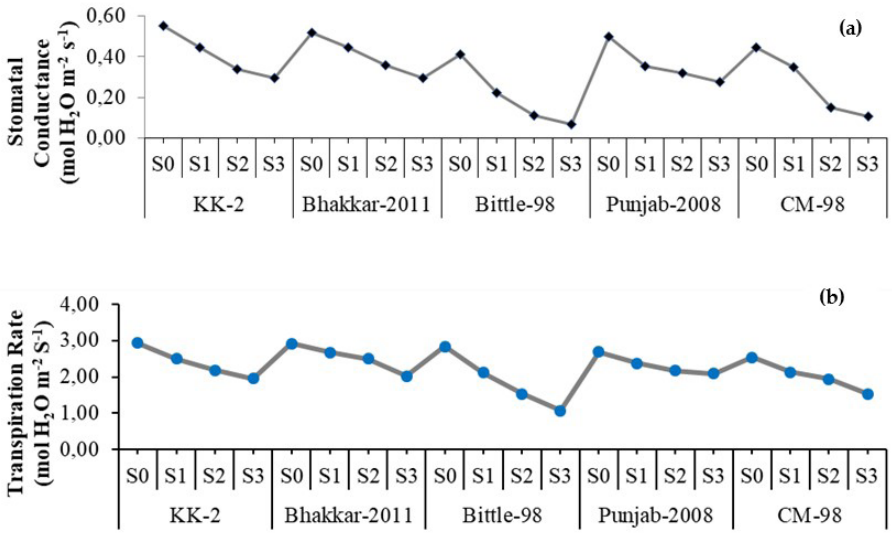


Figure 5. Effect of salinity stress on stomatal conductance (a), and transpiration rate (b) of chickpea genotypes: salinity levels=S0: 0 mM NaCl, S1: 50 mM NaCl, S2:100 mM NaCl, S3: 150 mM NaCl. Genotypes= KK-2, Bhakkar-2011, Bittle-98, Punjab-2008, CM-98. Error bar shows standard error.

The trend for transpiration rate was as Bhakkar-2011 > KK-2 > Punjab-2008 > CM-98> Bittle-98.

3.4. Ionic responses of chickpea genotypes under salinity stress

3.4.1. Nitrogen (N), phosphorus (P), and potassium (K)

The data regarding leaf N, P, and K contents as shown in Table 1, were affected by salinity application significantly at $p \leq 0.05$ while the genotypic effect was significant in N and K^+ whereas P was non-significant ($p > 0.05$). The results showed that the N contents ranged from 13.37 to 27.30 mg kg^{-1} dry weight $plant^{-1}$. The highest Leaf-N was measured in KK-2 and the lowest in Bittle-98. The data regarding Leaf-P revealed that the Leaf-P contents ranged from 1.84 to 2.47 mg kg^{-1} dry weight plant. The highest Leaf-P was measured in KK-2 and the lowest in Bittle-98. The data showed that the K contents ranged from 15.47 to 38.53 mg kg^{-1} dry weight $plant^{-1}$. The highest Leaf-K was measured in KK-2 and the lowest in the genotype Bittle-98. So conclusively for all three NPK, the genotype KK-2 possessed the highest and Bittle-98 as the lowest content of these elements (Table 1). Under the saline condition, K content decreased in all genotypes with increasing salinity levels. The lowest decrease was observed in KK-2 (7.9%) and Bhakkar-2011 (11%) at 50 mM salinity compared to their control.

3.4.2. Na^+ concentration

The Na^+ contents in chickpea were affected significantly by the salinity application, while the genotypic effect was non-significant ($p \leq 0.05$) (Table 1). The Na^+ contents ranged from 7.92 to 24.88 mg kg^{-1}. With the increase in salinity, Na^+ contents increased. The highest Na^+ contents (24.88) were recorded in KK-2 genotype at S_3 (150 mM), while the lowest Na^+ was found in CM-98 at the lowest level of salinity stress i.e., S_0 (0 mM). The highest Na^+ values were found in the S_3 level of salinity as compared to all other salinity levels. KK-2 and Bhakkar-2011 showed the lowest increase (20.3 and 0.48% respectively) at 50mM salinity compared to control.

3.4.3. K^+/Na^+ ratio

The K^+/Na^+ values of chickpea genotypes were affected significantly by salinity application (Table 1), but the genotypic effect was non-significant ($p \leq 0.05$). K^+/Na^+ ratio decreased with the increase in salinity level from S1 to S3. The K^+/Na^+ values ranged from 0.94 to 4.12. The highest K^+/Na^+ values were recorded in CM-98 at S_0 (0 mM), while the lowest K^+/Na^+ values were found in Bittle-98 at the

Table 1. Effect of different salinity levels on leaf N, P, K^+, Na^+ and K^+/Na^+ of chickpea genotypes.

