International Journal of Environment and Climate Change

Volume 12, Issue 12, Page 1795-1811, 2022; Article no.IJECC.96331
ISSN: 2581-8627
(Past name: British Journal of Environment & Climate Change, Past ISSN: 2231–4784)

Biochemical Response on Three Growth Phases of Chickpea under Graduated Salt Stress

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2022/v12i121627

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/96331

Original Research Article

Received: 16/11/2022
Accepted: 30/12/2022
Published: 31/12/2022

ABSTRACT

Chickpea *(Cicer arietinum L.)* is sensitive to salt sress, that affects its yield and there is need to identify the tolerant genotypes. The present study was conducted to evaluate the effect of NaCl salt stress on chickpea genotypes with specific biochemical attributes contributing to their adaptability to salt stress. Ten chickpea genotypes both desi (Annigeri 1, BGD103, NBeG47, JG11, GBM2, JAKI9218, ICC1431, ICC5003, ICCV96029) and *kabuli* (MNK 1) were evaluated for salinity tolerance. To determine the most tolerant genotype to salinity stress, an experiment was done at College of Agriculture, Vijayapur during 2019 as factorial form under completely block design (CRD) with three replications and 3 treatments, control and 2 NaCl salinity levels (3dS/m and 6dS/m) in 10 chickpea cultivars at 30, 60 and 90 days after sowing. Salinity is a serious abiotic stress, causing oxidative stress. Various biochemical parameters in chickpea genotypes were considered under varied NaCl concentrations. The results revealed that proline was significantly higher in JG 11 (33.42 mg g^{-1}fr. wt.) at 6 $dS.m^{-1}$ of salt as compared to other genotypes, because of high concentration of proline content enable JG11 to maintain low water potentials and tolerance to salt stress. Salt stress reduces the total chlorophyll content of leaves in salt susceptible plants and

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increases it in salt tolerant plants. The chlorophyll content decreased in all genotypes during the stress. Maximum decrease in chlorophyll content was observed with ICCV96029 and NBeG 47 among ten genotypes. Among biochemical parameters, the proline concentration was increased by high salinity, while total chlorophyll concentration was decreased in all tested genotypes. Hence proline and total chlorophyll content were more consistent with salt tolerance responses of the genotypes.

Keywords: NaCl; salt stress; chickpea; plant; genotypes.

1. INTRODUCTION

Chickpea is the second most important legume crop after dry beans [1]. The genus Cicer originated in South-Eastern Turkey and spread to other parts of the world. Chickpea is grown in 54 countries with nearly 90% of its area covered in developing countries [2]. The species is grouped into *desi* and *kabuli* type: *desi* generally have small, darker coloured seeds, where as Kabuli is usually producing large, cream-coloured ones.

Soil salinity is becoming more problematic due to the increase in irrigation around the world. The harmful impacts of salinity include low agricultural production, low economic returns due to high cost of cultivation, reclamation, management, ecological imbalance due to halophytes and marine life forms from fresh water to brackish water, poor human health due to toxic effects of accumulated elements [3]. Resistance to salt stress does not rely on a single trait but, on the contrary, it has a very complex nature as it depends upon various morphological and biochemical traits. Salinity affects germination, initial seedling establishment, growth, nitrogen fixation, flowering, pod development and seed filling of chickpea [4,5]. Salinity stress delayed flowering to a greater extent in the sensitive than tolerant genotypes due to higher concentrations of Na^+ in young leaves and the accumulations of $Na^+$and K^+ in old green leaves, in the sensitive than in the tolerant chickpea genotypes [6]. The concentration of numerous metabolites, including proline and glycine betaine, also increases under salt stress, providing defence against osmotic challenge by serving as compatible solutes [7,8].

The availability of water to the growing tissue becomes a limiting factor under saline conditions even in the presence of moisture in the soil resulting in what is termed as “Physiological Drought” [9,10]. Water uptake by plants hence, attains importance under saline conditions. Oxidative stress is responsible for the generation of reactive oxygen species (ROS) which are deleterious to plants [11,12]. ROS are highly reactive and cause damage to biomolecules such as lipids, proteins and nucleic acids [13]. Proline is considered as the only osmolyte which has been shown to scavenge singlet oxygen and free radicals including hydroxyl ions. It also serves as redox potential regulator and protects macromolecules such as proteins, DNA and reduces enzyme denaturation caused by heat, NaCl and other stresses [14]. Chickpea being sensitive to salinity needs considerable enhancement of salinity tolerance to be grown on natural saline soil. Keeping all the factors in mind the present investigation was formulated to study the effect of salt stress in chickpea genotypes at different crop development stages (30, 60 and 90 days after sowing). The tolerant chickpea (*Cicer arietinum* L.) genotypes will be identified on the basis of biochemical indices.

2. MATERIALS AND METHODS

The pot experiment was conducted at rain out shelter College of Agriculture, Vijayapura and laboratory study was conducted at Department of Crop Physiology. The College of Agriculture, Vijayapura is situated at 16°49’ N latitude and 76°34’ E longitude with an altitude of 678 meters above sea level. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. The 10 genotypes like Annigeri 1, JAKI 9218, BGD 103, MNK 1, JG11, GBM 2, NBeG 47, ICC 1431, ICC 5003 and ICCV96029 were used. There were three treatments including control and salinity levels were developed by using NaCl solution.

2.1 Salient Features of the Genotypes

In the experiment, seven genotypes were collected from chickpea scheme, Regional Agricultural Research Station (RARS), College of Agriculture, Vijayapura and three genotypes were collected from ICRISAT. The chickpea entries included both *desi* and *kabuli* types. The seed material had much of the genetic variability (Table 1). The genotypes include highly tolerant,

moderately tolerant, susceptible and highly susceptible to salt stress. Among the genotypes selected for the experiment, two genotypes ICC5003 and ICC1431 were tolerant to salt stress and the ICCV 96029 genotype was susceptible to salt stress (Vadez et al. 2007) were considered as checks (c).

2.2 Sowing and Salinity Treatments:+

The pots of uniform size (30x30 cm) were filled with 10 kg of air-dried soil and farmyard manure in 6:1 ratio. Before sowing, pots were irrigated with 2.5 liters of water (control) or salt solutions of different concentrations. The plants were subjected to three conditions *viz.* control (C1) and two salinity treatments (C2 and C3). Salt solutions were prepared by using NaCl salt. The salt concentrations of different solutions are given below.

