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GENETIC ANALYSIS OF SALINITY TOLERANCE IN CHICKPEA (CICER ARIETINUM L.)

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SUMMARY

The salinity tolerance in chickpea (*Cicer arietinum* L.) genotypes underwent scrutiny in saline and nonsaline conditions, as this study's aim. In the first experiment, 20 genotypes cultivated in pots sustained screening for salt tolerance. The second experiment involved a field study conducted on 10 selected genotypes planted under non-saline (Ismailia) and saline (El-Arish) soil conditions for two crop seasons (2022–2023 and 2023–2024). The results revealed a substantial variation among the genotypes under both salt stress conditions. Despite a considerable reduction in growth, yield, and biochemical traits in the saline condition, the chickpea genotypes Azad, Giza-1, FLIP-03-27C, and ICCV-10306 exhibited tolerance and demonstrated superior performance in most growth, yield, and biochemical traits. Genetic measures for seed weight, branch, filled pods, and total pod count demonstrated the highest phenotypic (PCV) and genotypic coefficient of variation (GCV) under both environments. The genetic gain demonstrated significance for relative water content, chlorophyll content, plant height, pod count, whole pod count, and seed weight under both environments. The heritability of most traits suggested that additive genetic action was significant in their determination, indicating the selection based on these traits could be helpful in breeding programs to improve the chickpea yield.

Keywords: Chickpea (*Cicer arietinum* L.), genotypes, salinity tolerance, genetic parameters, ISSR analysis, GCV and PCV, heritability, growth, and yield traits

Key findings: In screening of 20 chickpeas (*C. arietinum* L.) genotypes, Azad, Giza-1, FLIP-03-27C, and ICCV-10306 appeared as the most resilient to salinity. The results indicated substantial genetic diversity in genotypes, heritability, and genetic gain for key traits, suggesting potential improvement in chickpea under saline environments.

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INTRODUCTION

Salinity is a critical environmental element and constraint in the production of various crops globally. The soil salt deposition resulted from a dry environment, elevated temperatures, and inadequate irrigation management in Egypt (Corwin, 2021). The climatic variations also exacerbate soil salinization with time. The response mechanism to water stress is intricate; besides morphological, physiological, and metabolic variations, the interactions among these components are also crucial for resistance to water stress (Sahab et al., 2021). Several studies have progressed from plant breeders to identify tolerant genotypes exhibiting superior growth and vield characteristics in various crops.

Chickpea (Cicer arietinum L.) is an important pulse crop, native to the Middle East, and mostly grown in semi-arid regions globally. It is a considerable source of protein, carbohydrates, fibers, and other vital vitamins and minerals. Chickpea, being a legume crop, fixes the atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria called rhizobia found in root nodules. Atmospheric nitrogen fixation by chickpeas plays a vital role in sustaining soil fertility, especially in desert regions with limited precipitation (Koul et al., 2022). Furthermore, salinity stress has become one of the significant limiting factors in chickpea cultivation and production (Zawude and Shanko, 2017).

The chickpea nutritional aspects must address the rising food consumption and ensure the meals possess the highest nutrient content. This crop can improve the soil structure and quality, particularly in saline soils, by employing several breeding methods develop the salt-tolerant genotypes. to Screening crop genotypes is not а straightforward technique for selecting the optimal genotypes. The conventional screening procedure for studying salt tolerance typically relies on the grain yield in chickpeas and other crops (Kiani-Pouya and Rasouli, 2014; Shah et al., 2020).

Recently, the screening has transitioned to assessing physiological characteristics related to salt tolerance in different crops (Tao et al., 2021). Considerable genotypic diversity in growth, physiological, and biochemical features suggested these measures could serve as screening criteria for selection of salt-tolerant genotypes the (Alkahtani and Dwiningsih, 2023). Tolerant genotypes may prevent Na+ buildup in aerial tissues, hence enhancing photosynthetic efficiency and increasing the grain yield in barley (Mahlooji et al., 2018). The tolerant genotypes exhibited the best photosynthetic activity with a reduced transpiration rate in stress conditions compared with susceptible chickpea genotypes (Awari et al., 2017).

Induced salinity decreases the photosynthesis, respiration, and protein synthesis, and eventually decreases crop yield in Pisum sativum L. (Khan et al., 2022). From discussion, the above the presented investigations aimed to identify the salttolerant chickpea genotypes through seedling screening studies and characterization of morphological and physiological traits for salt tolerance in identified chickpea genotypes.

