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RESEARCH ARTICLE

Screening and evaluation of chickpea (*Cicer arietinum* L.) genotypes for salinity stress tolerance: A biochemical, physiological and yield assessment

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Abstract

Chickpea (*Cicer arietinum* L.), an important legume crop extensively cultivated across India, is adversely affected by a range of biotic and abiotic stresses, with soil salinity being a major factor that significantly reduces its productivity. Among several alternatives available for salinity management developing tolerant cultivars is the judicious and sustainable approach. The present investigation was undertaken to screen 20 chickpea genotypes in pots, under controlled conditions by treating with 60 mM & 120 mM NaCl concentration in split doses at sowing and 15 days to sowing (DAS). Observation on various physiological, biochemical and alternation in yield contributing parameters were recorded during the pod initiation stage and used for statistical analysis *i.e.* correlation and path analysis. All the traits show significant variation at both 1 % and 5 % level of significance. Results revealed that there was a significant decrease in total chlorophyll and relative water content in susceptible genotypes (ICC 249 & ICC 247) while salt tolerant genotypes (ICC 5439 & GNG 1581) showed significantly lower reduction in these traits. A significant increase was observed in lipid peroxidation and proline content while non-significant increase in protein content in tolerant genotypes as compared to sensitive genotypes. The results showed that the total proline content increased due to the production of stress-related proteins during salinity stress. Based on the results of the study, ICC 5439 and GNG 1581 are highly tolerant chickpea genotypes under salinity stress conditions. ICC 6050, ICC 251, ICC 252 and ICC 262 are medium-tolerant genotypes, while ICC 253, ICC 247 and ICC 249 are highly susceptible genotypes. The remaining are minimum tolerant and sensitive genotypes. The study further revealed significant direct associations among traits, emphasizing the feasibility of effective selection for improving chickpea characteristics. The findings offer valuable insights into the tolerant genotypes, guiding future breeding and selection programs.

Keywords: correlation; path analysis; proline; proteins; salinity stress; susceptible; tolerant

Introduction

Chickpea (*Cicer arietinum* L.) is an important food legume, with South Asia being the largest producer. It ranks third in the global production of pulses after common bean and field pea, with a total production of approximately 11.6 million tons, of which 80 % is desi and 20 % is Kabuli (1). India is the leading producer of chickpeas, accounting for 73 % of global production in 2020, followed by Turkey, Myanmar and Pakistan. In India, chickpea is a major pulse crop and contributes to 27-30 % of total pulse output (2). India has a total area of 9.55 million hectares dedicated to chickpea production, with a production of 9.94 million tons and productivity of 806 kg/ha. The productivity of chickpeas in Punjab is 700 kg/ha and in Rajasthan, it is 680 kg/ha (3). Chickpea is an important crop for food security in many countries and efforts to improve its productivity and tolerance to environmental stresses are important for

ensuring sustainable food production. Agriculture originated independently in different regions of the world, including at least 11 separate regions in the old and new Worlds (4). In India, agriculture began to take shape around 9000 BC, with the early domestication of crops and animals (5). Plants and animals were highly valued and considered essential for survival by Indians (6). This led to settled life and the use of improved agricultural techniques. The importance of agriculture and its impact on society continues to this day, as it remains a critical sector for ensuring food security and supporting livelihoods around the world (7, 8).

In North India, particularly in Haryana and Punjab salinity is a major concern. In the Western region, the salinity and water logging affect agricultural land. Salinity can adjust Phenological development and physiological adaptation (9). Furthermore, salinity stress can also lead to changes in the expression of genes related to stress response, ion balance and metabolism reduction in plant height, chlorophyll content, leaf area index, water status and increase in lipid peroxidation and proline content during the stress (10, 11). In addition, salinity stress can affect the microbial community in the soil, which can further influence plant growth and productivity (12). Reactive oxygen species (ROS) generated during stress results in oxidative damage by increases in lipid peroxidation and electrolyte leakage (13). Overall, managing salinity stress in agricultural lands is critical for sustainable crop production and food security. Chickpea is divided into Kabuli and Desi, which differ in their geographic distribution, seed size and plant type (11, 14). Chickpea seeds are composed of carbohydrates (50-58 %), protein (15-22 %), moisture (7-8 %), fat (3.8-10.20 %) and micronutrients (<1 %) (3). With an average protein content of almost 18 %, chickpeas have a higher protein content than lentils and field peas (15). Chickpeas are rich in lysine and arginine but have low levels of sulphur-containing amino acids such as cysteine and methionine (16).

The self-fertilizing diploid nature of all chickpea cultivars and their wild relatives, with 2n=2x=16 chromosomes and a genome size of 740 megabase pairs (Mbp) (17). Although there are rare reports of chickpea species with a 2n=14 chromosome number (18), the chromosomes of chickpeas are generally small, with an average length of 1.32-3.69 µm (micrometre) and mitotic metaphase chromosome length of 2.2 µm (19). Phenotyping crops for salinity tolerance is influenced by various environmental factors and developmental stages (20, 21). Chickpea productivity can be affected by various abiotic factors as well as biotic factors (22). The phenotypic coefficient assesses the environmental impact on the genotype, while the genotypic coefficient of variation estimates heritable variability. Effective selection will require consideration of heritability, selection intensity and genetic gain. Several research studies have explored these themes (23-25). This analysis aims to gather vital information regarding the behaviour of specific chickpea genotypes under salt stress, including correlations and pathways between yield and various phenological, morphological, biochemical and physiological traits. The research aspires to enhance understanding of salinity stress-related breeding programs for chickpeas.

Materials and Methods

Experimental site

This experiment was conducted during the Rabi season of 2020-2021 in a polyhouse at the Department of Genetics and Plant Breeding, Lovely Professional University, Phagwara (Punjab) situated at latitude 31°19'32'' N and longitude 75° 34'45"' E and at an altitude 243 m above the mean sea level. The experimental area occupied was uniform with respect to topography and climate.