C1 = Control
C2= 5 gram of NaCl salt dissolved in 1 liter of water for preparing 3 $dS.m^{-1}$
C3= 10 gram of NaCl salt dissolved in 1 liter of water for preparing 6 $dS.m^{-1}$
(Actual salinity values are expressed as $dS.m^{-1}$)

2.3 Observations to be Recorded

The observations recorded at specific intervals in different growth stages to assess the influence of salinity on chickpea growth and biochemical attributes. The details of observation taken and standard procedures were adopted, which are described in detail which is as follows.

Table 1. Salient features of chickpea genotypes used for experiment

Sl. No.	Variety	Features
1	Annigeri-1	High yielding variety with a duration of 90-110 days and an average yield is 8-10 q ha^{-1} in rainfed and 20-25 q ha^{-1} in irrigated condition.
2	JG-11 (ICCV93954)	High yielding crop with duration of 100-105 days and yields up to 20-25 q ha^{-1} and is resistant to wilt and moderately resistant to dry root rot.
3	MNK1	*Kabuli* seed type with duration of 95-110 days, extra large seeds and moderately resistant to wilt. Average yield of MNK1 genotype is 13 q ha^{-1}.
4	BGD-103	The genotype BGD-103 is bold seed genotype and is considered as moderately tolerant to salt stress. The genotype bears white flowers and medium maturing crop with a duration of 90-95 days. It is resistant to fusarium wilt.
5	JAKI9218	Average yield of JAKI 9218 is 18-20 q ha^{-1} with days to maturity is 93-125. It is resistant to wilt, root rot and color rot.
6	ICC1431	Moderately yielding variety with a duration of 90-100 days and *desi* seed type, the genotype considered as tolerant to salt stress.
7	ICCV96029	The genotype is small seed type and it is considered as susceptible to salt stress. The genotype bears very early flowering and *desi* seed type with a duration of 80-90 days. It is moderately resistant to wilt, blight and root rot and yield up to 12-13q ha^{-1}.
8	NBeG47	The genotype resistant to wilt and root rot with a duration 90 days and average yield up to 20-25 q ha^{-1}.
9	ICC5003	The genotype yield up to 18-20 q ha^{-1} with a duration of 90-100 days and *desi* seed type. Resistant to wilt, dry root rot and salt stress.
10	GBM 2	The days to maturity of the genotype is 100-120 and *desi* seed type. Its average yield is up to 20-22 q ha^{-1}.

2.4 Biochemical Studies

2.4.1 Proline content

The ninhydrin (2,2-dihydroxyindane-1,3-dione, CAS number 485-47-2) based colorimetric assay is extensively used to assay amino acids. At neutral pH ninhydrin destroys primary amino acids and also reacts with the released NH_3 to form a deep purple colour known as Ruhemann's purple, which has a maximum absorption at 570 nm. Further in this pH, reaction with proline and other imino acids forms yellow orange product. In the case of low pH, the colour is red with a peak of absorbance at 520 nm. At low pH also the purple is formed but rapidly loses an amine residue leads to colourless by-products. The levels of amino acids under stress condition are usually lower than proline levels [15].

Materials required:

- 3% Sulfosalicylic acid (3 g of 5-sulfocalicylic acid (2-hydroxy-5-sulfobenzoic acid) dissolved in 80 ml distilled water and make up to 100 ml).
- Acidic ninhydrin (1.25 g ninhydrin (1,2,3-indantrione monohydrate), 30 ml glacial acetic acid, and 20 ml of 6 M orthophosphoric acid were vortexed and with gentle warming. The solution could be stored at 4℃ for 1 week).
- Toluene
- L-proline
- Centrifuge
- Cuvette
- Spectrophotometer

Detailed methodology

1. Harvested plant samples were weighed (100 mg) for a reaction.
2. 3% sulfosalicylic acid (5 µl/mg fresh weight) was added and the sample was ground. The experiment was performed on ice.
3. The sample was centrifuged at maximum speed for 5 min at room temperature.
4. 100 µl of supernatant was collected and reaction mixture was prepared with 100 µl of 3% sulfosalicylic acid, 200 µl glacial acetic acid, 200 µl acidic ninhydrin.
5. The reaction mixture incubated for 1 h at 96℃. After incubation, the reaction was terminated on ice.
6. The samples were extracted with toluene by adding 1 ml of toluene to reaction mixture, the sample was vortexed for 30 s and left for 5 min to allow the separation of organic and water phases.
7. The chromophore containing toluene was removed into a fresh tube and the absorbance was measured at 520 nm using toluene as reference.
8. The concentration is expressed as micromole/g Fresh Weight.

$$\mu \text{ moles of proline per gram of tissue} = \frac{\mu g \text{ proline/ml} \times \text{ml toluene}}{\text{molecular weight of proline}} \times \frac{5}{\text{weight of sample}}$$

Where,

a. Molecular weight of proline is 115.5 g
b. Weight of the sample will be 0.1 g
c. Volume of toluene is 1 ml

2.5 Chlorophyll Content in Leaf

The chlorophyll content was measured at 30 DAS, 60 DAS and 90 DAS by following the method of Shoaf and Lixm [16]. Fresh leaf tissue (100 mg) was cut into small pieces and incubated in 10 ml of dimethyl sulfoxide (DMSO) in dark for 24 hours. After the incubation period, the sample was kept in a boiling water bath for five minutes. Later, the optical density was measured at 663 and 645 nm in UV-VIS Spectrophotometer. The care was taken to make the volume to 10 ml with DMSO, wherever the volume was reduced during boiling. Chlorophyll-*a,* chlorophyll-*b*, chlorophyll *a/b* ratio and total chlorophyll contents were calculated using the formulae given below and expressed in milligram per gram fresh weight of the sample (mg g^{-1}fr. wt).