MATERIALS AND METHODS

Twenty chickpeas (C. arietinum L.) genotypes first received assessment in 2022-2023 for their responses to germination and seedling development in salt stress (30 mM NaCl) in a pot experiment (Table 1). Chickpea seeds' sowing commenced on November 20, 2022, in 30-cm diameter plastic pots filled with 8 kg of soil, under saline and non-saline conditions, utilizing recommended fertilizer applications. Pots with perforations served for control circumstances, while pots without perforations were suitable for saline treatments. Each treatment obtained five seeds in every pot, with only three seedlings retained per pot upon germination at 20 days after sowing. The experiment continued in an outdoor setting featuring a rainout shelter. The saline treatment (30 mM NaCl), as administered in

No.	Names	Origin	No.	Names	Origin
1	Giza.1	Egypt	11	FLIP87-59	Iran
2	Giza.2	ICARDA	12	Azad	Iran
3	Giza.3	Egypt	13	ICCV10306	Iran
4	Giza.4	Egypt	14	ICCV10308	Iran
5	Giza.195	Egypt	15	ICCV10310	ICARDA
6	FLIP 03-27C	Iran	16	ILC482C	Iran
7	FLIP 05-67C	Iran	17	ILC3279	Iran
8	FLIP 06-64C	Iran	18	7932	ICARDA
9	FLIP 06-65C	Iran	19	117703	ICARDA
10	FLIP 06-86C	Iran	20	9584	ICARDA

Table 1. Origin of 20 chickpea genotypes used in the study.

Table 2. Soil composition of two regions (Ismailia and El-Arish farms).

Variables				
variables		Non saline	Saline	
Ec dS m ⁻¹		2.91	7.2	
рН		7.45	8.92	
Ca CO ₃ %		0.52	1.48	
	sand	96.65	83	
Particle size distribution %	silt	2.51	12	
	clay	0.84	5	
	Na ⁺	19.6	74.8	
	K ⁺	0.72	0.93	
Cations meq/L	Ca ⁺	5.2	29	
	Mg ⁺	3.9	39.6	
	Cl	15	13.1	
Anions meq/L	S04 ²⁻	8.1	33.73	
	HCO3 ⁻	2.1	3.4	

split doses, comprised the time of planting and 15 days after sowing, using an adequate amount to saturate the soil to field capacity. The control pots received no saline treatment, but only irrigation with tap water.

The pots' arrangement had a completely randomized design (CRD) with three replications. Their evaluations commenced at the germination (after 10 days) and early seedling stages (after 45 days) on the parameters: germination based percentage, shoot length (cm), root length (cm), dry root and shoot weight (g), and shoot/root ratio. The chickpea genotypes exhibited varying behavior regarding salt tolerance in the pot experiment during 2022-2023.

The cultivation of selected 10 genotypes began during 2023–2024 in two distinct soil types on November 20, 2023. One

was at the Faculty of Agriculture farm at Suez Canal University, Ismailia Governorate (nonsaline soil), and the other was at the farm in El-Arish city (saline soil with 7.2 dsm⁻¹), with recommended inputs and culture practices. Table 2 presents analyses of two soil types. For growth and yield assessment of the different chickpea genotypes, the data recording transpired on days to 50% flowering, plant height, number of filled pods/plant, 100-seed weight, chlorophyll content, and relative water content (RWC).

Leaf chlorophyll reading

Leaf chlorophyll content estimation used a hand-held SPAD-502 (plus-Minolta, Japan) at the flowering stage. Average SPAD Chl readings' calculation came from the third leaf (from the top) of the 10 individual chickpea

Name of Primer	Sequence 5'-3'
ISSR- 1	5'-AGAGAGAGAGAGAGAGAGYG-3'
ISSR- 2	5'-GTGTGTGTGTGTGTGTGTGTG3'
ISSR- 3	5'-CGCGATAGATAGATAGATA-3'
ISSR-4	5'-GACGATAGATAGATAGATA-3'
ISSR-5	5'-AGACAGACAGACAGACGC-3'
ISSR-6	5'-GATAGATAGATAGC-3'
ISSR- 7	5'-ACACACACACACACYA-3'
ISSR- 8	5'-AGAGAGAGAGAGAGAGYT-3'
ISSR- 9	5'-CTCCTCCTCCTCTT-3'
ISSR- 10	5'-CTCTCTCTCTCTCTRG-3'
ISSR- 11	5'-HVHCACACACACACAT-3'

Table 3. The sequence and names of ISSR primers used in the study.

A: Adenine, T: Thymine, G: Guanine, and C: Cytosine, Y: (C or T), V: (A or C or G), H: (A or C or T).

plants, measured from the tip to the leaf base, between 10:00 a.m. and 01:00 p.m., and then averaged (Minolta, 1989).

