Research Article

Growth, Physiological and Biochemical Response of Chickpea Cultivars to Different Levels of Salinity Stress

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Abstract | Salinity stress (SS) is a major environmental constraint that is limiting agricultural productivity across the globe. Therefore, this study aimed at to assess the effect of diverse SS levels on growth, physiological and biochemical traits of chickpea cultivars. The experiment comprised of different levels of salinity stress i.e., 0, 8 and 12 dsm^{-1} and different chickpea cultivars i.e., NIAB-2016, Bittle-2016 and Bhakar-2011. The maximum time to 50% emergence (T_{50}), and mean emergence time (MET) and minimum germination percentage (GP) and emergence index (EI) was recorded when high level of salt stress (12 dsm^{-1}) was imposed, while minimum, T_{50}, and MET and maximum GGP and EI was observed under control conditions. Cultivar Bhaker-2011 took less, T_{50}, and MET and had maximum GP and EI while cultivar NAIB-2016 took maximum, T_{50}, and MET time and had minimum GP and EI. Likewise, maximum plant height (PH: 68.20 cm), root length (RL: 7.70 cm), shoot length (SL: 16.67 cm), root fresh weight (RFW: 0.45 g) and shoot fresh weight (SFW: 5.22 g) were recorded in control condition while minimum was observed under high salt stress. Cultivars Bhaker-2011 had maximum PH (67.70 cm), SL (14.02 cm), and SFW (5.27 g) while cultivar NIAB-2016 had minimum PH (57.10 cm), SL (14.02 cm), and SFW (4.54 g) among the cultivars. The maximum chlorophyll a and b was recorded under normal conditions while lowest was observed under salt stress. Salty stress increased the Na^+ concentration and the activities of SOD, POD and CAT. Moreover, Bhaker-2011 had maximum chlorophyll a, b, and activities of SOD, POD and CAT among the cultivars. In conclusion, Bhaker-2011 appeared as a salt tolerant cultivar that was linked with improved growth, photosynthetic performance and antioxidant activities.

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Introduction

Plants faced different stress during growth cycle which considerably reduces their growth and productivity. Salinity stress (SS) is a challenging abiotic stress which considerably reduced the seed germination, plant growth and metabolic activities (Carbajal-Vázquez *et al*., 2022) and productivity (Rajabi *et al*., 2020). The high concentration of salts induced detrimental impacts on plant physiology and disturbs the ionic homeostasis, plant hormones balance and altered plants growth and subsequent development (Azzam *et al*., 2022; Yuan *et al*., 2022). Salinity stress reduces the synthesis of chlorophyll contents, photosynthetic efficiency and it disturbs plant water relationships, membrane stability and accumulation of different osmolytes (Dustgeer *et al*., 2021; Rehman *et al*., 2021). Moreover, SS also induced the reactive oxygen (ROS) production that cause damages to plant proteins, DNA membranes and enzymes (Rehman *et al*., 2021). Likewise, SS reduces uptake of nutrients and disturbs plant physiological and microbial activities in rhizosphere resulting in marked reduction in production (Tavakkoli *et al*., 2011; Chandra *et al*., 2020).

Different crops have developed their own defensive systems which preserved them salinity stress. The plant response to SS largely depends on the type of genotype and amount of salts in soil (Zahra *et al*., 2020). Like other pulse crops chickpea is also salt sensitive and salinity stress considerably reduced the final grain yield (Khan *et al*., 2013). The selection and identification of cultivars that have good tolerance abilities against the salinity stress can play an important role to overcome this problem. Likewise, germination and seedling related attributes are the most important criteria to select the cultivars having good tolerance against the salt stress (Jamil and Rha, 2004). Moreover, the germination percentage and growth rate of seedlings is an imperative characteristic being used for the selection of cultivars (Saboora *et al*., 2006; Khayatnezhad *et al*., 2010).

Chickpea (*Cicer arietinum* L.) is an indispensible legume crop grown on 12 mha of more than 45 countries (FAOSTAT, 2010; Hirich *et al*., 2014). The seeds of chickpea are enriched protein source particularly for the people of developing nations (Jukanti *et al*., 2012). Chickpea seeds contain 5% fat, 23% protein, 47% starch, 64% carbohydrates, 7mg/100 iron, 3mg/100g zinc and 140mg/g calcium (Sanjeewa *et al*., 2010). In addition, chickpea also maintains the soil fertility through biological nitrogen fixation in soil (Chang *et al*., 2011). Cultivars varied considerably against SS, thus this research was aimed to determine the effect of SS on growth, antioxidant activities, and photosynthetic pigments of chickpea genotypes.