Chlorophyll-*a* = 12.7 (A663) – 2.69 (A645) x $\frac{V}{1000\ x\ w\ x\ a}$

Chlorophyll-*b* = 22.9 (A645) – 4.68 (A663) x $\frac{V}{1000\ x\ w\ x\ a}$

Total Chlorophyll = 20.2 (A645) – 8.02 (A663) x $\frac{V}{1000\ x\ w\ x\ a}$

Where,

A645 = Absorbance of the extract at 645 nm
A663 = Absorbance of the extract at 663 nm

a = Path length of cuvette (cm)
w = Fresh weight of the sample (g)
v = Volume of extract (ml)

2.6 Chlorophyll Stability Index (CSI %)

Chlorophyll stability index was determined by Sairam et al. [17] and calculated as follows:

CSI = (total chlorophyll under stress / total chlorophyll under control) x 100

2.7 Malic Acid Content

Upper 4-5 compound leaves (2 g fresh wt.) from branches of different chickpea lines at the flowering stage were excised and crushed in 15 ml of hot distilled water (60-70°C) in a pestle and mortar. The pestle and mortar were washed with 5 ml of hot distilled water and the washing added to earlier 15 ml suspension. the total suspension was filtered through Whatman filter paper No. 42 and the filtrate made up to 25 ml with distilled water. Malic acid in the extract was determined using the method of Goodban and Stark [18] with minor modification.

Total mailc acid (mg g^{-1}fr.wt.) $\frac{T\ x\ 10\ x\ W1}{W\ x\ 5}$

Where,

T = titre value of 0.0 1 N NaOH in ml
W_1 = fresh weight taken for oven drying
W_2 = dry matter content after drying

3. RESULTS

Biochemical parameters like chlorophyll content in leaves, chlorophyll stability index, proline content, and malic acid content in leaves differed significantly with respect to genotypes, salinity concentration and their interactions.

3.1 Chlorophyll *a* Content

The chlorophyll *a* content values of leaves measured at 30, 60 and 90 days after sowing in chickpea genotypes. The chlorophyll a content differed significantly with respect to genotypes, salinity concentration and their interactions. Significantly higher chlorophyll a content was recorded under 0 dSm^{-1} at 30, 60 and 90 days after sowing. At 30 days after sowing significantly higher chlorophyll a content was recorded under 0 dSm^{-1} (1.444 mg g^{-1}fr. wt.) followed by 2 treatments, 3 $dSm^{-1}$and 9 dSm^{-1} (1.241 and 1.058 mg g^{-1}fr. wt., respectively). Similar trend was observed at 60 and 90 days after sowing. At 60 days maximum chlorophyll a content was recorded compared to 30 and 90 days and thereafter the chlorophyll a content decreased at 90 days. At 60 days maximum chlorophyll a content was recorded under 0 dSm^{-1} (1.656 mg g^{-1}fr. wt.) followed by 3 dSm^{-1} (1.284 mg g^{-1}fr. wt.) and least chlorophyll a (1.016 mg g^{-1}fr. wt.) content was observed under higher salinity level (6 dSm^{-1}).

Among the genotypes, at 60 days after sowing significantly higher chlorophyll *a* content was recorded in the genotype MNK 1 (1.433 mg g^{-1}fr. wt.) followed by JG 11 and BGD 103 (1.422 and 1.409 mg g^{-1}fr. wt., respectively). The genotypes ICC1431, JAKI 9218, Annigeri 1 and ICC5003 were found on par with each other. At 90 days after sowing, significantly higher chlorophyll *a* content was recorded in genotype JG 11 (1.218 mg g^{-1}fr. wt.) followed by BGD 103, MNK1, ICC1431, ICC5003, Annigeri 1 and JAKI 9218 (1.186, 1.174, 1.123 and 1.119 mg g^{-1}fr. wt., respectively). Among the genotypes the significantly least chlorophyll *a* was recorded in genotype ICCV96029 (0.979 mg g^{-1}fr. wt.).

3.2 Chlorophyll *b* Content

The chlorophyll *b* content increased from 30 to 60 days after sowing and thereafter the chlorophyll *b* content decreased at 90 days. The significantly maximum chlorophyll *b* content was recorded in genotype JG 11 (0.921 mg g^{-1}fr. wt.) followed by BGD 103 (0.885 mg g^{-1}fr. wt.). Genotypes, Annigeri 1, JAKI9218 and GBM 2 were found on par with each other (0.773, 0.0757 and 0.722 mg g^{-1}fr. wt., respectively) during 60 days after sowing .Among the genotypes ICC96029 and NBeG 47 were recorded significantly lower chlorophyll b content (0.543 and 0.577 mg g^{-1}fr. wt., respectively) and the genotypes Annigeri 1, ICC5003, ICC1431, JAKI9218 and GBM 2 (0.683, 0.681, 0.668, 0.620 and 0.605 mg g^{-1}fr. wt., respectively) were found on par with each other during 90 days after sowing.

Table 2. Effect of salinity stress on leaf chlorophyll "*a*" content (mg g^{-1}fr. wt.) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Chlorophyll "*a*" at 30 days				Chlorophyll "*a*" at 60 days				Chlorophyll "*a*" at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	1.488	1.194	1.046	**1.243**	1.689	1.278	1.028	**1.332**	1.328	1.041	0.887	**1.085**
JAKI 9218	1.403	1.183	1.034	**1.207**	1.585	1.253	1.000	**1.279**	1.303	1.033	0.831	**1.056**
BGD 103	1.542	1.365	1.174	**1.361**	1.743	1.377	1.142	**1.421**	1.405	1.134	1.020	**1.186**
MNK 1	1.525	1.331	1.142	**1.333**	1.831	1.369	1.100	**1.433**	1.414	1.096	1.012	**1.174**
JG11	1.599	1.388	1.182	**1.390**	1.847	1.406	1.172	**1.475**	1.440	1.175	1.033	**1.216**
GBM 2	1.398	1.176	0.969	**1.181**	1.563	1.230	0.973	**1.255**	1.295	1.027	0.827	**1.050**
NBeG 47	1.278	1.115	0.945	**1.112**	1.417	1.166	0.834	**1.139**	1.281	0.995	0.786	**1.021**
ICC 1431	1.413	1.321	1.133	**1.289**	1.709	1.328	1.072	**1.370**	1.363	1.063	0.942	**1.123**
ICC 5003	1.429	1.263	1.059	**1.250**	1.628	1.298	1.034	**1.320**	1.380	1.056	0.920	**1.119**
ICCV 96029	1.366	1.077	0.891	**1.111**	1.544	1.138	0.807	**1.163**	1.258	0.930	0.747	**0.979**
Mean	**1.444**	**1.241**	**1.058**		**1.656**	**1.284**	**1.016**		**1.347**	**1.055**	**0.901**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
Treatments (T)	0.004		0.010		0.004		0.012		0.002		0.006	
Genotypes (G)	0.012		0.033		0.015		0.040		0.007		0.019	
Interaction (T*G)	0.037		0.098		0.045		0.120		0.022		0.058	