ISSR - PCR Reactions

The genetic diversity study of the 10 chickpea genotypes (Giza-1, Giza-2, Giza-3, Giza-4, FLIP-03-27C, FLIP-06-65C, Azad, ICCV-10306, ICCV-10308, and ILC-3279) in saline conditions employed ISSR markers. A total of 11 ISSR primers used helped detect the polymorphism among the genotypes (Table 3). The PCR reaction consisted of 25 µl volume, including 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1 µM primers, 1 U Taq DNA polymerase, and 30 ng of template DNA (Table 2). Amplification involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation (94 °C for 1 min), annealing (50 °C for 1 min), and elongation (72 °C for 1.5 min), concluding with a final elongation at 72 °C for 7 min. Separating the PCR products used 1.5% agarose gel with ethidium bromide and visualized under UV light. Banding patterns' recording indicated the presence (1) or absence (0) of bands. Genetic similarity calculation engaged the Dice coefficient to generate a similarity matrix, then analyzed with the UPGMA clustering (Sneath and Sokal, 1973).

Statistical analysis

Two-way analysis of variance (ANOVA) with three replicates and error compatibility ensued on all the data based on various agronomic and biochemical properties recorded in two crop seasons of the chickpea. Comparing the chickpea genotypes in stress and non-stress environments used the AGRI-STAT software at a $P \le 0.05$ probability level. A set of genetic parameters (genotypic and phenotypic coefficient of variability, heritability in broad sense, and genetic gain) reached calculation and analysis using AGRI-STAT (Hallauer *et al.*, 2010).

RESULTS

Pots screening

The 20 chickpeas (C. arietinum L.) genotypes' watering with tap water and a 4500 ppm concentration of salinity started at the seedling stage for various growth traits (Figure 1). The results showed significant ($P \le 0.05$) variations among the genotypes for all traits. Ten chickpea genotypes (Giza-1, Giza-2, Giza-3, Giza-4, FLIP-03-27C, FLIP-06-65C, Azad, ICCV-10306, ICCV-10308, and ILC-3279) gave the highest mean values for germination (%), fresh and dry weight of shoot and root, and root/shoot ratio in a saline condition. The rest of the genotypes appeared with different degrees of decline for most growth traits.

Field evaluation

The seeds of selected 10 chickpea genotypes succeeded in growing in non-saline (Ismailia



Figure 1. Effect of salinity stress on germination (%), shoot length, root length, shoot and root dry weight, and root/shoot ratio in 20 genotypes of chickpea under normal and saline conditions.

Region) and saline (Al-Arish Region) soil conditions to evaluate them for growth, yield, and biochemical traits.

Early flowering and growth traits

Results revealed most studied chickpea genotypes appeared with early flowering in the saline condition compared with the non-saline condition in both seasons, with the reduction in flowering period being about 23.5% (Table 4). However, the genotypes differed more with long periods of earliness. The chickpea genotypes FLIP06-65C, Giza-4, Azad, and Giza-1 showed the shortest period up to 50% flowering in non-saline and saline conditions during both crop seasons.

The results further detailed significant $(P \le 0.05)$ differences among the genotypes and their responses to salinity conditions. Overall, the genotypes showed decreased plant height in the saline condition versus the nonsaline condition because of deficient water (Table 3). The chickpea genotypes ICCV-10306, FLIP-06-65C, Giza-1, and ILC-3279 demonstrated the tallest mean values for the