Genotype	Salinity Level	N	P	K^+	Na+	K^+/Na^+
		mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW	mg kg^{-1} DW	----
KK-2	S_0	27.30 ± 0.32 a	2.47 ± 0.01 a	38.5 ± 1.42 a	16.25±0.70 de	2.37±0.02 b-d
	S_1	25.43 ± 0.24 b	2.38 ± 0.02 b-d	35.4 ± 1.23 bc	19.55±0.50 b-d	1.82±0.10 d-h
	S_2	22.63 ± 1.10 de	2.28 ± 0.01 de	33.2 ± 0.50 cd	21.78±1.23 a-c	1.54±0.10 e-i
	S_3	19.70 ± 0.06 gh	2.12 ± 0.02 g-i	29.9 ± 0.70 gh	24.88±1.42 a	1.21±0.04 hi
Bhakkar-2011	S_0	26.80 ± 0.55 ab	2.45 ± 0.01 ab	36.8 ± 0.78 ab	15.65±0.81 de	2.36±0.10 b-d
	S_1	23.53 ± 0.76 d	2.24 ± 0.07 e	32.7 ± 1.11 de	15.72±0.58 de	2.09±0.13 d-f
	S_2	23.47 ± 0.38 d	2.37 ± 0.02 b-d	29.4 ± 0.58 gh	19.05±1.11 b-d	1.55±0.10 e-i
	S_3	20.10 ± 0.31 fg	2.25 ± 0.02 e	29.3 ± 0.81 gh	23.15±0.78 ab	1.27±0.04 g-i
Bittle-98	S_0	18.30 ± 0.42 h	2.15 ± 0.05 f-h	30.2 ± 1.10 e-g	13.52±0.38 e-g	2.23±0.06 de
	S_1	16.63 ± 0.52 i	2.00 ± 0.03 j	24.5 ± 1.35 jk	10.65±0.60 f-i	2.32±0.23 c-e
	S_2	15.07 ± 0.58 i	1.87 ± 0.04 k	19.1 ± 1.0 m	10.85±1.35 f-i	1.85±0.36 d-h
	S_3	13.37 ± 0.42 j	1.84 ± 0.04 k	15.5 ± 0.38 n	16.55±1.10 de	0.94±0.05 i
Punjab-2008	S_0	25.30 ± 0.25 bc	2.35 ± 0.02 bc	30.7 ± 0.52 e-g	9.82±0.49 g-i	3.15±0.21 b
	S_1	23.80 ± 0.29 cd	2.24 ± 0.04 ef	26.7 ± 0.73 ij	13.05±0.83 e-h	2.06±0.08 d-g
	S_2	21.70 ± 0.64 ef	2.20 ± 0.04 e-g	26.7 ± 0.83 ij	13.08±0.73 e-h	2.05±0.05 d-g
	S_3	18.63 ± 0.15 gh	2.11 ±0.04 hi	23.5 ± 0.49 kl	17.05±0.52 d-e	1.38±0.07 f-i
CM-98	S_0	23.70 ± 1.05 cd	2.24 ± 0.01 ef	32.4 ± 0.69 d-f	7.92±0.41 i	4.12±0.27 a
	S_1	19.50 ± 0.72 gh	2.08 ± 0.04 h-j	28.0 ± 0.45 hi	9.12±0.64 hi	3.11±0.22 bc
	S_2	19.13 ± 0.82 gh	2.06 ± 0.02 h-j	22.8 ± 0.64 kl	14.38±0.45 ef	1.58±0.05 d-i
	S_3	16.60 ± 0.60 i	2.04 ± 0.02 ij	21.6 ± 0.41 l	18.75±0.69 cd	1.15±0.05 hi

Values are means of replicates ± SE; values sharing the same letters are non-significant from each other. S0: 0 mM: S1:50 mM; 100 mM; 150 mM of NaCl. N = nitrogen; P = phosphorus; K^+ = potassium; Na = sodium; K^+/Na^+ = potassium to sodium ratio.

highest level of salinity stress i.e., S_3 (150 mM). Under salinity stress, KK-2, Bhakkar-2011, and Bittle-98 showed tolerance by the lowest decrease (23, 11, and -4%) in the K//Na ratio compared to its control while other genotypes showed more decrease compared to KK-2. The highest K^+/Na^+ values were found at the S_0 level of salinity as compared to all salinity levels (0 mM NaCl, 50 mM NaCl, 100 mM NaCl).

3.5. Relationship between traits of chickpea genotypes under salinity stress

Pearson's correlation analysis was carried out to examine the relationship between different parameters of chickpea genotypes. Seed yield had a moderate positive correlation with N (R^2=0.52), P (R^2=0.56,), stomatal conductance (R^2=0.50), and H_2O_2 (R^2=0.63), a significant relationship. Salinity tolerance was strongly associated with an increase in proline content. Proline contents were related moderately and positively to lipids peroxidation (R^2=0.80), protein (R^2=0.75), sugars (R^2=0.52), and H_2O_2 (R^2=0.59) while it was negatively correlated to stomatal conductance, SL, RL, R:S, K^+, and K^+/Na^+ ratio, however, this inverse relationship was non-significant. Potassium is the main determining factor for plant growth under saline conditions. Potassium was highly significant to N (R^2=0.86), P (R^2=0.86), Stomatal conductance (R^2=0.89), and transpiration rate (R^2=0.78) as well as moderately and positively related to RL (R^2=68) and R:S (R^2=0.61). A poorly negative correlation was found between potassium and biochemical as well as physiological parameters like lipids peroxidation and proteins. Na^+ is positively related to proline content as both are indicators of salinity tolerance. They are significantly affected at R^2=0.70. Both Na^+ and proline are negatively related to the K_+/Na_+ ratio.