Table 3. Effect of salinity stress on leaf chlorophyll "*b*" content (mg g^{-1}fr. wt.) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Chlorophyll "*b*" at 30 days				Chlorophyll "*b*" at 60 days				Chlorophyll "*b*" at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	0.925	0.666	0.611	**0.734**	0.928	0.796	0.595	**0.773**	0.877	0.606	0.565	**0.683**
JAKI 9218	0.840	0.656	0.585	**0.694**	0.916	0.783	0.571	**0.757**	0.740	0.585	0.536	**0.620**
BGD 103	1.025	0.832	0.673	**0.843**	1.078	0.871	0.705	**0.885**	0.892	0.687	0.645	**0.741**
MNK 1	0.942	0.823	0.656	**0.807**	1.030	0.853	0.691	**0.858**	0.911	0.659	0.624	**0.731**
JG11	0.982	0.836	0.708	**0.842**	1.132	0.898	0.733	**0.921**	0.929	0.710	0.686	**0.775**
GBM 2	0.798	0.626	0.576	**0.667**	0.863	0.754	0.550	**0.722**	0.729	0.574	0.511	**0.605**
NBeG 47	0.753	0.598	0.539	**0.630**	0.829	0.617	0.529	**0.659**	0.704	0.537	0.489	**0.577**
ICC 1431	0.876	0.793	0.641	**0.770**	1.001	0.841	0.672	**0.838**	0.768	0.636	0.600	**0.668**
ICC 5003	0.846	0.718	0.620	**0.728**	0.963	0.829	0.616	**0.803**	0.839	0.619	0.583	**0.681**
ICCV 96029	0.688	0.581	0.504	**0.591**	0.799	0.604	0.518	**0.640**	0.673	0.514	0.442	**0.543**
Mean	**0.868**	**0.713**	**0.611**		**0.954**	**0.785**	**0.618**		**0.806**	**0.613**	**0.568**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
Treatments	0.001		0.004		0.001		0.004		0.001		0.003	
Genotypes	0.004		0.012		0.005		0.013		0.003		0.009	
Interaction (T*G)	0.014		0.039		0.015		0.040		0.010		0.027	

Table 4. Effect of salinity stress on leaf total chlorophyll (mg g^{-1}fr. wt.) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Total Chlorophyll at 30 days				Total Chlorophyll at 60 days				Total Chlorophyll at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	2.413	1.860	1.657	**1.977**	2.617	2.074	1.623	**2.105**	2.205	1.647	1.452	**1.768**
JAKI 9218	2.243	1.839	1.619	**1.900**	2.502	2.036	1.571	**2.036**	2.043	1.619	1.368	**1.676**
BGD 103	2.567	2.197	1.847	**2.204**	2.821	2.248	1.847	**2.305**	2.297	1.821	1.665	**1.928**
MNK 1	2.467	2.154	1.798	**2.140**	2.861	2.222	1.791	**2.291**	2.325	1.755	1.636	**1.905**
JG11	2.581	2.224	1.890	**2.232**	2.979	2.304	1.904	**2.396**	2.369	1.885	1.719	**1.991**
GBM 2	2.196	1.802	1.545	**1.848**	2.426	1.985	1.523	**1.978**	2.024	1.602	1.338	**1.655**
NBeG 47	2.031	1.713	1.484	**1.743**	2.246	1.783	1.364	**1.798**	1.985	1.532	1.275	**1.597**
ICC 1431	2.289	2.114	1.774	**2.059**	2.709	2.169	1.745	**2.208**	2.131	1.699	1.542	**1.791**
ICC 5003	2.275	1.981	1.679	**1.978**	2.591	2.127	1.651	**2.123**	2.219	1.675	1.504	**1.799**
ICCV 96029	2.055	1.658	1.395	**1.703**	2.343	1.742	1.326	**1.804**	1.931	1.445	1.189	**1.522**
Mean	**2.312**	**1.954**	**1.669**		**2.610**	**2.069**	**1.634**		**2.153**	**1.668**	**1.469**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
Treatments	0.004		0.011		0.005		0.012		0.002		0.006	
Genotypes	0.014		0.036		0.015		0.041		0.008		0.020	
Interaction (T*G)	0.041		0.109		0.046		0.122		0.023		0.061	

Table 5. Effect of salinity stress on chlorophyll stability index at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Chlorophyll stability index at 30 days				Chlorophyll stability index at 60 days				Chlorophyll stability index at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	0.887	0.557	0.337	**0.594**	0.935	0.593	0.355	**0.628**	0.821	0.607	0.232	**0.553**
JAKI 9218	0.815	0.534	0.333	**0.561**	0.871	0.570	0.341	**0.594**	0.797	0.573	0.226	**0.532**
BGD 103	0.978	0.661	0.433	**0.691**	0.970	0.697	0.441	**0.703**	0.906	0.691	0.312	**0.636**
MNK 1	0.924	0.637	0.406	**0.656**	0.980	0.673	0.424	**0.692**	0.936	0.673	0.292	**0.634**
JG11	0.941	0.680	0.437	**0.686**	0.994	0.716	0.465	**0.725**	0.908	0.735	0.352	**0.665**
GBM 2	0.793	0.523	0.307	**0.541**	0.841	0.545	0.327	**0.571**	0.759	0.520	0.192	**0.490**
NBeG 47	0.723	0.493	0.287	**0.501**	0.791	0.529	0.306	**0.542**	0.732	0.487	0.170	**0.463**
ICC 1431	0.869	0.628	0.377	**0.625**	0.925	0.647	0.398	**0.657**	0.848	0.664	0.269	**0.594**
ICC 5003	0.842	0.566	0.357	**0.588**	0.898	0.602	0.383	**0.627**	0.813	0.637	0.242	**0.564**
ICCV 96029	0.754	0.472	0.276	**0.501**	0.829	0.508	0.290	**0.543**	0.742	0.447	0.156	**0.448**
Mean	**0.853**	**0.575**	**0.355**		**0.904**	**0.608**	**0.373**		**0.826**	**0.603**	**0.244**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
Treatments	0.001		0.003		0.001		0.002		0.002		0.004	
Genotypes	0.004		0.009		0.002		0.007		0.005		0.015	
Interaction (T*G)	0.011		0.028		0.007		0.020		0.016		0.044	