Experimental material

This study aimed to investigate the effect of NaCl salt with a concentration of 60 mM and 120 mM on the growth and development of 20 genotypes of chickpeas. The plants were grown in 25 cm diameter plastic pots filled with sandy loam soil and the experiment followed a Completely Randomized Design (CRD) with 3 replications under both saline (pots without holes) and control (pots with holes) conditions. In each pot, five seeds were planted and the experimental material consisted of 20 genotypes where 17 are collected from ICRISAT, Hyderabad i.e. ICC 6050, ICC 5003, ICC 263, ICC 262, ICC 258, ICC 5439, ICCL 86111, ICC 244, ICC 245, ICC 246, ICC 247, ICC 248, ICC 249, ICC 250, ICC 251, ICC 252 and ICC 253. Whereas 3 were collected from ARS, SriGanganagar i.e. GNG1488, GNG1581 and GNG1958.

Preparation of saline solution

Weights of 1.752 g and 3.504 g of NaCl were used, with each added to a separate volumetric flask containing approximately 800 mL of water. Once the NaCl had fully dissolved, water was added to each flask to bring the final volume up to 1000 mL, resulting in solutions with concentrations of 60 mM. Therefore, the 60mM NaCl treatment was applied in two split doses, one at the time of sowing and the other at 15 DAS. Effect of salinity in tolerant genotype GNG 1958 and ICC 1581 shown in Fig. 1 and Fig. 4 respectively whereas, effect of salinity in sensitive genotype ICC 247 and ICC 249 shown in Fig. 2 and Fig. 3 respectively.

Statistical analysis

The analysis includes the calculation of various statistical parameters such as mean, standard deviation, coefficient of variation and analysis of variance (ANOVA) to test the significance of differences between treatments. Statistical Package for Completely Randomized Design (CRD) developed at IASRI New Delhi will be followed for analysis of quantitative traits. ANOVA was used to test the significance of differences among the genotypes for various traits. The data were analyzed using appropriate methods of ANOVA and covariance (25). To determine the significance of the genotypes, the calculated value of 'F' was compared with the tabular value of 'F' at both 1 and 5 % levels of probability against error degree of freedom. This helps to identify the genotypes with superior performance for specific traits and can guide breeding programs aimed at improving chickpea productivity under salinity stress.

Estimation of correlations

The phenotypic correlation coefficient measures the correlation between two traits including both genetic and environmental influences. While the genotypic correlation coefficient measures the correlation between two traits due

0nm 60nm 120nm

Fig. 1. Effect of salinity in tolerant genotype - GNG 1958.

0nm 60nm 120nm

Fig. 2. Effect of salinity in sensitive genotype - ICC 247.

0nm 60nm 120nm

Fig. 3. Effect of salinity in sensitive genotype - ICC 249.

0nm 60nm 120nm

Fig. 4. Effect of salinity in tolerant genotype - ICC 1581.

to genetic factors only. When testing the significance of phenotypic and environmental correlation coefficients, the estimated values compared with the tabulated values of at n-2 degrees of freedom at two levels of probability, which are typically 5 % and 1 % (26).

Now, genotypic and phenotypic correlation coefficients were worked out according to the formula described below.

$$\text{Phenotypic correlation (rp)} = \frac{\text{PCOVxy}}{\sqrt{\text{PVx. PVy}}} \quad \text{.....(1)}$$

$$\text{Genotypic correlation(rg)} = \frac{\text{GCOVxy}}{\sqrt{\text{GVx. GVy}}} \quad \text{.....(2)}$$

$$\text{rxy} = \frac{\text{Cov (x, y)}}{\sqrt{\text{V}}(\text{x}) \text{ x } \sqrt{\text{V}}\ (\text{y})} \quad \text{.....(3)}$$

Where,

r_{xy} = Correlation coefficient between character x and y,

$Cov_{x,y}$ = Co-variance of character x and y,

V_x = Variance of character x

V_y = Variance of character y

rp = Phenotypic correlation

rg = Genotypic correlation.

Path coefficient analysis

Path coefficient analysis, as employed to determine the direct and indirect contributions of various traits toward the total correlation coefficient with grain yield (27-29). This analysis involves splitting the correlation coefficient into measures of direct and indirect effects, enabling the estimation of the contribution of each independent variable on the dependent variable as well as residual effects. The resulting information aids in determining the yield and yield-contributing traits. Path coefficients were evaluated based on the scales provided (30).

To estimate various direct and indirect effects, the following set of simultaneous equations were formed and solved.

$r_{1y}=P_{1y}+r_{12}P_{2y}+r_{13}P_{3y}+\ldots+r_{1I}P_{Iy}$ ..... (4)

$r_{2y}=r_{2y}P_{1y}+P_{2y}+r_{23}P_{3y}+\ldots+r_{2I}P_{Iy}$ .....(5)

$r_{Iy}=r_{I1}P_{1y}+r_{I2}P_{2y}+r_{I3}P_{3y}+\ldots+P_{Iy}$ .....(6)

Where,

r_{1y} to r_{Iy} = Coefficient of correlation between causal factor 1 to I and dependent character y,

r_{12} to $r_{I\text{-}1,I}$ = Coefficient of correlation among causal factors themselves and

P_{1y} to P_{Iy} = Direct effects of characters 1 to I on character y.

Residual effect, which measures the contribution of the characters not considered in the causal scheme, was obtained as:

Residual effect

Where,

$$(\text{PRY}) = \sqrt{1 - R^2} \quad \text{.....(7)}$$

$$\text{R2} = \sum_{ij} P_i{}^2 Y + 2 \sum_{\substack{i \neq j \\ i>j}} P_{iy}\ P_{jy} R_{ij} \quad \text{.....(8)}$$

Results and Discussion

Analysis of variance

The analysis of variance results indicates a significant effect of all parameters, except for protein, which was found to be non-significant. Additionally, all 20 chickpea genotypes demonstrated genetic diversity under salinity conditions. To ascertain the impact of salt stress on genotypes displaying varying degrees of tolerance, the study tracked several data, including biochemical, physiological and yield parameters.