Chicknes	Days to 50% flowering							Plant height (cm)					
	2022/2	023	2023/2	2024	Means		2022/20	023	2023/2024		Means		
genotypes	Ν	S	Ν	S	Ν	S	Ν	S	Ν	S	Ν	S	
Giza-1	71.0	51.3	70.3	52.7	70.7	52.0	66.8	61.9	66.6	64.3	66.7	63.1	
Giza-2	72.7	54.3	72.0	54.3	72.4	54.3	62.2	56.4	61.8	55.5	62.0	56.0	
Giza-3	74.7	52.0	74.3	51.3	74.5	51.7	54.6	49.1	55.0	48.5	54.8	48.8	
Giza-4	64.7	52.3	63.7	52.3	64.2	52.3	56.3	50.8	56.8	50.1	56.6	50.5	
FLIP-03-27C	71.0	54.3	70.0	54.7	70.5	54.5	53.0	47.5	53.5	47.0	53.3	47.3	
FLIP-06-65C	48.3	51.3	47.7	50.0	48.0	50.7	75.6	70.0	74.9	70.0	75.3	70.0	
Azad	70.3	53.3	69.3	52.3	69.8	52.8	59.3	53.9	59.8	53.5	59.6	53.7	
ICCV-10306	71.3	52.0	72.3	52.0	71.8	52.0	75.7	70.6	75.6	72.9	75.7	71.8	
ICCV-10308	73.7	56.0	74.3	55.0	74.0	55.5	61.2	55.9	60.2	58.6	60.7	57.3	
ILC-3279	72.7	52.3	73.7	50.7	73.2	51.5	63.2	57.7	64.1	59.1	63.7	58.4	
Means	69.0	52.9	68.8	52.5	68.9	52.7	62.8	57.4	62.8	58.0	62.8	57.7	
RD%	-23.4		-23.6		-23.5		-8.6		-7.8		-8.2		
P values	G=<0.0	001**	G=<0.001 ^{**}		G=<0.001 ^{**}		G=<0.001 ^{**}		G=<0.001 ^{**}		G=<0.001**		
	S=<0.001**		S=<0.001**		S=<0.001**		S=<0.001**		S=<0.001**		S=<0.001**		
	G*S=<	0.001**	G*S=<	:0.001**	G*S=<	:0.001**	G*S=<	0.001**	G*S=<	:0.001**	G*S=<	:0.001**	
		E	Branches	s per pla	nt Fil			lled pods per plant					
Giza-1	27.3	26.7	27.6	26.9	27.5	26.8	40.3	51.7	39.8	49.5	40.1	50.6	
Giza-2	21.5	20.7	21.8	18.5	21.7	19.6	64.7	53.7	61.2	53.3	63.0	53.5	
Giza-3	29.8	28.4	30.0	27.7	29.9	28.1	49.8	32.3	49.3	33.9	49.6	33.1	
Giza-4	26.1	25.0	26.4	23.3	26.3	24.2	67.4	39.6	66.7	41.6	67.1	40.6	
FLIP-03-27C	34.5	33.6	32.5	32.0	33.5	32.8	60.5	55.7	61.2	57.7	60.9	56.7	
FLIP-06-65C	29.4	28.9	29.0	29.2	29.2	29.1	45.7	25.9	45.3	24.6	45.5	25.3	
Azad	40.7	37.9	40.1	36.6	40.4	37.3	52.4	49.6	49.4	49.7	50.9	49.7	
ICCV-10306	27.6	25.9	26.6	27.6	27.1	26.8	54.2	44.9	52.1	46.5	53.2	45.7	
ICCV-10308	34.3	33.5	33.9	33.0	34.1	33.3	58.9	45.9	60.7	47.3	59.8	46.6	
ILC-3279	29.7	28.9	27.8	28.7	28.8	28.8	35.3	30.6	34.5	32.1	34.9	31.4	
Means	30.1	29.0	29.57	28.4	29.8	28.7	52.9	43.0	52.0	43.6	52.5	43.3	
RD%	-3.8		-4.1		-4.0		-18.7		-16.2		-17.5		
P values	G=<0.0	001**	G=<0.	001**	G=<0.	001**	G=<0.001**		G=<0.	001**	G=<0.	001^{**}	
	S=<0.0	01**	S=<0.	001**	S=<0.	001**	S=<0.001**		S=<0.001**		S=<0.	001**	
	G*S=<	0.001**	G*S=<	:0.001**	G*S=<	:0.001**	G*S=<	0.001**	G*S=<	:0.001**	G*S=<	:0.001**	

Table 4. Effect of salinity stress on days to 50% flowering, plant height, branches per plant, and filled pods per plant in 10 chickpea genotypes under non-saline and saline conditions for two crop seasons.

**: Significant at $P \le 0.01$, N: Non-saline, S: Saline, RD%: Reduction (%).

plant height in saline and non-saline conditions in both crop seasons. The Azad, ICCV-10308, FLIP-03-27C, and FLIP-06-65C exhibited the most number of branches per plant, while the chickpea genotype Giza-2 has substantially decreased for the said trait in saline conditions.

Yield and yield components

According to the number of filled pods per plant among the chickpea genotypes, a significant decline emerged in the saline condition compared with the non-saline. The genotypes FLIP03-27C, Giza-2, Azad, ICCV-10308, and Giza-1 showed the highest number of filled pods per plant in both salinity conditions, while the genotype FLIP-06-65C owned the least mean value for the said trait in the saline environment (Table 4).