4. Discussion

4.1. Effect of salinity stress on growth parameters of chickpea genotypes

Characterization of genetic diversity within the population of crop plants is essential for the selection of elite genotypes with superior agronomic traits (Simon et al., 2007). Among all genotypes, the highest SL occurred in the control treatment and the height declined as the salinity levels increased. Root length decreased at the highest salt level (S_3). Salinity decreased the root and SL in all genotypes (Figure 1a-1b). A decrease in RL and SL might occur because of decreased photosynthesis, tissue expansion, and cell division. Similar findings were reported in tomato plants by Zhang et al. (2016). Kafi and Rahimi (2011) also depicted that the main RL and total RL of purslane decreased with salt concentrations. Thus, our findings agree with Kafi and Rahimi (2011). In earlier reports, in beans (Kaymakanova, 2009), groundnut (Mensah et al., 2006), and chickpea (Al-Mutawa, 2003) decreased radicle lengths were observed when salinity was increased. A decrease in RL led to a reduced R:S ratio.

The results about grain yield revealed that the highest grain yield was found in Bhakkar-2011 at S_1 (50 mM), while the lowest grain yield was found in Bittle-98 at S_3 (150 mM). Grain yield decreased with the increase in salinity levels in all genotypes. This might occur because of decreased photosynthetic activity of plants to cope with stress caused by salinity. This decrease in photosynthetic activity leads to a decrease in RL and SL that ultimately results in a lower grain yield of chickpea genotypes. Our results are in line with the findings of Zhang et al. (2016), who recorded a significant reduction in the total yield of tomatoes at the above salinity levels of 5 dS m^{-1}, and this decrease was a 7.2% per unit increase in salinity.

4.2. Effect of salinity stress on biochemical characteristics of chickpea genotypes

In this study, several biochemical characters were studied in detail for the best comparison and selection of superior genotype (s). The proline contents ranged from 5.96 to 9.28 µg g^{-1} FW. The highest proline contents (µg g^{-1} FW) were recorded in KK-2 genotype at S_3 (150 mM), while the lowest proline contents were found in Bittle-98 at the lowest level of salinity stress i.e., S_0 (0 mM). The highest proline contents were found with S_3 level of salinity as compared to all salinity levels (0 mM NaCl, 50 mM NaCl, 100 mM NaCl). Proline protects cells from free radicals in plants under stress conditions (Bandeoğlu et al., 2004). Interestingly, it was noted that the proline contents were produced more where salinity level was higher as compared to the control treatment among all genotypes. Our results also demonstrated a marked increase in proline contents in the shoot tissue under salt stress. Similar results were communicated by Turan et al. (2009). The application of NaCl caused an increase in proline content. Bandeoğlu et al. (2004) argued that proline accumulation in the shoot and root tissue of lentils increased under salt stress. Khan et al. (2002) also reported that proline accumulation increased in root tissue that was exposed to salt stress in rice. The evidence of the study clearly showed that proline accumulation is higher in genotypes that were less tolerant to salinity as compared to genotypes that produced lower proline. Beyaz and Kir (2020) also depicted that the activities of antioxidant enzymes (SOD, CAT (except in shoot), GR, and APX), MDA, and proline accumulation enhanced when subjected to 14 days of salt stress in a medium containing 100 mM NaCl.

It has been verified that salt treatment raised lipid peroxidation or induced oxidative stress in plant tissues. Lipid peroxidation requires active O_2 uptake and involves the production of superoxide radicals (O^{-2}). The other highly reactive chemical species are involved like singlet oxygen ($^1O_2$), hydroxyl free radical (OH), and H_2O_2 all of which initiate lipid peroxidation (Bor et al., 2003). It was prominent that lipid peroxidation was more produced where salinity level was higher as compared to control treatment in all genotypes. The fluctuations in lipid peroxidation in chickpea genotypes under salt stress, which probably come from an increased capacity for

oxygen radical scavenging and maintenance of cellular membranes designate the association between salt tolerance and antioxidant defense system. The highest H_2O_2 was generated in S_3 and S_2 levels of salinity as compared to the other salinity levels (0 mM NaCl, 50 mM NaCl) in our studies. The lower enzyme activity at higher NaCl treatments in many genotypes could be attributed to the inactivation of H_2O_2 produced in different cellular compartments where SOD catalysis the dismutation of superoxide radicals (Yamaguchi et al., 1995).

Protein, Sugar, and carbohydrate content increased with the increase in salinity. However, the protein results were not significant statistically. The genotypic difference was also found in protein sugar and carbohydrate contents in response to salinity. Compatible osmolytes accumulation such as carbohydrates protects plants from stress conditions (Parida et al., 2002). The decreased carbohydrate and sugar usage because of decreased photosynthesis rate might be the cause of their accumulation under saline stress (Khoyerdi et al., 2016). An increase in carbohydrates was reported in Pistachio rootstock (Goharrizi et al., 2020), bermudagrass (Yu et al., 2015), and sorghum (Sayyad-Amin et al., 2016). Results related to protein content showed a non-significant increase. On contrary to this, a decrease in protein content was reported earlier (Goharrizi et al., 2020). They reported that ROS might cause deleterious effects on the proteins, damaging the photosynthetic pigments, and cell membranes (Ahmad et al., 2010). A diffusible molecule, Hydrogen peroxide can enter the cell membrane rapidly and cause cell destruction (Kordrostami et al., 2017).