Different parameters used in the analysis	
Physiological parameters	Total chlorophyll content, Relative water content and Lipid peroxidation
Biochemical parameters	Proline and Protein content
Yield attributing parameters	Number of pods per plant and seed yield per plant

In the experiment, 20 chickpea genotypes were evaluated using a completely randomized design with three replications for 19 different parameters. Mean squares for both the replications and treatments for all parameters can be found in Table 1. The study showed that the variation due to replications was non-significant for all characters. However, the variation due to treatments was significant for all the characters under saline (60mM and 120mM) and non-saline conditions.

Mean performance of genotypes for different parameters

The Mean performance of 20 genotypes for physiological, biochemical and yield parameters was tabulated in Table 2.

Physiological parameters

Total Chlorophyll Content (mg/g FW)

Salinity negatively influenced total chlorophyll content, with a significant reduction at 120 mM NaCl compared to 60 mM and non-saline conditions. Under non-saline conditions, genotype ICC 252 exhibited the highest chlorophyll content (6.44), while ICC 250 and ICC 247 had 3.69 mg/g FW. At 60 mM NaCl, ICC 252 maintained the highest chlorophyll content (5.62), whereas ICC 247 had the lowest (2.52). At 120 mM NaCl, genotype ICC 248 had the highest chlorophyll content (3.97) and ICC 251 the lowest (1.13 mg/g FW).

Several researchers have noted that Na^+ accumulation can lead to K^+ content in the leaves, resulting in chlorophyll degradation and disruption of the thylakoid membrane (31). The decline in chlorophyll concentration under saline conditions may be attributed to both enhanced degradation and inhibited synthesis of the pigment (31). Chlorophyll inhibition results from the suppression of particular enzymes that aid in the creation of green pigments (34). A similar result of difference in total chlorophyll content due to salinity levels was also obtained by (35-37).

Relative Leaf Water Content (RWC)

Osmotic stress from salinity reduces RWC, with the most significant decline at 120 mM NaCl. Under control conditions, ICC 5439 had the highest (84.77 %) and GNG 1958 was the lowest (59.33 %). At 60 mM NaCl, ICC 247 showed the highest RWC (65.31 %), while ICC 249 had the lowest (35.31 %). At 120 mM NaCl, ICC 247 again had the highest (62.76 %) and ICC 249 was the lowest (29.03 %).

Table 1. ANOVA for various characters in chickpea under 60 mM, 120 mM and non-saline condition

Characters	Replication		Treatment		Replication		Treatment		Replication		Treatment	
	MSS	f-value	MSS	f-value	MSS	f-value	MSS	f-value	MSS	f-value	MSS	f-value
	60mM saline condition				120mM saline condition				Non-saline condition			
Total chlorophyll content	0.01	0.20	2.31	63.79**	0.02	1.68	2.23	165.28**	0.14	1.60	2.11	23.60**
Relative water content	1.09	1.15	169.94	179.32**	0.12	0.17	190.16	261.18**	2.37	1.67	98.91	69.86**
Lipid per oxidation	0.03	0.68	4.12	81.72**	0.02	0.35	11.80	203.02**	0.10	0.91	5.44	45.33**
Proline content	0.01	2.51	0.43	187.80**	0.01	1.95	0.62	177.44**	0.00	0.52	0.17	57.11**
Total Protein	1.21	0.08	282.83	20.58**	6.29	0.41	298.79	19.93**	2.79	0.55	402.61	78.89**
Numbers of pod per plant	4.86	2.79	40.95	23.48**	5.60	2.65	51.68	24.51**	5.42	2.69	29.35	14.57**
Seed yield per plant	0.01	0.69	3.57	218.24**	0.01	0.10	3.54	228.57**	0.02	0.94	2.90	93.62*

*, ** significant at 5 % and 1 % probability levels respectively

df for replication and treatment- 2 & 19 respectively

A similar increase in saline irrigation was reported to decrease the relative water content of chickpeas (38). RWC was reduced in salinity-affected plants and obtained similar results as the degree of reduction increased with the increase in salinity level (39).

Lipid Peroxidation

Lipid peroxidation, measured as malondialdehyde (MDA) µmoles/g DW, varies with salt concentration. Under non-saline conditions, it ranged from 13.2 to 22.68. At 60 mM NaCl, the highest lipid peroxidation was in ICC 244 (19.84) and the lowest in ICC 251 (13.36). At 120 mM NaCl, ICC 244 again had the highest (22.32) and ICC 251 the lowest (14.85). Under control environments, ICC 245 had the highest lipid peroxidation (14.63) and ICC 5439 was the lowest (9.85). At 120 mM NaCl, the highest was in ICC 245 (18.51 and the lowest in ICC 244 (8.42). These results was in consistent with previous findings, which reported that with increasing salinity, a minimal increment was observed across all genotypes under trail (39). The salt stress has significantly influenced the lipid peroxidation in soybean leaf and roots (40).

Biochemical parameters

Proline content (mg/g DW)

Proline content increased under stress conditions, with the highest accumulation observed at 120 mM NaCl compared to 60 mM and control conditions. At the pod initiation stage, genotype GNG 1488 showed the maximum proline accumulation (2.24) under control treatment. At 60 mM NaCl, GNG 1488, ICC 250 and ICC 248 had the highest proline content (2.82), while ICC 248 reached the highest level at 120 mM NaCl (3.28). The minimum proline content was found in genotype ICC 249, with (1.49) under control, (1.63) at 60 mM and (1.74) at 120 mM NaCl.

A similar result has been stated in the resistance chickpea genotype under salinity stress in chickpeas (42). In sensitive genotypes, proline levels drop during the chickpea germination stage when exposed to salt stress (43). Proline accumulates significantly in groundnut lines treated with NaCl which aids in osmotic pressure (44).

