Original Research Article

Effect of NaCl Salt Stress on Biochemical parameters of Chickpea*(cicerarietinum* l.) Genotypes.

ABSTRACT

Chickpea *(Cicer arietinum L.)* is sensitive to salt sress, that affects its yield and there is need toidentify 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 chickpeagenotypes both desi (Annigeri1,BGD103,NBeG47,JG11,GBM2,JAKI9218,ICC1431,ICC5003,ICCV96029) and *kabuli*(MNK 1) were evaluated forsalinity 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 three NaCl salinity levels (0, 3dS/m and 6dS/m) in 10 chickpea cultivars. 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 increasing salt concentration resulted in higher membrane injury index ; among genotypes, ICCV96029 with 15.14% and MNK1 with 12.21% had the highest and lowest value, respectively at 90 days after sowing. 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. Total leaf chlorophyll content decreased in all genotypes during the stress. Maximum decrease in chlorophyll content was observed with ICCV96029 and NBeG 47 among tengenotypes. Over all, proline and total chlorophyll content were more consistent with salt tolerance responses of the genotypes.

Introduction :

~~Chickpea (*Cicer arietinum* L.) is a legume crop and belongs to the family Fabaceae. It is self pollinated, diploid (2n=2x=16) with a genome size of 740 Mbp. It is an annual crop that can complete its life cycle in 90 to 180 days depending on the prevailing meteorological conditions.~~ It is the second most important legume crop after dry beans (Varshney *et al*., 2012). 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 (Gaur *et al*., 2012). 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. It is cultivated in an area of 9.6 million hectares (a record in the last 50 year) with a production of 9.37 million tonnes and productivity of 974 kg per ha (Anon, 2016-17).

Soil salinity is becoming more problematic due to theincrease in irrigation around the world. The harmful impacts of salinity include low agricultural production,low economic returns due to high cost of

Comment [P1]: This article is interesting, but unfortunately the author only presents the results, without any explanation why one genotype is more tolerant than the other. This article discusses the biochemical content in several growth phases. It is interesting to be able to discuss and conclude the right time to measure each biochemical so that it describes the salt stress experienced by chickpea plants.

Title suggestion: biochemical response on three growth phases of chickpea under graduated salt stress

Comment [P2]: You mention the date after sowing in the text but not in the abstract

Comment [P3]: In the text there is no measurement related with membrane injury

Comment [P4]: So???? What is means? They have different mechanism?

Comment [P5]: What you mean? Consistent to detect? Or all genotypes have similar response? Please clarify the sentence

Comment [P6]: This information is not related to the topic of study

Comment [P7]: The genotype studied is kabuli or desi?

cultivation,reclamation, management, ecological imbalance due tohalophytes and marine life forms from fresh water tobrackish water, poor human health due to toxic effectsof accumulated elements (Hu and Schmidhalter, 2002). Resistance to salt stress does not rely on a single traitbut, on the contrary, it has a very complex nature as itdepends upon various morphological and biochemicaltraits. Salinity affects germination, initial seedling establishment, growth, nitrogen fixation, flowering, pod development and seed filling of chickpea (Toker*et al*., 2007; Al-Mutawa, 2003). 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 (Pushpavalli*et al*., 2016). The concentration of numerous metabolites, including proline and glycinebetaine, also increases under salt stress, providing defence against osmotic challenge by serving as compatible solutes (Sanchez *et al.*, 2008; Wu *et al*., 2013).

The availability of water to the growing tissuebecomes a limiting factor under saline conditions evenin the presence of moisture in the soil resulting in whatis termed as "Physiological Drought". Water uptake byplants hence, attains importance under salineconditions. Oxidative stress is responsible for the generation of reactive oxygen species (ROS) which aredeleterious to plants (Azooz*et al.,* 2011, Ahmad *et al.,*2012). ROS are highly reactive and cause damage tobiomolecules such as lipids, proteins and nucleic acids(Tuteja*et al.,* 2009). Proline is considered as the onlyosmolyte which has been shown to scavenge singletoxygen and free radicals including hydroxyl ions. Italso serves as redox potential regulator and protectsmacromolecules such as proteins, DNA and reducesenzyme denaturation caused by heat, NaCl and otherstresses (Kumar *et al.,* 2010). Chickpea being sensitiveto salinity needs considerable enhancement of salinitytolerance to be grown on natural saline soil. Thereforean understanding of the mechanisms involved in salttolerance of the chickpea plant is crucial to select salttolerant genotypes or to engineer salt sensitivegenotypes with genetic traits to include salt tolerance.Keeping all the factors in mind the present investigation was formulated to study the effect of salt stress inchickpea genotypes at different crop developmentstages (30, 60 and 90 days after sowing). The tolerant chickpea(*Cicer arietinum* L.) genotypes will be identified onthe basis of biochemical indices.