4.3. Effect of salinity stress on physiological parameters of chickpea genotypes

Stomatal conductance and transpiration rate are important physiological indicators showing the effects of salinity stress on photosynthetic activity. Photosynthetic activity is compromised and shown by the decrease in stomatal conductance and transpiration rate with the increase in salinity level in all genotypes. The number of stomata decreased with NaCl salinity which might reduce the stomatal conductance. These results were also proved by Qiu et al. (2007), who described those stomata factors are the main ones that reduce the transpiration rate with the stress of salinities by decreasing CO_2 conductance.

4.4. Effect of salinity stress on ion concentrations in chickpea genotypes

The highest Leaf-N was measured in KK-2 and the lowest was in Bittle-98. Our findings, however, do not agree with the findings of van Hoorn et al. (2001), who depicted in their studies that the nitrogen content of chickpea was not affected by salinity and variety and showed for stems and leaves an increase till the start of pod formation and then a decrease, similar to soybean and broad bean. In our studies, the highest Leaf-P was measured in KK-2 and the lowest in Bittle-98. This uptake was increased as Abd_Allah et al. (2018) demonstrated that B. subtilis-induced amelioration of salinity stress in plants was directly linked with efficient nutrient uptake (N, P, and K) and extrusion of toxic ions, including Na^+. The excess salt concentration impeded the uptake of essential mineral elements, including N, P, and K, while B. subtilis helped enhance the nutrient uptake in chickpea plants. The highest Leaf K^+ was measured in KK-2 and the lowest was in Bittle-98. With the increase in salinity levels, K^+ levels decreased and Na^+ levels increased. However, different genotypes varied in the K^+ and Na^+ concentrations in their leaves. It was noted that leaf K was present in the high amounts where growth was low as compared to low K^+ bearing genotypes. Chickpea was 'excluding' Na^+ to keep shoot concentrations at a lower level, a common response in crop plants (Rauf and Tester, 2008), but the shoots still showed sensitivity to Na^+ toxicity. Similar results were found by Turner et al. (2013) that 55 genotypes of chickpea were subjected to 0, 40, or 60 mM NaCl added to the soil to determine the variation in salt tolerance, and they found that the sensitive genotypes not only contained higher tissue Na^+ but also slightly more K^+. Variation was also evident for leaf Na^+ concentrations in the two chickpea genotypes under NaCl treatments in salinized soil (Kotula et al., 2015) and hydroponics (Khan et al., 2015).

In chickpea genotypes, the K^+/Na^+ ratio decreased with an increase in salinity, but the decreasing percentage was low in KK-2 and Bhakkar-11 compared to other genotypes. The importance of maintaining an optimal K^+/Na^+ ratio for plant salt tolerance is hardly surprising and is well discussed in the literature (Cuin et al., 2003). It is also obvious that such an optimal ratio can be maintained by either restricting Na^+ accumulation in plant tissues or by preventing K^+ loss from the cells (Garthwaite et al., 2005). The maintenance of high K^+ concentrations in shoots or higher cytosolic K^+/Na^+ ratios contribute to salt tolerance (Kronzucker and Britto, 2011). As a result, while Na+ 'exclusion' is an important mechanism for chickpeas to lower the danger of Na+ toxicity in leaves, it cannot account singularly for the variations in salt tolerance between these genotypes. Other biochemical, physiological, growth, yield, and ionic parameters can be used as an indicator for testing the tolerance of plants to salinity.

4.5. Relationship between traits of chickpea genotypes under salinity stress

Pearson's correlation analysis was carried out to examine the relationship between different parameters of chickpea genotypes (Table 2). Seed yield had a moderate positive correlation with N (R^2=0.52), P (R^2=0.56,), stomatal conductance (R^2=0.50), and H_2O_2 (R^2=0.63), a significant relationship. Salinity tolerance was strongly associated with an increase in proline content (Table 2). Proline contents were related moderately and positively to lipids peroxidation (R^2=0.80), protein (R^2=0.75), sugars (R^2=0.52), and H_2O_2 (R^2=0.59) while it was negatively related to stomatal conductance, SL, RL, R:S, K^+, and K^+/Na^+ however this inverse relationship was non-significant (Table 2).

Table 2. Relationship between growth, yield, physiological and biochemical parameters of chickpea.