Protein content (mg/g DW)

Protein content increased with higher NaCl concentrations, with the highest levels under 120 mM NaCl. Genotype ICC 253 exhibited the maximum protein content: (163.65) under 0 mM, (159.87) under 60 mM and (171.36) under 120 mM. The minimum protein content was found in genotype ICC 249, with (122.06) under 0 mM and genotype ICC 251 with (123.33) under 60 mM and (134.36) under 120 mM.

With an increase in the salt level, there is an increased absorbance of the some elements that help in activation of the some enzymes that are required for the synthesis of protein (44). Tolerant chickpea genotypes have a higher accumulation of protein & nucleic acid than the sensitive ones (45). Similar results have shown that the NaCl effect can be beneficial in the case of protein content (46).

Yield parameters

Number of Pods per Plant

Under non-saline conditions, the number of pods per plant ranges from 22 to 34 across all genotypes. The minimum variation was observed in ICC 6050 (11, 9.89) and ICC 5003 (17.0, 23.16), while the maximum variation occurred in ICC 249 (40.9, 50.0) and ICC 5439 (34.78, 50.74) under 60mM and 120 mM NaCl treatments compared to the control. The highest number of pods per plant was observed in genotype ICC 252, with 34.00 under 0 mM, 28.00 under 60 mM and 26.00 under 120 mM salt concentrations. The lowest number of pods per plant was reported in genotype ICC 249, with 22.00 under 0mM, 13.00 under 60 mM and 11.00 under 120 mM NaCl.

Seed Yield per Plant

The yield of seed per plant ranges from 2.6 to 7.11 g across all the genotypes under control conditions. The least reduction in seed yield was observed in ICC 248 (0.73, 0.78) and ICC 252 (0.72, 0.67), while the highest reduction was observed in ICC 5439 (1.61, 1.76) and ICC 253 (1.27, 1.35), followed by ICC 249 (1.4, 1.52) under 60 mM and 120 mM NaCl treatments. The seed yield per plant was maximum in genotype ICC 262, with 7.11 g under 0 mM, 5.92 g under 60 mM and 5.64 g under 120 mM NaCl whereas, minimum seed yield per plant was

Table 2. Mean of various genotypes of chickpea under control and saline (60 mM and 120 mM) conditions

	Total chlorophyll content			Relative Leaf Water Content			Lipid Peroxidation			Proline content			Total Protein			No. of pods per plant			Seed yield per plant		
Genotypes	**Ctrl**	**60mM**	**120mM**	**Ctrl**	**60mM**	**120 mM**	**Ctrl**	**60mM**	**120 mM**	**Ctrl**	**60mM**	**120mM**	**Ctrl**	**60mM**	**120mM**	**Ctrl**	**60mM**	**120 mM**	**Ctrl**	**60mM**	**120 mM**
ICC 6050	5.8	4.09	3.32	71.97	60.25	56.49	13.07	15	17	1.73	2.06	2.24	137.59	134.39	137.68	27.00	24.00	24.33	3.93	2.57	2.40
ICC 5003	4.02	3.31	1.61	67.85	57.98	54.6	12.15	14.13	16.23	1.57	1.9	2.25	156.59	149.78	158.28	27.33	22.67	21.00	3.73	2.71	2.10
ICC 263	4.41	3.62	1.92	69.94	55.91	52.76	12.48	13.43	15.42	1.79	2.18	2.19	143.22	144.82	141.4	32.00	24.67	25.00	4.43	3.14	3.07
ICC 262	4.25	3.64	2.05	67.01	47.25	42.28	13	14.5	16.58	2.11	2.58	2.84	149.65	141.87	146.5	31.00	25.00	24.33	7.11	5.95	5.64
ICC 258	3.85	3.14	1.51	68.6	60.31	56.68	12.5	14.31	16.49	1.6	2.08	2.27	152.58	147.67	149.9	30.00	24.00	23.00	4.80	3.57	3.54
ICC 5439	4.07	2.56	1.67	84.77	58.86	53.43	9.85	14.24	16.72	1.52	1.68	1.81	149.29	144.94	145.92	23.00	15.00	11.33	2.96	1.35	1.20
GNG 1488	4.84	3.84	2.04	77.53	64.03	58.63	13.68	14.63	16.63	2.24	2.82	3.11	150.27	147.24	150.28	28.00	22.00	18.67	4.03	3.01	2.73
GNG 1581	4.08	3.35	1.68	72.66	61.67	58.64	12.02	13.09	15.19	1.89	1.91	2.04	150.18	145.71	148.2	32.00	25.33	23.00	4.35	3.57	3.49
ICCL 86111	6.07	5.18	3.36	69.94	60.52	57.18	13.52	14.55	16.73	2.08	2.2	2.42	140.6	135.34	146.17	27.33	20.00	18.33	4.06	3.03	2.81
GNG 1958	5.13	4.23	2.4	59.33	46.5	47.91	12.23	13.29	15.47	2.07	2.2	2.55	137.32	131.6	142.59	29.00	21.00	19.00	5.22	4.16	3.99
ICC 253	4.01	3.43	1.74	68.1	60.31	57	14.49	15.06	16.57	2.13	2.67	3.01	163.65	159.87	171.36	27.00	21.00	17.00	2.60	1.33	1.25
ICC 244	4.49	3.68	1.84	67.42	57.76	51.32	14.57	10.42	8.42	1.65	2.13	2.47	153.87	148.19	162.59	28.00	22.00	20.67	3.82	2.82	2.72
ICC 245	5.01	3.29	2.59	73.18	64.63	61.31	14.63	16.3	18.51	2.17	2.44	2.72	157.15	152.49	153.89	29.00	22.00	20.00	4.00	2.64	2.60
ICC 246	4.03	3.29	1.45	78.01	63.15	59.38	11.24	14.36	16.65	1.96	2.43	2.64	155.82	148.68	153.55	30.00	24.00	23.00	3.40	2.54	2.41
ICC 252	6.44	5.62	3.86	64.61	57.11	54.5	14.1	14.98	16.91	1.7	2.58	3.03	148.56	143.65	152.42	34.00	28.00	26.00	4.75	4.03	4.08
ICC 251	3.74	2.75	1.13	68.31	49.95	43.77	12.58	15.34	17.53	1.98	2.37	2.46	128.81	123.33	134.36	33.00	27.00	24.00	4.92	3.61	3.49
ICC 250	3.69	3	1.45	75.21	64.69	60.49	12.91	13.92	15.99	2.03	2.82	3.14	160.87	157.15	168.73	30.00	23.00	21.00	3.76	2.34	2.26
ICC 249	4.78	3.37	1.42	63.06	35.31	29.03	10.02	14.61	16.78	1.49	1.63	1.74	122.06	125.51	136.01	22.00	13.00	11.00	3.00	1.60	1.48
ICC 248	5.69	5.47	3.97	67.93	60.35	58.04	13.9	13.31	14.58	2.11	2.82	3.28	125.06	133.24	145.8	33.00	27.00	24.67	4.85	4.12	4.07
ICC 247	3.69	2.52	1.27	74.46	65.31	62.76	13.39	14.42	16.36	2.02	2.8	3.11	141.14	145.16	156.27	31.00	25.00	22.67	3.63	2.16	2.15
CD 5 %	**0.49**	**0.31**	**0.19**	**1.97**	**1.61**	**1.41**	**0.57**	**0.37**	**0.4**	**0.09**	**0.08**	**0.1**	**6.4**	**6.4**	**6.4**	**2.35**	**2.18**	**2.40**	**0.29**	**0.21**	**0.21**
CV %	**6.5**	**5.19**	**5.5**	**1.69**	**1.69**	**1.59**	**2.7**	**1.58**	**1.5**	**2.89**	**2.06**	**2.31**	**2.58**	**2.58**	**2.58**	**4.86**	**5.80**	**6.95**	**4.23**	**4.25**	**4.33**