3.3 Total Chlorophyll Content

The total chlorophyll content recorded significantly higher values at 60 days after sowing compared to 30 and 90 days. Among the salinity levels significantly higher total chlorophyll content was recorded under 0 dSm^{-1} (2.312 mg g^{-1}fr. wt.) followed by 3 dSm^{-1} and 6 dSm^{-1} (1.954 and 1.669 mg g^{-1}fr. wt., respectively) at 30 days after sowing (Table 3). Further, at 60 days after sowing 0 dSm^{-1} recorded highest total chlorophyll content (2.610 mg g^{-1}fr. wt.) followed by 3 dSm^{-1} and 6 dSm^{-1} (2.069 and 1.634 mg g^{-1}fr.wt, respectively). however, among the interaction effect the genotype JG 11 recorded significantly higher total chlorophyll at 0 dSm^{-1} (2.979 mg g^{-1}fr. wt.) and the genotype ICCV96029 (1.326 mg g^{-1}fr. wt.) recorded significantly lower total chlorophyll content at 6 dSm^{-1} during 60 days after sowing. The genotype JG11and BGD 103 showed maximum total chlorophyll content at 0 dSm^{-1} (2.369 and 2.325 mg g^{-1}fr. wt., respectively) and least total chlorophyll was recorded by the genotype ICCV96029 (1.189 mg g^{-1}fr. wt.) followed by NBeG47, GBM2, JAKI9218 and Annigeri 1 (1.275, 1.338, 1.368 and 1.452 mg g^{-1}fr. wt., respectively) under 6 dSm^{-1} during 90 days after sowing.

3.4 Chlorophyll Stability Index (CSI)

The genotypes, salinity levels and their interactions for chlorophyll stability index at 30, 60 and 90 days after sowing. Among the genotypes significantly higher chlorophyll stability index was recorded during 60 days after sowing in genotype JG 11 (0.725) followed by genotype BGD 103, MNK 1, ICCV1431, Anniger 1 and ICC5003 (0.703, 0.692, 0.657, 0.628 and 0.627, respectively) . Similarly, at 90 days after sowing, higher chlorophyll stability index was recorded in genotypes JG 11 and BGD 103 (0.665 and 0.636, respectively) and the least chlorophyll stability index was recorded in genotypes ICCV96029 and NBeG 47 (0.448 and 0.463, respectively). Further, the genotypes ICC1431, ICC 5003, Annigeri 1, JAKI 9218 and GBM 2 (0.594, 0.564, 0.553, 0.532 and 0.490) were found on par with each other during 90 days after sowing.

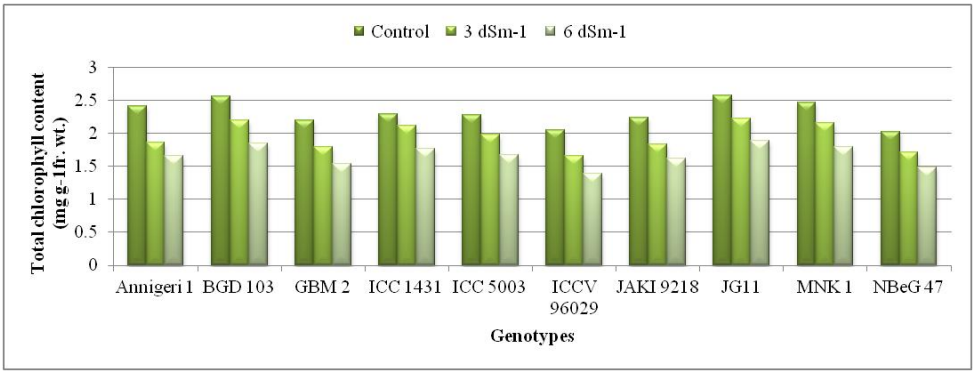

Fig. 1. Effect of salinity on total chlorophyll content of chickpea genotypes at 30 days after sowing

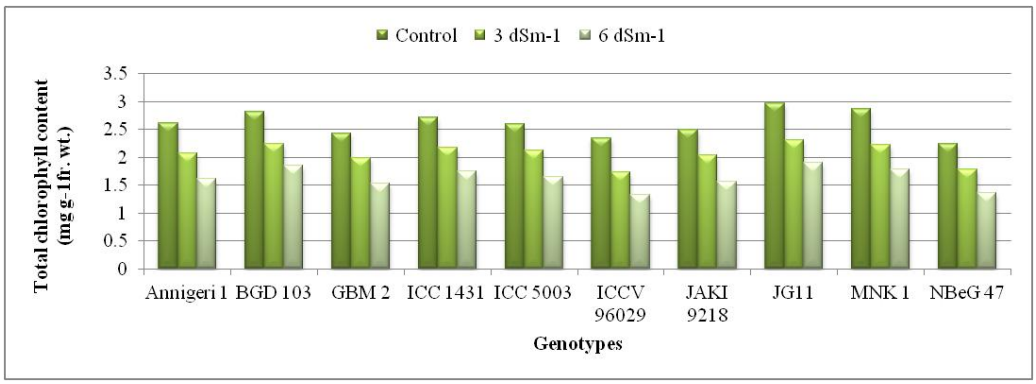

Fig. 2. Effect of salinity on total chlorophyll content of chickpea genotypes at 60 days after sowing

Table 6. Effect of salinity stress on leaf proline content (mg g^{-1}fr. wt.) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Proline content at 30 days				Proline content at 60 days				Proline content at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	15.89	18.24	22.88	**19.00**	19.82	22.31	27.10	**23.08**	22.16	25.68	29.92	**25.92**
JAKI 9218	18.43	21.81	26.51	**22.25**	22.33	25.17	30.01	**25.84**	24.33	27.97	32.83	**28.38**
BGD 103	15.48	19.28	24.28	**19.68**	19.64	24.45	29.25	**24.45**	22.31	27.25	32.50	**27.35**
MNK 1	15.20	17.86	22.36	**18.47**	19.65	23.15	26.38	**23.06**	21.99	25.95	29.87	**25.94**
JG11	16.97	21.86	23.88	**20.90**	21.22	25.67	30.27	**25.72**	23.82	28.13	33.42	**28.46**
GBM 2	15.88	17.62	20.32	**17.94**	19.83	22.49	25.69	**22.67**	22.17	25.63	28.51	**25.44**
NBeG 47	14.74	17.11	21.71	**17.85**	18.87	21.87	24.70	**21.81**	21.07	24.67	27.18	**24.31**
ICC 1431	17.24	18.54	22.95	**19.58**	21.32	23.42	27.24	**23.99**	24.10	26.55	30.39	**27.01**
ICC 5003	15.46	19.35	21.85	**18.89**	19.43	24.01	25.86	**23.10**	21.87	26.81	28.68	**25.79**
ICCV 96029	13.71	16.00	20.16	**16.62**	17.89	20.45	23.68	**20.67**	20.09	23.25	26.16	**23.17**
Mean	**15.90**	**18.77**	**22.69**		**20.00**	**23.30**	**27.02**		**22.39**	**26.19**	**29.94**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
EC	0.05		0.13		0.06		0.15		0.06		0.16	
Genotypes	0.16		0.43		0.19		0.52		0.20		0.53	
Interaction (E*G)	0.49		1.30		0.58		1.55		0.60		1.59	