For 100-seed weight, the chickpea genotypes revealed notable ($P \leq 0.05$) differences in both salinity environments. The genotypes Azad, Giza-1, Giza-2, and Giza-3 occurred with the supreme 100-seed weight in non-saline and saline conditions. For seed yield per plant, the chickpea genotypes provided significant ($P \leq 0.05$) variances in both salinity environments. The four genotypes Azad, Giza-1, FLIP-03-27C, and ICCV-10306 exhibited the highest seed yield per plant over both crop seasons (Table 5).

	100-seed weight (g)						Seed yield per plant (g)					
Chickpea genotypes	2022	2/2023	2023/2024		Means		2022/2023		2023/2024		Means	
	Ν	S	Ν	S	Ν	S	Ν	S	Ν	S	Ν	S
Giza-1	23.0	22.8	23.3	23.0	23.2	22.9	15.07	11.33	15.94	13.11	15.5	12.2
Giza-2	20.3	22.5	20.5	21.7	20.4	22.1	15.59	10.07	16.47	10.04	16.0	10.1
Giza-3	24.3	21.4	24.1	21.4	24.2	21.4	25.48	11.32	24.97	10.59	25.2	11.0
Giza-4	16.8	14.4	16.2	12.8	16.5	13.6	18.84	9.69	17.3	10.28	18.1	10.0
FLIP-03-27C	23.0	20.0	21.4	21.4	22.2	20.7	20.27	11.2	20.05	12.17	20.2	11.7
FLIP06-65C	18.9	16.5	17.5	15.1	18.2	15.8	16.81	10.87	16.26	11.65	16.5	11.3
Azad	25.2	23.4	25.5	23.2	25.4	23.3	24.28	15.97	23.55	16.27	23.9	16.1
ICCV-10306	14.5	12.8	14.3	14.3	14.4	13.6	19.95	11.92	22.86	11.24	21.4	11.6
ICCV-10308	17.8	15.4	17.2	14.9	17.5	15.2	13.3	11.05	14.51	11.62	13.9	11.3
ILC-3279	15.9	18.5	15.7	18.4	15.8	18.5	18.9	9.6	18.97	9.49	18.9	9.5
Means	20.0	18.8	19.6	18.6	19.8	18.7	18.8	11.3	19.1	11.6	19.0	11.5
RD%	-6.00		-5.10		-5.56		-39.9		-39.3		-39.5	
P values	G=<0.001**		G=<0.001**		G=<0.001**		G=<0.001**		G=<0.001**		G=<0.001**	
S=<0.001**		001**	S=<0.001**		S=<0.001**		S=<0.001**		S=<0.001**		S=<0.001**	
	G*S=<	0.001**	G*S=<	<0.001**	G*S=<	:0.001**	G*S=<	0.001**	G*S=<	0.001**	G*S=<	:0.001**
		C	Chloroph	nyll conte	nt			RWC (at 60 days)				
Giza-1	66.9	51.3	65.0	51.6	66.0	51.5	73.6	61	71.6	58.1	72.6	59.6
Giza-2	64.2	53.6	62.0	52.9	63.1	53.3	64.7	55.9	62.8	56.6	63.8	56.3
Giza-3	61.9	54.1	60.9	52.0	61.4	53.1	84.4	81.5	83.8	81.6	84.1	81.6
Giza-4	71.3	48.9	70.0	46.4	70.7	47.7	74.2	70.9	70.9	72.3	72.6	71.6
FLIP-03-27C	61.1	46.1	59.1	43.5	60.1	44.8	76.3	79.8	74.7	78.3	75.5	79.1
FLIP-06-65C	53.9	54.0	54.5	54.3	54.2	54.2	81.1	65.0	80.0	64.1	80.6	64.6
Azad	63.8	49.1	63.0	51.5	63.4	50.3	77.1	74.5	76.3	72.2	76.7	73.4
ICCV-10306	56.2	53.6	54.8	51.7	55.5	52.7	83.5	79.5	80.8	77.9	82.2	78.7
ICCV-10308	61.1	52.5	61.0	50.7	61.1	51.6	84.7	83.5	81.3	80.6	83.0	82.1
ILC-3279	53.9	51.0	54.2	49.3	54.1	50.2	59.8	56.3	58.3	56.3	59.1	56.3
Means	61.4	51.4	60.5	50.4	60.9	50.9	75.9	70.8	74.1	69.8	75.0	70.3
RD%	-16.3		-16.7		-16.4		-6.7		-5.8		-6.3	
P values	G=<0.0	001**	G=<0.	001**	G=<0.	001**	G=<0.001**		G=<0.001**		G=<0.	001**
	S=<0.0	001**	S=<0.	001**	S=<0.0	001**	S=<0.0	001**	S=<0.0	001**	S=<0.	001**
	G*S=<	0.001**	G*S=<	<0.001**	G*S=<	:0.001**	G*S=<	0.001**	G*S=<	0.001**	G*S=<	:0.001**

Table 5. Effect of salinity stress on 100-seed weight, seed yield per plant, chlorophyll content, and RWC (at 60 days) in 10 chickpea genotypes under non-saline and saline conditions for two crop seasons.