observed in genotype ICC 253, with 2.60 g under 0mM and in genotype ICC 5439, with 1.33 g under 60 mM and 1.20 g under 120 mM NaCl.

There are 20 genotypes under study all genotypes have their response to salinity stress, in yield parameters such as no. of pod/plant and seed yield/plant we can see the reduction. The no. of pods per plant was found at maximum in the genotypes ICC 252, ICC 252 and ICC 252 while the minimum no. of pods per plant was found in the genotypes ICC 249 under 0mM, 60 mM and 120 mM salt concentrations. The seed yield per plant was found minimum in genotypes ICC 5439 (1.20) under 0 mM, 60 mM and 120 mM salt concentrations. Salinity (NaCl) interferes with assimilate translocation which lowers enzyme activity and impacts production. Mung beans grown in salt had lower yields because of increased flower shedding and decreased photosynthetic efficiency in seed development (47). This resulted in fewer seeds and pods on the plant, which further impacted production.

Correlation between various traits under study at saline (60 mM and 120 mM) and non-saline conditions

Correlation indicates the association between the pair of characters and it forms the basis for the selection index for crop improvement. The estimates of phenotypic & genotypic correlation between 7 characters *viz*., TCC, RLWC, LP, PC, TP, NPP, SYP of physiological, biochemical and yield traits tabulated in Table 3 for 60 mM, Table 4 for 120 mM and Table 5 for non-saline (control) condition respectively.

Correlation between physiological, biochemical and yield traits under 60 mM conditions

The seed yield per plant at 60mM saline condition exhibited significantly high and positive correlations both at genotypic and phenotypic levels with a number of pod per plant (r_g = 0.615, r_p = 0.604). It also manifested a significant positive correlation at both genotypic and phenotypic levels with Total chlorophyll content (r_g = 0.435, r_p = 0.432). The relative water content exhibited highly significant and positive correlations both at genotypic and phenotypic levels with total protein (r_g = 0.680, r_p = 0.663). It also manifested a positive, but significant correlation with the number of pods per plant (r_g = 0.380) and Proline content (r_g = 0.442, r_p = 0.439). The proline content exhibited highly significant and positive correlations both at genotypic and phenotypic levels with a number of pods per plant (r_g = 0.580, r_p = 0.565).

Correlation between physiological, biochemical and yield traits under 120 mM conditions

The seed yield per plant at 120 mM saline condition exhibited highly significant and positive correlations both at genotypic and phenotypic levels with a number of pods per plant (r_g = 0.675, r_p = 0.656). It also manifested a significant but negative correlation at the genotypic level with Relative water content (r_g = -0.438). The relative water content exhibited highly significant and positive correlations both at genotypic and phenotypic levels with total protein (r_g = 0.542, r_p = 0.531). It also manifested a positive, but significant correlation with proline content (r_g = 0.467, r_p = 0.464). The proline content exhibited highly significant and positive correlations both at

Table 3. Correlation coefficient analysis of various traits under 60 mM saline condition

60mM at both genotypic and phenotypic level								
Characters		**TCC**	**RLWC**	**LP**	**PC**	**TP**	**NPP**	**SYP**
TCC	**rg**	1.000						
	rp	1.000						
RLWC	**rg**	-0.038	1.000					
	rp	-0.039	1.000					
LP	**rg**	-0.085	0.037	1.000				
	rp	-0.086	0.038	1.000				
PC	**rg**	0.217	0.4428*	0.177	1.000			
	rp	0.216	0.4391*	0.174	1.000			
TP	**rg**	-0.275	0.6802**	-0.054	0.313	1.000		
	rp	-0.269	0.6634**	-0.047	0.302	1.000		
NPP	**rg**	0.274	0.3809*	-0.014	0.5805**	0.073	1.000	
	rp	0.269	0.373	-0.012	0.5652**	0.072	1.000	
SYP	**rg**	0.4356*	-0.224	-0.119	0.252	-0.298	0.6151**	1.000
	rp	0.4326*	-0.222	-0.118	0.251	-0.287	0.6046**	1.000

Table 4. Correlation coefficient analysis of various traits under 120 mM saline condition