Materials and Methods

Experimental site

The present study was carried in wire house of Department of Agronomy, UAF. The soil was collected with spade and brought to lab and sieved in order to fill the pots. The soil was analyzed by standard procedures of Homer and Pratt (1961) and it was recognized as sandy loam with pH 7.89 and contained organic matter 0.81%, N 0.043%, P 6.98 mg kg^{-1} and K 195 mg kg^{-1}.

Growth conditions

The soil collected from agronomy farm was sieved and quantity of salt (NaCl) was added into the soil according to the treatments in order to maintain the salinity levels. After that pots having size of 380.00 cm^2 was filled with 5 kg soil and ten seeds sown in every pot. The pots were daily visited and irrigations were applied as per crop needs on the basis of visual observations. The weeds grown in pots were manually up-rooted and no attack of insects and disease were reported.

Experimental details

The study contained SS levels i.e., 0, 8 and 12 dsm^{-1} and different chickpea cultivars i.e., NIAB-2016, Bittle-2016 and Bhakar-2011. The current study was executed in completely randomized design having factorial combination.

Data collection and measurements

The mean emergence time (MGT) was measured with the procedures of Ellis and Robets (1981), whereas, the emergence index (EI) was calculated with the procedures of AOSA (1983). Moreover, T50 and final emergence percentage were calculated by the standard protocols of Farooq *et al*. (2005). Three plants were collected from each pot and plant height (PH) was measured and the average was taken. Similarly, the same three plants were taken, root length (RL), root fresh weight (RFW), shoot length (SL), and shoot fresh weight (SFW) was taken and the average

was worked out. The concentration of total soluble proteins (TSP) was determined with the procedures of Bradford (1976). The activity of SOD was determined by the methods of Zhang (1992), whereas the POD and CAT activities were determined by the procedures of Chance and Maehly (1955) and Guan *et al.* (2009).

Statistical analysis

The observations on growth, physiology and biochemical characteristics were analyzed by using the analysis of variance technique (Steel *et al.*, 1997) and LSD at 0.05 probability level was used to measure significance among mean values.

Results and Discussion

SS significantly affected all the tested germination traits (Table 1). The results indicated that maximum T50, MET, and minimum GP, and minimum EI were recorded in stronger SS (12 dsm-1), whilst minimum T50, MET, and maximum GP and EI was noticed under no salt stress (Table 1). Cultivars also behaved differently under salt stress. The results indicated that maximum T50, MET was taken by the NIAB-2016 as compared to other cultivars, while minimum T50 and MET were taken by the Bhaker-2011 (Table 1). Likewise, minimum GP and EI were also noted in Bhaker-2011 whilst maximum GP and EI were noticed in cultivar Bhaker-2011 (Table 1). The results indicated that SS the emergence and reduced the GP and EI. The delayed emergence due to SS be attributed to reduced water uptake and specific ion toxicity (SIT) which considerably increased the time to start emergence. These findings are the same as the outcomes of Munns and Tester (2008) they also noted that salt stress delayed the emergence. Salt stress increased the time MET owing to osmotic stress which resulted in a reduction in water take which consequently increased the T50 and MET. Likewise, Rajabi *et al.* (2020) and Ashagre *et al.* (2013) also noted that salt stress increased the MET. The differences among cultivars for germination traits can be the due to difference in their genetic makeup and their ability to cope with SS (Khodarahmpour *et al.*, 2012).

The maximum plant height, RL, RFW, SL, and SFW was recorded in control conditions, whereas minimum PH, RL, RFW, SL, SFW was recorded in strong SS (12 dSm^{-1}) (Table 2). In the case of cultivars; Bhaker-2011 performed better and had maximum PH, RL, RFW, SL and SFW whereas, NIAB-2016 had minimum PH, RL, RFW, SL and SFW (Table 2). Salt stress reduced the water uptake and nutrients translocation and therefore it considerably reduced the plant height (Hossein and Kasra, 2011). The differences amid genotypes for PH could be due to differences in the genetic makeup of plants. Previously Hassan *et al.* (2018) also stated that plant height is a genetic character and it differed significantly among cultivars. The reduction in RL and SL under SS might be due to the reduced availability of water owing to osmotic stress caused by SS (Sultan *et al.*, 2021). SS decreased the RFW which could be due to a reduction in the hydrolysis of food reservoirs and its movement to growing plant parts (Gholizadeh *et al.*, 2021).