**: Significant at $P \le 0.01$, N: Non-saline, S: Saline, RD%: Reduction (%).

Table 6. Genetic parameters of the studied traits in chickpea genotypes under non-saline and saline conditions.

Barameters		Non-salin	ne conditio	n		Saline condition				
Falaneters	PCV	GCV	h²	GA	PCV	GCV	h²	GA		
Days to 50% flowering	13.25	13.22	99.49	15.97	14.40	14.38	99.66	17.35		
Plant height	11.52	11.44	98.69	16.13	3.20	2.79	76.12	2.64		
Branches per plant	0.93	0.70	56.28	1.57	1.69	1.48	76.56	3.71		
Filled pods per plant	16.88	16.80	99.07	20.02	21.55	21.48	99.38	20.97		
100-seed weight	19.20	19.09	98.87	7.70	20.93	20.91	99.76	8.08		
Seed yield per plant	14.21	13.89	97.68	8.23	15.38	15.12	98.62	8.17		
Chlorophyll content	8.71	8.65	98.66	10.79	5.63	5.59	98.37	5.81		
RWC	11.24	11.07	97.03	16.84	14.83	14.74	98.79	21.21		
PCV (Phenotypic Coefficient	of Variatio	n)								
GCV (Phenotypic Coefficient of Variation)										
h ² (Heritability)										
GA (Genetic Advance)										

Chlorophyll content

On physiological analysis, the chickpea genotypes indicated significant ($P \le 0.05$) differences for chlorophyll content for both salinity environments (Table 5). The genotypes FLIP06-65C, Giza-2, Giza-3, and ICCV-10306 showed the highest mean values of chlorophyll content in the saline condition during both seasons. Meanwhile, the genotypes ICCV-10308, Giza-1, Azad, ILC-3279, Giza-4, and FLIP-03-27C indicated the moderate response to salinity and showed the least chlorophyll content for both environments.

Relative water content

For all chickpea genotypes, the relative water content (RWC) showed a significant decreasing order in the saline condition compared with the non-saline soil condition (Table 5). However, the degree of RWC decrease differed among the genotypes. The genotypes ICCV-10308, Giza-3, FLIP-03-27C and ICCV-10306 were visible with the highest relative water content for the salt-stress condition.

Genetic parameters

In the case of genetic parameters, the phenotypic (PCV) exceeded the genotypic coefficient of variation (GCV) in non-saline and saline conditions (Table 6). The saline condition revealed increased values of PCV and GCV for all variables, except branches per plant. The chickpea traits seed number, 100-seed weight, pods per plant, and days to maturity had significant values of PCV and GCV. Heritability (Broad sense) ranged from 56.28 (branches per plant) to 99.49 (days to 50% flowering) in non-saline circumstances. Under the saline condition, the broad sense heritability ranged from 76.12 (plant height) to 99.76 (100-seed weight). Characteristics behave genetically similar in most contexts. The highest genetic gain was evident among the chickpea genotypes for the traits filled pods per plant, days to 50% flowering, and RWC in non-saline and saline environments.

Identification of ISSR primers

Ten chickpeas (C. arietinum L.) genotypes (Giza-1, Giza-2, Giza-3, Giza-4, FLIP-03-27C, FLIP-06-65C, Azad, ICCV-10306, ICCV-10308, and ILC-3279) attained selection from the salinized habitat. For the said research, using 11 primers yielded reliable and measurable polymorphism. The primers were noticeable with many bands, comprising 4-13 amplified DNA fragments and 1-9 polymorphic fragments (Figure 2). The minimum of four fragments bore amplification with ISSR-1 and ISSR-5, while the maximum of 14 fragments with the ISSR-8 and ISSR-10. The 105 amplified fragments had 49 polymorphic bands, showing 45.6% polymorphism. The results also revealed seven different bands and monomorphic bands. The amplified 49 fragments ranged from 100 to 1500 base pairs (Figure 3).

Genetic similarity

Among the 10 chickpea genotypes, the genetic similarity assessment used the scored ISSR data matrix. The similarity matrix utilized helped construct a dendrogram with the UPGMA algorithm (Table 7). The ISSR data analysis indicates genetic similarity among the chickpea genotypes varied from 78% to 92%. Alongside ISSR analysis, the highest similarity level (92%) was notable between genotype 4 (Giza-4) and genotype 5 (FLIP-06-65 C), indicating a close relationship. The lowest genetic similarity (78%) appeared between genotype 1 (Giza-1) and genotype 10 (ILC-3279).