Table 7. Effect of salinity stress on malic acid (mg g^{-1}fr.wt) at 30, 60 and 90 DAS in chickpea genotypes

Genotypes	Malic acid at 30 days				Malic acid at 60 days				Malic acid at 90 days			
	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean	0 dSm^{-1} (Control)	3 dSm^{-1}	6 dSm^{-1}	Mean
Annigeri 1	7.93	8.23	8.58	**8.25**	14.96	17.67	18.73	**17.12**	12.74	15.17	16.23	**14.71**
JAKI 9218	6.76	7.09	7.40	**7.08**	14.30	16.52	17.55	**16.12**	11.56	13.53	14.93	**13.34**
BGD 103	7.22	7.72	8.10	**7.68**	15.30	16.80	18.46	**16.85**	13.13	14.30	16.12	**14.52**
MNK 1	8.10	8.48	8.72	**8.43**	13.34	17.64	18.43	**16.47**	13.42	15.14	16.32	**14.96**
JG11	5.89	7.18	7.77	**6.95**	13.77	16.49	19.46	**16.57**	11.35	13.99	16.96	**14.10**
GBM 2	6.17	6.40	6.72	**6.43**	14.25	15.62	17.65	**15.84**	12.42	13.63	15.22	**13.76**
NBeG 47	5.76	6.05	6.35	**6.05**	13.55	15.33	16.33	**15.07**	12.34	13.46	15.36	**13.72**
ICC 1431	6.75	7.16	7.54	**7.15**	14.84	16.35	17.59	**16.26**	10.59	12.83	13.83	**12.42**
ICC 5003	6.25	6.62	7.27	**6.71**	14.11	15.91	17.57	**15.86**	10.43	13.41	15.34	**13.06**
ICCV 96029	6.10	6.53	6.99	**6.54**	13.54	15.41	17.24	**15.40**	9.64	13.11	14.74	**12.50**
Mean	**6.69**	**7.15**	**7.54**		**14.20**	**16.37**	**17.90**		**11.76**	**13.86**	**15.50**	
	SEm±		**LSD @5%**		**SEm±**		**LSD @5%**		**SEm±**		**LSD @5%**	
EC	0.018		0.046		0.017		0.044		0.015		0.041	
Genotypes	0.054		0.144		0.055		0.147		0.051		0.135	
Interaction (E*G)	0.160		0.424		0.166		0.441		0.153		0.406	

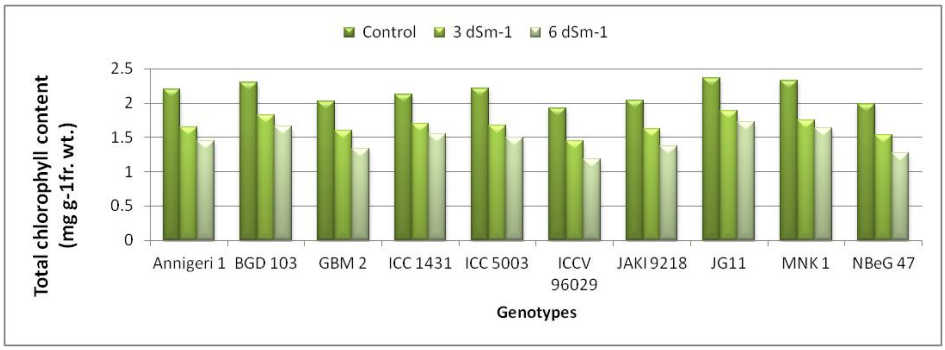

Fig. 3. Effect of salinity on total chlorophyll content of chickpea genotypes at 90 days after sowing

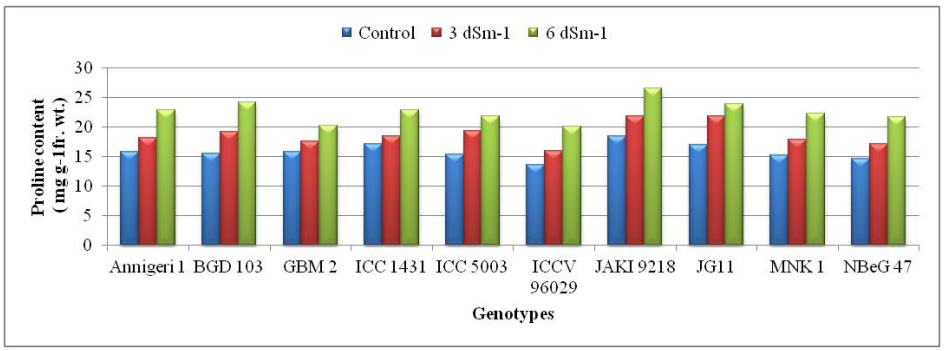

Fig. 4. Effect of salinity on proline content of chickpea genotypes at 30 days after sowing

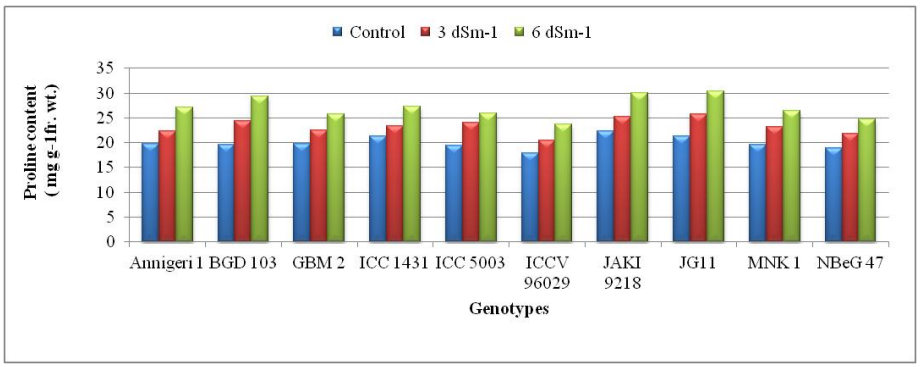

Fig. 5. Effect of salinity on proline content of chickpea genotypes at 60 days after sowing