Table 1: *Effect of different levels of salinity stress on germinations traits of chickpea cultivars.*

Salinity stress	TSE (days)	MET (days)	EI (EI)	T_{50} (days)	FEP (%)
S_1 (control)	7.2B	12.1C	74.6A	9.6A	93.0A
S_2 (8 dsm^{-1})	9.4A	12.8B	61.7B	10.7B	76.2B
S_3 (12 dsm^{-1})	10.0A	13.6A	51.9C	11.3C	63.7C
LSD≤0.05P	0.68	0.63	1.46	0.54	1.83
Cultivars (CV)					
CV_1 (NIAB-2016)	9.8A	13.3A	59.0C	11.1A	70.8C
CV_2 (Bittle-2016)	9.3A	13.0B	62.2B	10.7A	76.7B
CV_3 (Bhakar-2011)	8.6B	12.1C	66.9A	9.0B	85.4A
LSD≤0.05P	0.68	0.63	1.46	0.54	1.83
S × CV					
$S_1 \times CV_1$	8.3	12.7	71.3	10.0cd	87.0c
$S_1 \times CV_2$	8.3	12.3	73.7	9.7de	93.0b
$S_1 \times CV_3$	7.7	11.3	78.7	9.0d	99.0a
$S_2 \times CV_1$	10.0	13.3	59.0	11.3ab	69.3e
$S_2 \times CV_2$	9.7	13.0	61.3	11.0b	74.7d
$S_2 \times CV_3$	8.7	12.0	64.7	9.7de	84.7c
$S_3 \times CV_1$	10.7	14.0	46.7	12.0a	56.0g
$S_3 \times CV_2$	10.0	13.7	51.7	11.3ab	62.3f
$S_3 \times CV_3$	9.3	13.0	57.3	10.7bc	72.7d
LSD≤0.05P	NS	NS	NS	0.93	3.16

TSE: time to start emergence; MET: mean emergence time; EI: emergence index; T50: time to 50% emergence; FEP: final emergence percentage. Means having different letters showing significance at 0.05 P.

The results indicated that SS imposed a negative impact on photosynthetic pigments (Table 3). In the case of cultivars, Bhakar-2011 had maximum values for chlorophyll and carotenoid contents whereas the NIAB-2016 had maximum values for the aforementioned photosynthetic pigments (Table 3). In the present study, salinity stress considerably reduced the photosynthetic pigments (Table 2). The

excessive concentration of Na^+ owing to salinity stress causes the production of ROS that denatures enzymes required for the synthesis of chlorophyll contents thereby substantially reduced the chlorophyll contents (Alzahib *et al*., 2021).

Table 2: *Effect of different levels of salinity stress on growth attributes of chickpea cultivars.*

Salinity stress	PH (cm)	RL (cm)	RFW (g)	SL (cm)	SFW (g)
S_1 (control)	68.2A	7.7A	0.45A	16.66A	5.52A
S_2 (8 dsm^{-1})	56.2B	6.2B	0.45AB	14.29B	4.81B
S_3 (12 dsm^{-1})	51.8C	4.8C	0.42B	12.99C	4.34C
LSD≤0.05P	1.83	0.22	0.026	0.86	0.47
Cultivars (CV)					
CV_1 (NIAB-2016)	57.1B	5.9C	0.37C	14.02B	4.54B
CV_2 (Bittle-2016)	58.4AB	6.6A	0.49A	14.52A	4.86AB
CV_3 (Bhakar-2011)	67.7A	6.3B	0.46B	15.39A	5.27A
LSD≤0.05P	1.83	0.22	0.026	0.86	0.47
S × CV					
S_1× CV_1	63.7	6.9c	0.38	15.73	4.68cd
S_1× CV_2	65.0	8.4a	0.44	16.53	5.68ab
S_1× CV_3	76.0	7.8b	0.54	17.70	6.20a
S_2× CV_1	58.3	6.1de	0.44	13.60	4.77cd
S_2× CV_2	56.7	6.0e	0.46	14.40	4.50cd
S_2× CV_3	53.7	6.5d	0.44	14.87	5.17bc
S_3× CV_1	49.3	4.6g	0.29	12.73	4.18d
S_3× CV_2	53.7	5.3f	0.59	12.63	4.39cd
S_3× CV_3	52.3	4.6g	0.39	13.60	4.44cd
LSD≤0.05P	NS	0.39	NS	NS	0.82

PH: Plant height; RL: root length; RFW: root fresh weight; SFW: shoot fresh weight. Means having different letters showing significance at 0.05 P.

The concentration of TSP and anti-oxidant activities was significantly enhanced under SS. The maximum TSP, SOD, POD and CAT activities were noted in high level of salt stress, whilst minimum TSP and antioxidant activities were noted in normal conditions (Table 4). Amongst cultivars Bhaker-2011 had maximum TSP and antioxidant activities were activities, whereas the cultivar NIAB-2016 had minimum TSP and antioxidant activities were (Table 4). The increase in protein under salt stress can be ascribed to increase in protein synthesis and conversation of nitrogen in proteins (Ashraf, 2003). The anti-oxidant activities were considerably increased under the SS, likewise, Sultan *et al*. (2021) also found a marked increase in SOD activity under salt stress. Likewise, Khan *et al*. (2022) noted a significant increase in SOD and CAT are considerably increased under the activity under salt stress.