A dendrogram categorized the 10 genotypes chickpea into two principal groupings (Figure 3). In cluster one, one subcluster had two chickpea genotypes, i.e., 7 (Azad) and 4 (Giza-4). Another subcluster, separated into two groups, included 5 (Flip-03-27C), 1 (Giza-1), 3 (Giza-3), and 8 (ICCV-10306). The second cluster, subdividing into subclusters, comprised the first subcluster with 10 (ILC-3279) and 2 (Giza-2), while the second subcluster consisted of 9 (ICCV-10308) and 6 (Flip-06-65C).



Figure 2. Amplification of the 10 chickpea genotypes with ISSR-PCR primers No. 1, 2, 7, and 11. M: marker, 1: Giza-1, 2: Giza-2, 3: Giza-3, 4: Giza-4, 5: FLIP-03-27C, 6: FLIP-06-65 C, 7: Azad, 8: ICCV-10306, 9: ICCV-10308, and 10: ILC-3279.



Figure 3. Dendrogram generated based on the UPGMA clustering method and Jacquard`s coefficient using ISSR analysis among the 10 chickpea genotypes.

Principal coordinate analysis (PCoA)

The genetic similarity matrix based on Jaccard's similarity coefficient also underwent the PCoA, for better visualization of the relationship among the chickpea genotypes. A PCA variable showed clustering in four quadrants' variables (Figure 4). PCA analysis indicated that genotypes 4 (Giza-4) and 7 (Azad) resulted in quadrant I. The genotype 6 (FLIP-06-65C) surfaced in quadrant II on a distance from the other studied genotypes. The genotypes 9 (ICCV-10308), 10 (ILC-3279), and 2 (Giza-2) appeared in quadrant III. The chickpea genotypes 8 (ICCV-10306), 1 (Giza-1), 3 (Giza-3), and 5 (FLIP-03-27 C) remained in quadrant IV.

	Giza1	Giza 2	Giza 3	Giza 4	FLIP 03-27 C	FLIP 06-65 C	Azad	ICCV 10306	ICCV 10308
	1	2	3	4	5	6	7	8	9
1	1								
2	0.86	1							
3	0.88	0.85	1						
4	0.85	0.85	0.85	1.00					
5	0.84	0.84	0.86	0.92	1.00				
6	0.86	0.86	0.85	0.84	0.83	1.00			
7	0.86	0.85	0.86	0.87	0.85	0.85	1.00		
8	0.85	0.85	0.87	0.85	0.85	0.85	0.87	1.00	
9	0.80	0.81	0.85	0.86	0.87	0.80	0.83	0.85	1.00
10	0.78	0.83	0.82	0.84	0.85	0.84	0.81	0.83	0.87

Table 7. Genetic similarity among the 10 chickpea genotypes as estimated using ISSR-PCR data using 11 primers.



Figure 4. PCA plot explained by two axes (Coordinates), with four quadrants defined.

DISCUSSION

Salinity stress is one of the prominent abiotic stresses, caused by excessive salt accumulation in the soil. Salinity tolerance is a specific developmental stage mostly affected environmental factors; hence, by its phenotyping is guite difficult. Several studies have advanced to understand the complexities of chickpea salinity tolerance with physiological and genetic perspectives (Kotula et al., 2020).

In the pot experiment, 10 out of 20 genotypes showed the highest mean values for germination (%), fresh and dry weight of shoots and roots, and root/shoot percentage in the saline condition. Results were greatly analogous to past findings, which revealed salt

stress (0, 50, 100, and 150 mM) provided a significant decrease in almost all growth traits: shoot and root lengths (31.3% and 30%), respectively, and dry weight of shoots and roots (34.9% and 34.1%), respectively (Zawude and Shanko, 2017). Salinity caused a reduction in plant growth, which could be due to the inhibition of cell division and expansion (Atta *et al.*, 2023).

The results showed most studied genotypes of the chickpea appeared early flowering in the saline condition in both seasons compared with the non-saline condition, which could be because of the stress condition caused by saline soils. However, such traits are considerably crucial from a breeder's viewpoint. Such behavior helps crop plants avoid or reduce stress conditions caused by other abiotic and biotic factors. These traits can also be beneficial as an indicator to select tolerant genotypes (Touchan and Basal, 2022).