120 mM at both genotypic and phenotypic level								
Characters		**TCC**	**RLWC**	**LP**	**PC**	**TP**	**NPP**	**SYP**
TCC	**rg**	1.000						
	rp	1.000						
RLWC	**rg**	0.198	1.000					
	rp	0.197	1.000					
LP	**rg**	0.025	0.030	1.000				
	rp	0.026	0.030	1.000				
PC	**rg**	0.295	0.4673*	0.008	1.000			
	rp	0.294	0.4642*	0.010	1.000			
TP	**rg**	-0.184	0.5428**	-0.282	0.5146*	1.000		
	rp	-0.181	0.5313**	-0.275	0.4983*	1.000		
NPP	**rg**	0.292	0.355	-0.071	0.4384*	-0.027	1.000	
	rp	0.285	0.351	-0.071	0.4234*	-0.017	1.000	
SYP	**rg**	0.365	-0.4382*	-0.074	0.289	-0.310	0.6752**	1.000
	rp	0.363	-0.132	-0.074	0.288	-0.301	0.6567**	1.000

Table 5. Correlation coefficient analysis of various traits under non-saline or control condition

		Control at both genotypic and phenotypic levels						
		TCC100	RLWC100	LP100	PC100	TP	NPP	SYP
TCC	rg	1.000						
	rp	1.000						
RLWC	rg	-0.334	1.000					
	rp	-0.329	1.000					
LP	rg	0.334	-0.227	1.000				
	rp	0.323	-0.220	1.000				
PC	rg	0.073	0.059	0.5572**	1.000			
	rp	0.071	0.059	0.5435**	1.000			
TP	rg	-0.368	0.371	0.278	0.106	1.000		
	rp	-0.359	0.367	0.272	0.103	1.000		
NPP	rg	0.037	-0.201	0.4911*	0.3958*	-0.001	1.000	
	rp	0.046	-0.184	0.4621*	0.3906*	0.002	1.000	
SYP	rg	0.161	-0.4162*	0.201	0.276	-0.196	0.6208**	1.000
	rp	0.158	-0.409	0.199	0.274	-0.193	0.5928**	1.000

genotypic and phenotypic levels with the Number of pods per plant (r_g = 0.580, r_p = 0.565). It also manifested a positive, but significant correlation with Relative water content (r_g = 0.442, r_p = 0.439).

Correlation between physiological, biochemical and yield traits under Control conditions

The seed yield per plant at non-saline conditions exhibited highly significant and positive correlations both at genotypic and phenotypic levels with a number of pods per plant (r_g = 0.621, r_p = 0.593). Seed yield per plant exhibited only significant but negative correlations at genotypic with Relative water content (r_g = -0.416). The lipid peroxidation only exhibited highly significant and positive correlations both at genotypic and phenotypic levels with proline content (r_g = 0.558, r_p = 0.544). The Lipid peroxidation exhibited significant and positive correlation at both genotypic and phenotypic level with number of Pod per Plant (r_g = 0.491, r_p = 0.462). It also manifested a positive, but significant correlation with the number of pods per plant (r_g = 0.396, r_p = 0.391).

Seed yield has been observed to have a positive and significant correlation with pods per plant and seed weight (48). Additionally, a positive correlation between yield and pods per plant has been reported (49). Under salinity conditions, seed yield shows a positive correlation with total chlorophyll, relative water content and filled pods, indicating that these traits contribute to salinity tolerance (50). Correlation tells the degree and direction of association traits that have significant correlation with yield and may be used as an indirect parameter for selecting higher-yielding lines.

Path analysis for various traits under study at saline (60 mM and 120 mM) and non-saline conditions

In the present study, the dependent character was seed yield per plant and all other remaining six characters were *viz.,* total chlorophyll content, relative water content, lipid peroxidation, proline content, total protein and number of pod per plant considered as independent characters tabulated in Table 6 for 60 mM, Table 7 for 120 mM and Table 8 for non-saline (control) condition respectively.

Path coefficient analysis at both genotypic and phenotypic levels under 60 mM saline condition

Genotypic level under 60 mM saline condition

Relative water content has a negative and very high direct effect (-1.179) on seed yield per plant. It exhibited negative and high indirect effects on seed yield per plant through proline content (-0.522), total protein (-0.802) and number of pods per plant (-0.449). Total protein content has a positive and high direct effect (0.433) on seed yield per plant. It exhibited negative and low indirect effects on seed yield per plant through total chlorophyll content (-0.119) while positive and low indirect effects on seed yield per plant through proline content (0.135). The number of pods per plant has a positive and high direct effect (0.566) on seed yield per plant. It exhibited a positive and moderate indirect effect on seed yield per plant through relative water content (0.216). The residual effect at genotypic level (0.123) at 60 mM saline condition was too low which indicates that most of the characters contributing to the seed yield were taken in the present study.

Phenotypic level under 60 mM saline condition

Total chlorophyll content had a positive and low direct effect (0.117) on seed yield per plant. It exhibited negligible indirect effect on seed yield per plant through all parameters taken for study. Lipid peroxidation had a positive and high direct effect (0.305) on seed yield per plant. Proline content had a positive and low direct effect (0.191) on seed yield per plant. It exhibited positive and low indirect effects on seed yield per plant through the number of pods per plant (0.108). The number of pods per plant has a positive and high direct effect (0.653) on seed yield per plant. It exhibited a positive and low indirect effect on seed yield per plant through total chlorophyll content (0.176). It exhibited a positive and moderate indirect effect on seed yield per plant through relative water content (0.244) and while positive and high indirect effect on seed yield per plant through proline content (0.369). The residual effect at phenotypic level (0.0987) at 60 mM saline condition was too low which indicates that most of the characters contributing to the seed yield were taken in the present study.