Table 3: *Effect of different levels of salinity stress on photosynthetic attributes of chickpea cultivars.*

Salinity stress	Chlorophyll a	Chlorophyll b	Carotenoids
S_1 (control)	9.67A	3.41A	0.69A
S_2 (8 dsm^{-1})	7.61B	2.45B	0.58B
S_3 (12 dsm^{-1})	6.62C	1.76C	0.59B
LSD≤0.05P	0.28	0.16	0.02
Cultivars (CV)			
CV_1 (NIAB-2016)	6.76C	2.32B	0.60B
CV_2 (Bittle-2016)	7.82B	2.61A	0.61B
CV_3 (Bhakar-2011)	9.32A	2.69A	0.65A
LSD≤0.05P	1.83	0.16	0.02
S × CV			
S_1× CV_1	8.43	3.10c	0.65b
S_1× CV_2	9.53	3.40b	0.66b
S_1× CV_3	11.03	3.73a	0.77a
S_2× CV_1	6.40	2.23e	0.57cd
S_2× CV_2	7.47	2.57d	0.59cd
S_2× CV_3	8.97	2.55d	0.58cd
S_3× CV_1	5.43	1.62f	0.57cd
S_3× CV_2	6.47	1.86f	0.59cd
S_3× CV_3	7.97	1.78f	0.61c
LSD≤0.05P	NS	0.28	0.03

Means having different letters showing significance at 0.05 P.

Table 4: *Effect of different levels of salinity stress on soluble proteins and anti-oxidant activities of chickpea cultivars.*

Salinity stress	TSP (mg/g FW)	SOD (U/mg FW)	POD (U/µg protein)	CAT (U/mg protein)
S_1 (control)	69.39B	53.83C	6.08C	30.49B
S_2 (8 dsm^{-1})	70.08B	168.83B	15.77B	53.77A
S_3 (12 dsm^{-1})	72.46A	236.79A	18.16A	55.24A
LSD≤0.05P	1.68	2.01	0.88	1.50
Cultivars (CV)				
CV_1 (NIAB-2016)	69.10B	147.33C	12.12B	42.86B
CV_2 (Bittle-2016)	69.88B	151.66B	12.47B	47.64A
CV_3 (Bhakar-2011)	72.94A	160.47A	15.41A	49.0A
LSD≤0.05P	1.68	2.01	0.88	1.50
S × CV				
S_1× CV_1	67.60	50.27	4.87f	26.0
S_1× CV_2	68.80	52.97	6.27ef	31.20
S_1× CV_3	71.77	58.27	7.10e	33.57
S_2× CV_1	68.70	162.07	14.50d	50.03
S_2× CV_2	69.63	169.63	14.40d	55.10
S_2× CV_3	71.90	174.80	18.40b	56.17
S_3× CV_1	71.00	229.67	17.0bc	51.83
S_3× CV_2	71.20	232.37	16.73c	56.63
S_3× CV_3	75.17	248.33	20.73a	57.27
LSD≤0.05P	NS	3.49	1.53	NS

TSP: total soluble proteins; SOD: superoxide dismutase; POD: peroxidase; CTA: catalase. Means having different letters showing significance at 0.05 P.

Conclusions and Recommendations

The increase in salt stress linearly decreased the germination, growth, and photosynthetic pigments however, salinity significantly increased antioxidant activities. Cultivars behaved differently in terms of salt stress tolerance. Cultivar Bhakker-2016 is characterized as the most tolerant cultivar owing to better germination, growth, and antioxidant activities as compared to other cultivars. Therefore, the cultivar, Bhakker-2016 can be used in future breeding programs to develop salt-tolerant cultivars.

Novelty Statement

Cultivars varied considerably against salinity stress, thus this research was aimed to characterize the best salt tolerant cultivars.

Author's Contribution

Muhammad Umer Chattha: Conceived and planned the experiment and write the original draft.
Muhammad Ilyas: Review and editing
Imran Khan: Conceived and planned the experiment and write the original draft.
Ambreen Fatima: Helped in Data collection
Athar Mahmood, Muhammad Bilal Chattha, Muhammad Iqbal, Muhammad Tahir Akbar, Muhammad Mahmood Iqbal, Faran Muhammad, Muhammad Talha Aslam and Muhammad Umair Hassan: Reviewed and edited the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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