This study determined filled pods per plant and 100-seed weight are the vital yield components that manage the seed yield in chickpeas. Most genotypes showed significant decreases in yield and its components in saline conditions compared with non-saline conditions, despite high mean values for two seasons. For a stress condition, some plants can reach and finish the vegetative stage but not reach the reproductive stage since the plants need more nutrients, metabolism enzymes, and enough water (Buttar et al., 2020). Sobh et al. (2023) reported that soil saline conditions lowered the number of full pods per plant, and in salt-sensitive chickpea genotypes, the pod abortion increased. Salinity stress lowers plant growth and development and production by causing osmotic effects, ionspecific stress, ionic imbalance, and oxidative stress (Kumar et al., 2021).

The chickpea genotypes revealed significant differences for seed yield per plant for both environments due to a large seed size compared with other genotypes. The presented results agreed with past findings illustrating remarkable correlations between dry matter and seed yield (Mahlooji et al., 2018). With the gradual accumulation of toxic ions in shoots, most of the biomass production occurred during the vegetative growth stage due to low accumulation. However, salt the seed production formed mainly at the end of the plant life, when also the maximum rate of salts accumulated.

For chlorophyll content, the chickpea genotypes showed significant differences in both environments. The potential cause may be because of increasing destructive enzymes chlorophyllase. called Pigments' system reduction is due to the weakening of the protein-pigment-lipid complex induction and elevated chlorophyllase enzyme activity (Muhammad et al., 2024). The higher chlorophyll content in saline conditions could provide an index for the superior grain yield (Mahlooji et al., 2018).

Relative water content significantly decreased in the saline condition for all chickpea genotypes. These genotypes may be more tolerant of stress because it can keep more water in cells. These results agree with Kaur et al. (2014) in chickpeas and Fariduddin et al. (2012) in tomatoes, who reported the leaf water potential showed linear decreases as the concentrations of NaCl rose. Mahlooji et al.'s (2018) findings exhibited the tolerant genotype rather than the sensitive genotype gave a higher RWC in salinity stress. Arefian and Shafaroudi (2015) observed the higher maintenance of leaf RWC in chickpea genotypes, which indicated the said genotype could be more efficient in water absorption, avoiding tissue dehydration. It could also involve an adaptation of plant growth for the saline condition.

The PCV exceeded the GCV values for all attributes in both non-saline and saline conditions. Given their varied genetic make-up, revealed the aenotypes considerable differences. Any plant attribute connected to seed production has its own genetic system and depends on heredity and nature, recording distinct environmental influences on the yield components in chickpeas (Mohammadi and Talebi, 2015). High genetic gain was evident for the traits filled pods per plant, 100-seed weight, and RWC in non-saline and saline environments. These results showed additive genes affect trait expression and can be applicable in breeding programs to select the stress-tolerant genotypes. In chickpeas, additive and non-additive genetic variations influenced the quantitative trait inheritance (Kadir et al., 2017; Hegde et al., 2018; Qulmamatova, 2023).

The ISSR analysis revealed a high level polymorphism among the chickpea of genotypes. Lenka et al.'s (2015) findings also demonstrated primers produced reliable and reproducible banding patterns, and the number, amplified DNA fragments' size, and the percentage of generated polymorphic bands varied among the primers. Tahir and Karim (2011) reported 6.8 marker primers using 27 ISSR primer pairs. The current ISSR analysis can successfully determine the phylogenetic relationship among the studied chickpea genotypes. These results also gained support from past studies, which reported considerable genetic diversity and relationship in the chickpea genotypes using RAPD and ISSR markers (Tahir and Karim, 2011; Choudhary *et al.*, 2013; Ansabayeva and Akhmetbekova, 2024).

Talebi *et al.* (2008) mentioned the 2D principal component analysis (PcoA)assessment used the first two principal coordinates that provided the reliable grouping of accessions in the coordinate system and evaluated the genetic relationship among the chickpea accessions. Choudhary *et al.* (2013) reported the two-dimensional PCoA plot separated all the chickpea accessions into two major clusters, and the wild accessions appeared on the right side as a separate cluster, whereas most cultivated accessions appeared on the left side.

CONCLUSIONS

The promising research identified the chickpea (C. arietinum L.) genotypes Azad, Giza-1, FLIP-03-27 C, and ICCV-10306 with considerable tolerance to saline conditions and demonstrated superior growth and yield traits. These genotypes revealed substantial genetic variation for key traits related to salt tolerance, with high heritability, indicating a significant genetic influence. Targeted selection and breeding on these resilient genotypes could enhance chickpea production and promote agricultural sustainability in saline regions.

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