Path coefficient analysis at both genotypic and phenotypic levels under 120 mM saline condition

Genotypic level under 120 mM saline condition

Total chlorophyll content has a negative and very high direct effect (-1.456) on seed yield per plant. Lipid peroxidation has a positive and high direct effect (0.305) on seed yield per plant. Proline content had a positive and high direct effect (0.493) on seed yield per plant. It exhibited positive and moderate indirect effects on seed yield per plant through relative water content (0.230), total protein (0.254) and number of pods per plant

Table 6. Path-coefficient analysis of various traits under 60 mM saline condition

Path coefficient analysis at genotypic level under 60mM						
	TC 60mM	**RWC 60mM**	**LP 60mM**	**PC 60mM**	**TP 60mM**	**NPP 60mM**
TC 60mM	**0.0675**	-0.0026	-0.0057	0.0146	-0.0186	0.0185
RWC 60mM	0.0451	**-1.1788**	-0.0433	-0.5219	-0.8019	-0.449
LP 60mM	0.0051	-0.0022	**-0.0597**	-0.0106	0.0032	0.0008
PC 60mM	0.0005	0.0009	0.0004	**0.0021**	0.0007	0.0012
TP 60mM	-0.1189	0.2943	-0.0234	0.1354	**0.4326**	0.0316
NPP 60mM	0.1552	0.2155	-0.0078	0.3284	0.0414	**0.5657**
SYP 60mM	0.4356	-0.2242	-0.119	0.2521	-0.2981	0.6151
Partial R^2	**0.0294**	**0.2643**	**0.0071**	**0.0005**	**-0.1289**	**0.348**
R SQUARE = 0.9847 RESIDUAL EFFECT = 0.1235						

Path coefficient analysis at phenotypic level under 60mM						
	TC 60mm	**RWC 60mM**	**LP 60mM**	**PC 60mM**	**TP 60mM**	**NPP 60mM**
TC 60mM	**0.1168**	-0.0046	-0.01	0.0252	-0.0315	0.0314
RWC 60mM	0.0047	**-0.1187**	-0.0045	-0.0521	-0.0787	-0.0443
LP 60mM	-0.0262	0.0116	**0.305**	0.053	-0.0144	-0.0037
PC 60mM	0.0413	0.084	0.0332	**0.1912**	0.0578	0.108
TP 60mM	0.0597	-0.1471	0.0105	-0.0671	**-0.2217**	-0.016
NPP 60mM	0.1758	0.2439	-0.0079	0.3691	0.047	**0.6532**
SYP 60mM	0.4326	-0.2222	-0.1183	0.2505	-0.2872	0.6046
Partial R^2	**0.0505**	**0.0264**	**-0.0361**	**0.0479**	**0.0637**	**0.3949**
R SQUARE = 0.9904 RESIDUAL EFFECT = 0.0978						

Table 7. Path-coefficient analysis of various traits under 120 mM saline condition

Path coefficient analysis at genotypic level under 120mM						
	TC 120mM	**RWC 120mM**	**LP 120mM**	**PC 120mM**	**TP 120mM**	**NPP 120mM**
TC 120mM	**-1.456**	-0.2878	-0.0363	-0.43	0.2676	-0.4244
RWC 120mM	-0.0327	**-0.1657**	-0.0049	-0.0774	-0.0899	-0.0588
LP 120mM	0.0076	0.009	**0.305**	0.0025	-0.0859	-0.0216
PC 120mM	0.1455	0.2302	0.0041	**0.4926**	0.2535	0.216
TP 120mM	0.0643	-0.19	0.0986	-0.1802	**-0.3501**	0.0093
NPP 120mM	0.1707	0.2077	-0.0415	0.2568	-0.0155	**0.5857**
SYP 120mM	0.3649	-0.133	-0.074	0.2887	-0.3102	0.6752
Partial R^2	-0.5314	0.022	-0.0226	0.1422	0.1086	0.3954
R SQUARE = 0.9676 RESIDUAL EFFECT = 0.18						

Path coefficient analysis at phenotypic level under 120mM						
	TC 120mM	**RWC 120mM**	**LP 120mM**	**PC 120mM**	**TP 120mM**	**NPP 120mM**
TC 120mM	**1.007**	0.1983	0.0264	0.2964	-0.1821	0.2871
RWC 120mM	-0.0762	**-0.3867**	-0.0116	-0.1795	-0.2054	-0.1356
LP 120mM	-0.0072	-0.0082	**-0.2747**	-0.0026	0.0756	0.0196
PC 120mM	-0.0264	-0.0417	-0.0009	**-0.0899**	-0.0448	-0.0381
TP 120mM	-0.0111	0.0327	-0.0169	0.0307	**0.0616**	-0.001
NPP 120mM	-0.0842	-0.1035	0.021	-0.125	0.005	**-0.2952**
SYP 120mM	0.3626	-0.132	-0.0736	0.2883	-0.3008	0.6566
Partial R^2	0.3652	0.051	0.0202	-0.0259	-0.0185	-0.1938
R SQUARE = 0.9823 RESIDUAL EFFECT = 0.1332						

Table 8. Path-coefficient analysis of various traits under non-saline or control condition

Path coefficient analysis at genotypic level under Control (Ctrl)						
	TC 100 Ctrl	**RWC 100 Ctrl**	**LP 100 Ctrl**	**PC 100 Ctrl**	**TP Ctrl**	**NPP Ctrl**
TC 100 Ctrl	**0.022**	-0.0074	0.0073	0.0016	-0.0081	0.0008
RWC Ctrl	-0.1172	**0.3505**	-0.0796	0.0208	0.1299	-0.0704
LP Ctrl	-0.0428	0.0291	**-0.1282**	-0.0714	-0.0356	-0.0629
PC Ctrl	-0.0072	-0.0059	-0.055	**-0.0987**	-0.0105	-0.0391
TP Ctrl	-0.0931	0.0938	0.0703	0.0268	**0.2532**	-0.0002
NPP Ctrl	0.0044	-0.0236	0.0576	0.0465	-0.0001	**0.1174**
SYP Ctrl	0.1613	-0.4162	0.201	0.2755	-0.1963	0.6208
Partial R^2	0.0035	-0.1459	-0.0258	-0.0272	-0.0497	0.0729
R SQUARE = 1.0041 RESIDUAL EFFECT =SQRT. (1- 1.0041)						