V	N	P	K^+	Pr	LP	H_2O_2	Prot	S	StC	T	Y	Car	SL	RL	R:S	K^+/Na^+	Na^+
N	**1**																
P	**0.91**	**1**															
K^+	**0.86**	**0.86**	**1**														
Pr	-0.09	-0.07	-0.17	**1**													
LP	-0.12	-0.06	-0.10	**0.80**	**1**												
H_2O_2	0.39	0.40	0.20	**0.59**	**0.59**	**1**											
Prot	-0.11	0.01	-0.09	**0.76**	**0.84**	**0.58**	**1**										
S	0.28	0.40	0.36	**0.52**	**0.57**	**0.50**	**0.64**	**1**									
StC	**0.86**	**0.83**	**0.89**	-0.34	-0.24	0.23	-0.24	0.16	**1**								
T	**0.72**	**0.76**	**0.78**	-0.40	-0.30	0.19	-0.26	0.12	**0.85**	**1**							
Y	**0.52**	**0.56**	0.37	0.12	0.10	**0.63**	0.18	0.29	**0.50**	0.47	**1**						
Car	0.12	0.22	0.16	**0.73**	**0.83**	**0.62**	**0.79**	**0.69**	0.00	-0.07	0.34	**1**					
SL	**0.54**	0.48	0.40	-0.05	-0.07	0.50	-0.08	0.07	**0.52**	**0.53**	0.43	0.03	**1**				
RL	**0.76**	**0.70**	**0.68**	-0.40	-0.35	0.17	-0.37	0.04	**0.81**	**0.79**	0.52	-0.13	**0.62**	**1**			
R:S	**0.62**	**0.58**	**0.61**	-0.47	-0.38	-0.09	-0.40	0.01	**0.69**	**0.68**	0.37	-0.16	0.16	**0.87**			
K^+/Na^+	0.42	0.31	0.42	**-0.64**	**-0.59**	-0.17	**-0.63**	-0.28	**0.55**	**0.50**	0.04	-0.47	0.48	**0.66**	**0.51**	**1**	
Na^+	0.04	0.16	0.15	**0.70**	**0.70**	0.34	**0.73**	**0.60**	-0.10	-0.17	0.10	**0.73**	-0.25	-0.36	-0.28	**-0.76**	**1**

V = variable; Pr = proline; LP = lipid peroxidation; Prot = protein; S = sugar; Stc = stomatal conduction; T = transpiration; Y = yield; Car = carbohydrates. Correlation values ≥ 0.5 are significant at a 5% level of significance.

5. Conclusions

Salinity reduced the Growth and yield of all genotypes, but the rate of decrease was different among the genotypes. Further, an increase in salinity level increased the proline content, crude protein, H_2O_2, Lipid peroxidation, Carbohydrates, crude protein, and Na^+ concentration, while a decrease in K concentration, K^+/Na^+ ratio, transpiration rate, stomatal conductance, N, and P was observed. The relative decrease in K^+/Na^+ ratio and K^+ concentration, the relative increase in Na+ accumulation, proline content, and other biochemical parameters were lower in salt tolerant cultivars. Salinity at 50mM is recommended as a threshold value for chickpea genotypes. Genotype CM-98 showed sensitivity to salinity stress among all genotypes. Although in these findings, proline content, Na^+, and K^+ accumulations are used as an indication of salinity tolerance, these genotypes should be tested further through genetic analysis.

Acknowledgements

The authors are thankful to the Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar for the provision of lab facilities.

References

ABD_ALLAH, E.F., ALQARAWI, A.A., HASHEM, A., RADHAKRISHNAN, R., AL-HUQAIL, A.A., AL-OTIBI, F.O.N., MALIK, J.A., ALHARBI, R.I. and EGAMBERDIEVA, D., 2018. Endophytic bacterium Bacillus subtilis BERA 71 improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *Journal of Plant Interactions*, vol. 13, no. 1, pp. 37-44. http://dx.doi.org/10.1080/17429145.2017.1414321.

AHMAD, P., JALEEL, C.A., SALEM, M.A., NABI, G. and SHARMA, S., 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Critical Reviews in Biotechnology*, vol. 30, no. 3, pp. 161-175. http://dx.doi.org/10.3109/07388550903524243. PMid:20214435.

AHMAD, P., KHALID, U.R.H., ASHWANI, K., MUHAMMAD, A. and NUDRAT, A.A., 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard Brassica juncea L. *African Journal of Biotechnology*, vol. 11, no. 11, pp. 2694-2703.

AL-MUTAWA, M.M., 2003. Effect of salinity on germination and seedling growth of chickpea Cicer arietinum L. genotypes. *International Journal of Agriculture and Biology*, vol. 5, no. 3, pp. 226-229.

ASHRAF, M. and WAHEED, A., 1993. Responses of some local/exotic accessions of lentil Lens culinaris Medic. to salt stress. *Journal Agronomy & Crop Science*, vol. 170, no. 2, pp. 103-112. http://dx.doi.org/10.1111/j.1439-037X.1993.tb01063.x.

AZOOZ, M.M., ASHRAF, M.Y. and PARVAIZ, A., 2011. Evaluation of salicylic acid SA application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. *International Journal of Plant Physiology and Biochemistry*, vol. 3, no. 14, pp. 253-264.

BANDEOĞLU, E., EYIDOĞAN, F., YÜCEL, M. and AVNI ÖKTEM, H., 2004. Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regulation*, vol. 42, no. 1, pp. 69-77. http://dx.doi.org/10.1023/B:GROW.0000014891.35427.7b.

BATES, L.S., WALDREN, R.P. and TEARE, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, vol. 39, no. 1, pp. 205-207. http://dx.doi.org/10.1007/BF00018060.