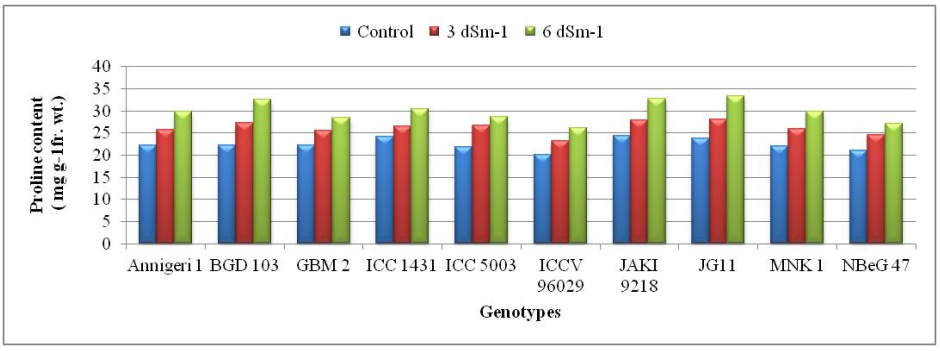

Fig. 6. Effect of salinity on proline content of chickpea genotypes at 90 days after sowing

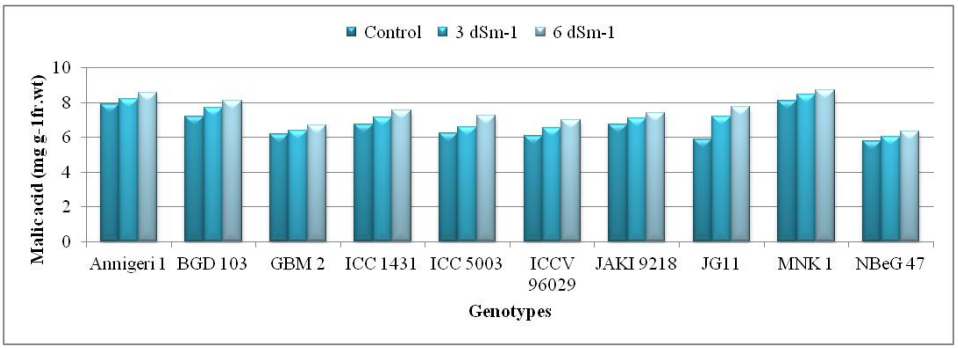

Fig. 7. Effect of salinity on malic acid content of chickpea genotypes at 30 days after sowing

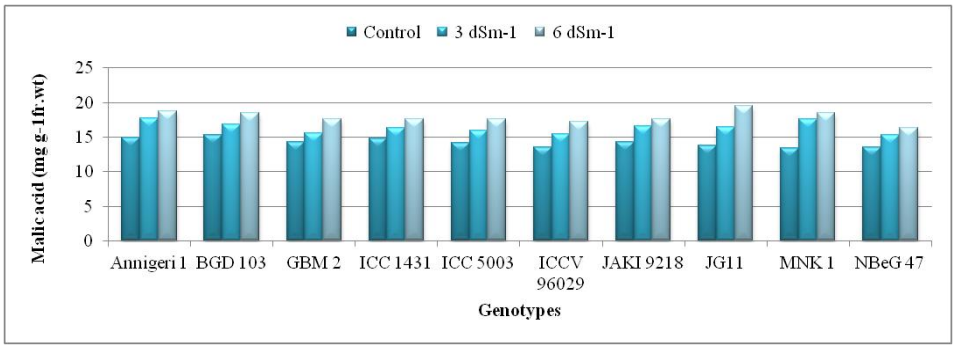

Fig. 8. Effect of salinity on malic acid content of chickpea genotypes at 60 days after sowing

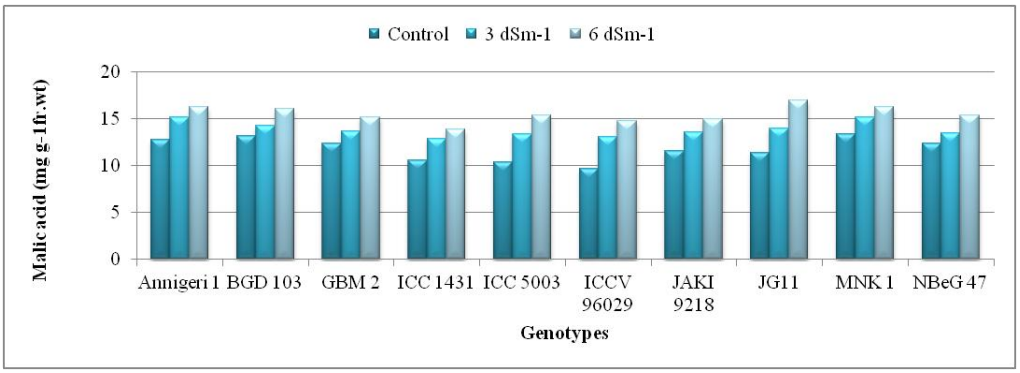

Fig. 9. Effect of salinity on malic acid content of chickpea genotypes at 90 days after sowing

3.5 Proline Content

Proline content differed significantly with genotype, salinity levels and their interactions. At 90 days after sowing the maximum proline content was recorded under 6 dSm^{-1} (29.94 mg g^{-1} fresh weight) and minimum proline content was observed under 0dSm^{-1} (22.39 mg g^{-1} fresh weight). Among the interaction levels, genotype JG11 (33.42 mg g^{-1} fresh weight) recorded maximum proline content followed by JAKI9218 and BGD 103 (32.83 and 32.50 mg g^{-1} fresh weight, respectively) at 6 dSm^{-1} at 90 days after sowing. Further, the content of proline was significantly higher in genotypes JG 11, JAKI9218 and BGD103 under 6 dSm^{-1} (30.27, 30.0 and 29.25 mg g^{-1}fresh weight, respectively) at 60 days after sowing. While the genotype ICC-96029 was recorded minimum proline content (20.09 mg g^{-1} fresh weight) at 0dSm^{-1} followed by NBeG 47, ICC5003 and MNK 1 under same salinity level (21.07, 21.87 and 21.99 mg g^{-1} fresh weight, respectively) at 90 days after sowing.