Path coefficient analysis at phenotypic level under Control (Ctrl)						
	TC 100 Ctrl	**RWC 100 Ctrl**	**LP 100 Ctrl**	**PC 100 Ctrl**	**TP Ctrl**	**NPP Ctrl**
TC 100 Ctrl	**-0.0349**	0.0115	-0.0113	-0.0025	0.0125	-0.0016
RWC Ctrl	0.0414	**-0.1258**	0.0277	-0.0075	-0.0462	0.0231
LP Ctrl	0.0864	-0.0588	**0.2673**	0.1453	0.0726	0.1235
PC Ctrl	-0.0027	-0.0023	-0.0205	**-0.0377**	-0.0039	-0.0147
TP Ctrl	0.0571	-0.0584	-0.0431	-0.0164	**-0.1588**	-0.0003
NPP Ctrl	-0.009	0.0358	-0.0902	-0.0762	-0.0003	**-0.1951**
SYP Ctrl	0.1578	-0.4087	0.1989	0.2736	-0.1926	0.5928
Partial R^2	-0.0055	0.0514	0.0532	-0.0103	0.0306	-0.1157
R SQUARE = 0.9994 RESIDUAL EFFECT = 0.0251						

(0.216). Total protein content has a negative and high direct effect (-0.350) on seed yield per plant. The number of pods per plant has a positive and high direct effect (0.586) on seed yield per plant. The residual effect at genotypic level (0.18) at 120 mM saline condition was too low which indicates that most of the characters contributing to the seed yield were taken in the present study.

Phenotypic level under 120 mM saline condition

Total chlorophyll content has a positive and very high direct effect (1.007) on seed yield per plant. Relative water content has a negative and high direct effect (-0.387) on seed yield per plant. Lipid peroxidation has a negative and moderate direct effect (-0.275) on seed yield per plant. Total protein content has a positive and negligible direct effect (0.062) on seed yield per plant. It exhibited negligible indirect effect on seed yield per plant through all parameters taken for study. The number of pods per plant has a negative and moderate direct effect (-0.295) on seed yield per plant. It exhibited a negative and low indirect effect on seed yield per plant through relative water content (-0.104) and proline content (-0.125). The residual effect at phenotypic level (0.1332) at 120 mM saline condition was too low which indicates that most of the characters contributing to the seed yield were taken in the present study.

Path coefficient analysis at both genotypic and phenotypic levels under control condition

Genotypic level under non-saline condition

Total chlorophyll content has a positive and negligible direct effect (0.022) on seed yield per plant. Relative water content has a positive and high direct effect (0.351) on seed yield per plant. Lipid peroxidation has a negative and low direct effect (-0.128) on seed yield per plant. Total protein content has a positive and moderate direct effect (0.253) on seed yield per plant. The number of pods per plant has a positive and low direct effect (0.117) on seed yield per plant. The residual effect at genotypic level (1.004) at non-saline conditions was too low which indicates that most of the characters contributing to the seed yield were taken in the present study.

Phenotypic level under non-saline condition

Total chlorophyll content has a negative and negligible direct effect (-0.035) on seed yield per plant. Relative water content as a negative and low direct effect (-0.126) on seed yield per plant. Lipid peroxidation has a positive and moderate direct effect (0.267) on seed yield per plant. Total protein content has a negative and low direct effect (-0.159) on seed yield per plant. It exhibited negligible indirect effect on seed yield per plant through all parameters taken for study. The number of pods per plant has a negative and low direct effect (-0.195) on seed yield per plant. The residual effect at phenotypic level (0.0251) at non-saline condition was too low which indicate that most of the characters contributing to the seed yield were taken in the present study.

Genotypic path coefficient analysis based on seed yield per plant as a dependent variable revealed that drought tolerance score, plant height and pods per plant exhibited high positive direct effects (51). Therefore, this research suggests that drought tolerance score and pod per plant can be good selection criteria for improving seed yield per plant in chickpeas for drought-stress environments. Path analysis under non-saline conditions, the number of filled pods; seed number and 100-seed weight had a moderate direct positive contribution on seed yield while a total number of pods had a moderate indirect positive effect on seed yield through number of filled pods and seed number (40). Likewise, the number of filled pods had a moderate indirect positive effect on seed yield through seed number. Under salinity, the number of filled pods and seed number had a moderate positive direct effect on seed yield while 100-seed weight had a weak positive direct effect on seed yield. While number of total pods had a moderate indirect positive effect on seed yield through number of filled pods and seed number, filled pods had a moderate indirect positive effect on seed yield through seed number.

Conclusion

In conclusion, the screening and evaluation of chickpea genotypes under salinity stress revealed a decline in relative water content, total chlorophyll and yield parameters with increasing salt levels. Conversely, lipid peroxidation, proline and total protein content increased in response to salinity. The rise in lipid peroxidation is attributed to elevated hydrogen peroxide (H_2O_2) levels in the roots under high salinity conditions. Proline accumulation results from the enhanced activity of proline biosynthetic enzymes, such as pyroline-5-carboxylase reductase, while the activity of proline degrading enzyme proline oxidase decreases, helping to mitigate oxidative damage. The increase in total protein content can be linked to the upregulation of stress-related proteins in response to salinity. Yield parameters were found to improve under non-saline conditions but decreased significantly under stress. These findings provide valuable insights into the characteristics of salinity-tolerant genotypes, which will inform future breeding and selection programs aimed at enhancing chickpea resilience to salinity stress.

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Disclaimers

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Conflict of interest

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Authors' contributions

AT carried out the experiment, took observations and analyzed the data. B, PJ guided the research by formulating the research concept and ZAD, AAL approved the final manuscript. RK, SK participated in the design of the study and performed the statistical analysis reviewed the. MR, KS[1], KS[2], NY, SUL, S contributed by imposing the experiment and helped edit, summarise and revise the manuscript. B and RK helped summarize and revise the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

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Ethical issues: None

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