BERGMEYER, H.U. and BERNT, E., 1974. UV-assay with pyruvate and NADH. In: H.U. BERGMEYER and K. GAWEHN, eds. *Methods of enzymatic analysis*. New York: Academic Press, vol. 2, pp. 574-579. http://dx.doi.org/10.1016/B978-0-12-091302-2.50010-4.

BEYAZ, R. and KIR, H., 2020. Physio-biochemical analyses in seedlings of sorghum-sudangrass hybrids that are grown under salt stress under in vitro conditions. *Turkish Journal of Biochemistry*, vol. 45, no. 2, pp. 177-184. http://dx.doi.org/10.1515/tjb-2018-0552.

BOR, M., ÖZDEMIR, F. and TÜRKAN, I., 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. *Plant Science*, vol. 164, no. 1, pp. 77-84. http://dx.doi.org/10.1016/S0168-9452(02)00338-2.

BRADFORD, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

CUIN, T.A., MILLER, A.J., LAURIE, S.A. and LEIGH, R.A., 2003. Potassium activities in cell compartments of salt-grown barley leaves. *Journal of Experimental Botany*, vol. 54, no. 383, pp. 657-661. http://dx.doi.org/10.1093/jxb/erg072. PMid:12554708.

GARTHWAITE, A.J., VON BOTHMER, R. and COLMER, T.D., 2005. Salt tolerance in wild Hordeum species is associated with restricted entry of Na+ and Cl− into the shoots. *Journal of Experimental Botany*, vol. 56, no. 419, pp. 2365-2378. http://dx.doi.org/10.1093/jxb/eri229. PMid:16014366.

GAUR, P.M., JUKANTI, A.K. and VARSHNEY, R.K., 2012. Impact of genomic technologies on chickpea breeding strategies. *Agronomy*, vol. 2, no. 3, pp. 199-221. http://dx.doi.org/10.3390/agronomy2030199.

GOHARRIZI, K.J., BAGHIZADEH, A., KALANTAR, M. and FATEHI, F., 2020. Combined effects of salinity and drought on physiological and biochemical characteristics of pistachio rootstocks. *Scientia Horticulturae*, vol. 261, p. 108970. http://dx.doi.org/10.1016/j.scienta.2019.108970.

HIRICH, A., OMARI, H., JACOBSEN, S.E., LAMADDALENA, N., HAMDY, A., RAGAB, R., JELLOUL, A. and CHOUKR-ALLAH, R., 2014. Chickpea 'Cicer arietinum'L. physiological, chemical and growth responses to irrigation with saline water. *Australian Journal of Crop Science*, vol. 8, no. 5, pp. 646-654.

HU, H., ZHANG, M., ZHANG, Y., FU, M., CHEN, J., LI, G., ZHAI, M., MING, X. and NING, T., 2021. Emergetic and cosmic exergy-based ecological assessments of long-term raised field eco-farming systems in saline–alkaline lands. *Ecological Indicators*, vol. 125, p. 107531. http://dx.doi.org/10.1016/j.ecolind.2021.107531.

JAMIL, A., RIAZ, S., ASHRAF, M. and FOOLAD, M.R., 2011. Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences*, vol. 30, no. 5, pp. 435-458. http://dx.doi.org/10.1080/07352689.2011.605739.

KAFI, M. and RAHIMI, Z., 2011. Effect of salinity and silicon on root characteristics, growth, water status, proline content and ion accumulation of purslane Portulaca oleracea L. *Soil Science and Plant Nutrition*, vol. 57, no. 2, pp. 341-347. http://dx.doi.org/10.1080/00380768.2011.567398.

KAUR, P., KAUR, J., KAUR, S., SINGH, S. and SINGH, I., 2014. Salinity induced physiological and biochemical changes in chickpea

Cicer arietinum L. genotypes. *Journal of Applied and Natural Science*, vol. 6, no. 2, pp. 578-588. http://dx.doi.org/10.31018/jans.v6i2.500.

KAYMAKANOVA, M., 2009. Effect of salinity on germination and seed physiology in bean Phaseolus vulgaris L. *Biotechnology, Biotechnological Equipment*, vol. 23, suppl. 1, pp. 326-329. http://dx.doi.org/10.1080/13102818.2009.10818430.

KHAN, H.A., SIDDIQUE, K.H., MUNIR, R. and COLMER, T.D., 2015. Salt sensitivity in chickpea: growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes. *Journal of Plant Physiology*, vol. 182, pp. 1-12. http://dx.doi.org/10.1016/j.jplph.2015.05.002. PMid:26037693.

KHAN, M.H., SINGHA, K.L. and PANDA, S.K., 2002. Changes in antioxidant levels in Oryza sativa L. roots subjected to NaCl-salinity stress. *Acta Physiologiae Plantarum*, vol. 24, no. 2, pp. 145-148. http://dx.doi.org/10.1007/s11738-002-0004-x.

KHOYERDI, F.F., SHAMSHIRI, M.H. and ESTAJI, A., 2016. Changes in some physiological and osmotic parameters of several pistachio genotypes under drought stress. *Scientia Horticulturae*, vol. 198, pp. 44-51. http://dx.doi.org/10.1016/j.scienta.2015.11.028.