3.6 Malic Acid Content

The malic acid content differed significantly with respect to dates of genotypes, salinity levels and

their interactions. The malic acid content in chickpea genotypes increased with increasing soil salinity concentration. Significantly higher malic acid content was recorded under 6 dSm^{-1} (7.54 mg g^{-1}fr. wt.) followed by 3 dSm^{-1} and 0 dSm^{-1} (7.15 and 6.69 mg g^{-1}fr. wt., respectively) during 30 days after sowing and similar trend was followed at 60 and 90 days after sowing. Under 30 days after sowing significantly least malic acid content was recorded compared to 60 and 90 days after sowing. Among the genotypes significantly higher malic acid content was recorded in genotypes MNK 1 (14.96 mg g^{-1}fr. wt.) followed by Annigeri 1, BGD103, JG 11 and GBM 2 (14.71, 14.52, 14.10 and 13.76 mg g^{-1}fr. wt., respectively) and least malic acid content was recorded in genotype ICC1431 (12.42 mg g^{-1}fr. wt.) at 90 days after sowing. All the genotypes recorded lower malic acid content under lowest salinity concentration (0 dSm^{-1}) compared to 3 dSm^{-1} and 6 dSm^{-1}.

4. DISCUSSION

In photosynthetic activity the chlorophyll content plays an important role. the higher chlorophyll content increases photosynthetic rate [19]. The data on chlorophyll a (Table 2), chlorophyll b (Table 3) and total chlorophyll content (Table 4) in chickpea genotypes as influenced by genotypes and their interactions differed significantly. The chlorophyll content in chickpea crop increased with growth period from 30 days to 60 days after sowing, there after the chlorophyll content decreased. According to the study conducted by Hassanein et al. [20] the content of chlorophyll a (chl a), chl b, carotenoids, chl a+b and total pigments gradually decreased with increase in salinity concentration and highest reduction of photosynthetic pigments were recorded at 200mM NaCl level. Among the genotypes significantly maximum total chlorophyll content was recorded at 60 days after sowing by the genotype JG 11and BGD 103 (Fig. 2).Similar findings were noticed by Taibi et al. [21] reported that the lipid peroxidation of chloroplast during salt stress decrease the chlorophyll pigments and in all genotypes the increa levels reducd the drysed salinity mass and chlorophyll pigments and increased malondialdehyde content. Kaur et al. [22] Observed that salt sress (20 and 30Mm NaCl) decreased the chlorophyll a, chlorophyll b and total chlorophyll content in chickpea genotypes at vegetative (65 days after sowing), flowering (90DAS) and pod initiation (110DAS) stage.

Osmolytes like proline plays a major role in protecting the membrane bound proteins and enzymes apart from its basic role of osmoprotection. These compounds lower the osmotic potential of the cell sap, thereby regaining the water potential gradient. This leads to uptake of more water from the saline root zone, which may buffer the immediate effect of water deficiency within the crop so that the crop can perform its metabolic activities more efficiently during the stress [23]. Al-saady et al. (2012) reported that proline accumulation not a reaction to damage caused by salt stress , it appears to be a plant response associated with salt tolerance. The proline content believed to be function as a compatible solutes in balancing vacuolar and cytoplasm water potential and tolerant genotypes showed higher accumulation of proline content [24].

The results obtained from the proline content at different growth stages (30, 60 and 90 days after sowing) in chickpea genotypes. Maximum proline content was recorded under 6 dSm^{-1} followed by 3dSm^{-1} and 0 dSm^{-1} at 30 days after sowing and similar trend was followed during 60 and 90 days after sowing. The compatible solutes like proline accumulated in the cytoplasm to balance the solute and ion accumulation and acts as a protective agent against stress induced cellular damage [25]. The genotype JG11 (33.42 mg g^{-1} fresh weight) recorded maximum proline content followed by JAKI9218 and BGD 103 (32.83 and 32.50 mg g^{-1} fresh weight, respectively) at 6 dSm^{-1} at 90 days after sowing.

Guo et al. [26], Observed that the alkaline salt stress caused, increasing the levels of malic acid, aconitic acid, succinic acid and fumaric acid and the increased levels of organic acids might be contributed to the maintainance of intracellular ion balance in plants. Significantly higher malic acid content was recorded under 6 dSm^{-1} (7.54 mg g^{-1}fr. wt.) followed by 3dSm^{-1} and 0 dSm^{-1} (7.15 and 6.69 mg g^{-1}fr. wt., respectively) during 30 days after sowing and similar trend was followed at 60 and 90 days after sowing. Under 30 days after sowing significantly least malic acid content was recorded compared to 60 and 90 days after sowing.

It was suggested that the total amount of organic acids present in the leaf and stem tissues was found to be maximum in the tolerant chickpea genotypes than in the susceptible genotype [27]. However, higher amount of malic acid content was observed in genotype JG11 (16.96 mg g^{-1}fr.

wt.) under 6 dSm^{-1} followed by genotype MNK 1, Annigeri 1 and BGD 103 under same salt concentration at 90 days after sowing. Scagel et al. [28] reported that the polyphenolic and organic acid concentration influenced by the levels salinity and 25mM NaCl had no effect on biomass, malic acid and concentration of phenolics, whereas 50mM NaCl (higher salinity concentration) reduced biomass, increased malic acid and concentration of phenolics.

5. CONCLUSION

In the present investigation, screening of chickpea genotypes for salt tolerance was carried out with ten chickpea genotypes, which include nine *desi* and one *kabuli* type under control and 2 salinity levels *viz*., 3 and 6 dSm^{-1} at 30, 60 and 90 DAS. The genotypes JG11, BGD 103, MNK 1, ICCV1431 and Annigeri 1 were found to be superior for Chlorophyll stability index at 60 days after sowing. Both proline and malic acid content, which are said to be indicators of stress tolerance, showed high values in JG 11 and the genotypes MNK 1, Annigeri 1 and BGD 103 also showed higher malic acid content at 90 days after sowing. From the study it was observed that the genotypes JG 11, BGD 103, MNK 1 and ICC 1431 were tolerant to salinity while, ICCV 96029 and NBeG 47 were found to be salt sensitive genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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