KORDROSTAMI, M., RABIEI, B. and KUMLEH, H.H., 2017. Biochemical, physiological and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage. *Physiology and Molecular Biology of Plants*, vol. 23, no. 3, pp. 529-544. http://dx.doi.org/10.1007/s12298-017-0440-0. PMid:28878492.

KOTULA, L., KHAN, H.A., QUEALY, J., TURNER, N.C., VADEZ, V., SIDDIQUE, K.H., CLODE, P.L. and COLMER, T.D., 2015. Salt sensitivity in chickpea (Cicer arietinum L.): ions in reproductive tissues and yield components in contrasting genotypes. *Plant, Cell & Environment*, vol. 38, no. 8, pp. 1565-1577. http://dx.doi.org/10.1111/pce.12506. PMid:25615287.

KRONZUCKER, H.J. and BRITTO, D.T., 2011. Sodium transport in plants: a critical review. *The New Phytologist*, vol. 189, no. 1, pp. 54-81. http://dx.doi.org/10.1111/j.1469-8137.2010.03540.x. PMid:21118256.

KUMAR, V., SHRIRAM, V., KISHOR, P.B.K., JAWALI, N. and SHITOLE, M.G., 2010. Enhanced proline accumulation and salt stress tolerance of transgenic indica rice by over-expressing P5CSF129A gene. *Plant Biotechnology Reports*, vol. 4, no. 1, pp. 37-48. http://dx.doi.org/10.1007/s11816-009-0118-3.

LAZOF, D.B. and BERNSTEIN, N., 1998. The NaCl induced inhibition of shoot growth: the case for disturbed nutrition with special consideration of calcium. *Advances in Botanical Research*, vol. 29, pp. 113-189. http://dx.doi.org/10.1016/S0065-2296(08)60311-0.

MELONI, D.A., OLIVA, M.A., MARTINEZ, C.A. and CAMBRAIA, J., 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, vol. 49, no. 1, pp. 69-76. http://dx.doi.org/10.1016/S0098-8472(02)00058-8.

MENSAH, J.K., AKOMEAH, P.A., IKHAJIAGBE, B. and EKPEKUREDE, E.O., 2006. Effects of salinity on germination, growth and yield of five groundnut genotypes. *African Journal of Biotechnology*, vol. 5, no. 20, pp. 1973-1979.

MUNNS, R., 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment*, vol. 25, no. 2, pp. 239-250. http://dx.doi.org/10.1046/j.0016-8025.2001.00808.x. PMid:11841667.

OHKAWA, H., OHISHI, N. and YAGI, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, vol. 95, no. 2, pp. 351-358. http://dx.doi.org/10.1016/0003-2697(79)90738-3. PMid:36810.

PARIDA, A., DAS, A.B. and DAS, P., 2002. NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, Bruguiera parviflora, in hydroponic cultures. *Journal of Plant Biology*, vol. 45, no. 1, pp. 28-36. http://dx.doi.org/10.1007/BF03030429.

QIU, D.-L., LIN, P. and GUO, S.Z., 2007. Effects of salinity on leaf characteristics and CO_2/H_2O exchange of Kandelia candel L. Druce seedlings. *Journal of Forest Science*, vol. 53, no. 1, pp. 13-19. http://dx.doi.org/10.17221/2081-JFS.

RAO, D.L.N., GILLER, K.E., YEO, A.R. and FLOWERS, T.J., 2002. The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea Cicer arietinum. *Annals of Botany*, vol. 89, no. 5, pp. 563-570. http://dx.doi.org/10.1093/aob/mcf097. PMid:12099530.

RAUF, R. and TESTER, M., 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, vol. 59, no. 1, pp. 651-681. http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911. PMid:18444910.

ROBERTS, F.S., 1985. *Measurement theory*. Cambridge: Cambridge University Press.

ROUT, N.P. and SHAW, B.P., 2001. Salt tolerance in aquatic macrophytes: ionic relation and interaction. *Biologia Plantarum*, vol. 44, no. 1, pp. 95-99. http://dx.doi.org/10.1023/A:1017978506585.

SADDIQ, M.S., IQBAL, S., HAFEEZ, M.B., IBRAHIM, A.M.H., RAZA, A., FATIMA, E.M., BALOCH, H., WOODROW, J.P. and CIARMIELLO, L.F., 2021. Effect of salinity stress on physiological changes in winter and spring wheat. *Agronomy*, vol. 11, no. 6, p. 1193. http://dx.doi.org/10.3390/agronomy11061193.

SAIRAM, R.K. and TYAGI, A., 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*, vol. 86, no. 3, pp. 407-421.

SAYYAD-AMIN, P., BORZOUEI, A., JAHANSOOZ, M.R. and PARSAEIYAN, M., 2016. Root biochemical responses of grain and sweet-forage sorghum cultivars under saline conditions at vegetative and reproductive phases. *Brazilian Journal of Botany*, vol. 39, no. 1, pp. 115-122. http://dx.doi.org/10.1007/s40415-015-0221-3.

SHAHBAZ, M. and ASHRAF, M., 2013. Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences*, vol. 32, no. 4, pp. 237-249. http://dx.doi.org/10.1080/07352689.2013.758544.

SHAHEENUZZAMN, M.D., 2015. Screening of chickpea genotypes against salinity stress. *Bangladesh Journal of Agricultural Research*, vol. 39, no. 4, pp. 605-619. http://dx.doi.org/10.3329/bjar.v39i4.22539.

SHRIVASTAVA, P. and KUMAR, R., 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, vol. 22, no. 2, pp. 123-131. http://dx.doi.org/10.1016/j.sjbs.2014.12.001. PMid:25737642.

SIMON, M.V., BENKO-ISEPPON, A.M., RESENDE, L.V., WINTER, P. and KAHL, G., 2007. Genetic diversity and phylogenetic relationships in Vigna Savi germplasm revealed by DNA amplification fingerprinting. *Genome*, vol. 50, no. 6, pp. 538-547. http://dx.doi.org/10.1139/G07-029. PMid:17632575.

SINGLA, R. and GARG, N., 2005. Influence of salinity on growth and yield attributes in chickpea cultivars. *Turkish Journal of Agriculture and Forestry*, vol. 29, no. 4, pp. 231-235.

STEEL, R.G.D., TORRIE, J.H. and DICKEY, D.A., 1997. *Principles and procedures of statistics: a biometrical approach*. New York: McGraw-Hill. Analysis of variance II: multiway classifications, pp. 204-252.

TURAN, M.A., ELKARIM, A.H.A., TABAN, N. and TABAN, S., 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research*, vol. 4, no. 9, pp. 893-897.

TURNER, N.C., COLMER, T.D., QUEALY, J., PUSHPAVALLI, R., KRISHNAMURTHY, L., KAUR, J., SINGH, G., SIDDIQUE, K.H. and VADEZ, V., 2013. Salinity tolerance and ion accumulation in chickpea Cicer arietinum L. subjected to salt stress. *Plant and Soil*, vol. 365, no. 1-2, pp. 347-361. http://dx.doi.org/10.1007/s11104-012-1387-0.

TUTEJA, N., AHMAD, P., PANDA, B.B. and TUTEJA, R., 2009. Genotoxic stress in plants: shedding light on DNA damage, repair and DNA repair helicases. *Mutation Research*, vol. 681, no. 2-3, pp. 134-149. http://dx.doi.org/10.1016/j.mrrev.2008.06.004. PMid:18652913.

VAN HOORN, J.W., KATERJI, N., HAMDY, A. and MASTRORILLI, M., 2001. Effect of salinity on yield and nitrogen uptake of four grain legumes and on biological nitrogen contribution from the soil. *Agricultural Water Management*, vol. 51, no. 2, pp. 87-98. http://dx.doi.org/10.1016/S0378-3774(01)00114-7.

VARSHNEY, R.K., SONG, C., SAXENA, R.K., AZAM, S., YU, S., SHARPE, A.G., CANNON, S., BAEK, J., ROSEN, B.D., TAR'AN, B., MILLAN, T., ZHANG, X., RAMSAY, L.D., IWATA, A., WANG, Y., NELSON, W., FARMER, A.D., GAUR, P.M., SODERLUND, C., PENMETSA, R.V., XU, C., BHARTI, A.K., HE, W., WINTER, P., ZHAO, S., HANE, J.K., CARRASQUILLA-GARCIA, N., CONDIE, J.A., UPADHYAYA, H.D., LUO, M.C., THUDI, M., GOWDA, C.L., SINGH, N.P., LICHTENZVEIG, J., GALI, K.K., RUBIO, J., NADARAJAN, N., DOLEZEL, J., BANSAL, K.C., XU, X., EDWARDS, D., ZHANG, G., KAHL, G., GIL, J., SINGH, K.B., DATTA, S.K., JACKSON, S.A., WANG, J. and COOK, D.R., 2013. Draft genome sequence of chickpea Cicer arietinum provides a resource for trait improvement. *Nature Biotechnology*, vol. 31, no. 3, pp. 240-246. http://dx.doi.org/10.1038/nbt.2491. PMid:23354103.

YADAV, S.S., LONGNECKER, N., DUSUNCELI, F., BEJIGA, G., YADAV, M., RIZVI, A.H., MANOHAR, M., REDDY, A.A., XAXIAO, Z. and CHEN, W., 2007. Uses, consumption and utilization. In: S.S. YADAV, ed. *Chickpea breeding and management*. Wallingford: CABI, pp. 101-142. http://dx.doi.org/10.1079/9781845932138.004.

YAMAGUCHI, K., MORI, H. and NISHIMURA, M., 1995. A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. *Plant & Cell Physiology*, vol. 36, no. 6, pp. 1157-1162. http://dx.doi.org/10.1093/oxfordjournals.pcp.a078862. PMid:8528608.

YU, J., SUN, L., FAN, N., YANG, Z. and HUANG, B., 2015. Physiological factors involved in positive effects of elevated carbon dioxide concentration on Bermudagrass tolerance to salinity stress. *Environmental and Experimental Botany*, vol. 115, pp. 20-27. http://dx.doi.org/10.1016/j.envexpbot.2015.02.003.

ZHANG, P., SENGE, M. and DAI, Y., 2016. Effects of salinity stress on growth, yield, fruit quality and water use efficiency of tomato under hydroponics system. *Reviews in Agricultural Science*, vol. 4, pp. 46-55. http://dx.doi.org/10.7831/ras.4.46.