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 themeansea level (MSL).The experiment was laid out in a complete randomized block 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.Theirwere three treatments including control and salinity levels were developed by using NaCl solution.

Comment [P8]: In the discussion you didn't mention any mechanism

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Comment [P10]: RCBD? Please use capital at the beginning of each abbreviated word

Comment [P11]: Addthe each genotype information. Such as pedigree, source, etc.

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Sowing and salinity treatments:

Earthen pots of uniform size (30x30 cm) were filled with 10 kg of air-dried soil and farmyard manure in 6:1 ratio. Each pot received a fertilizer dose of 120, 60 and 60 kg/ha of N, P and K, respectively. 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 three salinity treatments (C2 and C3). Salt solutions were prepared by using Naclsalt. The salt concentrations of different solutions are given below .

C1= 5 gram of NaCl salt dissolved in 1 liter of water for preparing 3 EC

C2= 10 gram of NaCl salt dissolved in 1 liter of water for preparing 6 EC

Actual salinity values are expressed as EC determined at 3 stages and mean of these was taken as salinity at C1, C2 and C3 levels.

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.

Biochemical studies:

Proline content

Proline content in leaves was determined following the method of (Bates *et al*., 1973). The protocol was based on the formation of red coloured formazone by proline with ninhydrin in acidic medium, which is soluble in organic solvents like toluene. Leaf sample (0.5 g) was homogenized in 5.0 ml of sulphosalycylic acid (3%) using mortar and pestle. The homogenate is filtered through whatman No. 1 filter paper and filterate was collected, which was used for the estimation of proline content. Two ml of extract was taken in test tube and to it 2 ml of glacial are added.

The reaction mixture was heated in boiling water both at 100 ^{0}C for 30 minutes, brick red colour develop after cooling the reaction mixture, 6 ml of toluene was added and then transferred to a separating funnel.

~~After mixing the chromophore containing toluene through mixing~~ the chromophore containing toluene was separated and its absorbance read at 520 nm in spectrophotometer against toluene blank. Concentrate of proline was estimated by referring to a standard curve made from known as concentration of proline and expressed in milligram per gram fresh weight of the sample (mg g^{-1}fr. wt.).

Comment [P13]: ?? is it number of pot?

Comment [P14]: Is it dose of NPK or Urea, KCl?

Comment [P15]: Before sowing, ...

Comment [P16]: Only two salt treatments

Comment [P17]: NaCl→see standard for writing chemical compounds. Please check others

Comment [P18]: Do you use the laboratory standard NaCl? Please mention the brand

Comment [P19]: Is it control? The control also include the salt in it?

Comment [P20]: What is it? (mention the abbreviation for first time

Comment [P21]: When?

Comment [P22]: How long the stress treatments given?

Comment [P23]: The method was different with Bates along I know. Do you read the original article? Or please mention the part of the modification

Comment [P24]: Fresh leaf..

Comment [P25]: Glacial what? Glacial = 100% content of...

Comment [P26]: What the standard used?

Chlorophyll content in leaf

The chlorophyll content was measured at 35 DAS and 45 DAS by following the method of Shoaf and Lixm (1976). 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).

$$\text{Chlorophyll-}a = 12.7\,(A663) - 2.69\,(A645) \times \frac{V}{1000 \times w \times a}$$

$$\text{Chlorophyll-}b = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times w \times a}$$

$$\text{Total Chlorophyll} = 20.2\,(A645) - 8.02\,(A663) \times \frac{V}{1000 \times w \times a}$$

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)

Chlorophyll stability index (CSI %)

Chlorophyll stability index was determined by Sairam et al. (1997) and calculated asfollows:

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

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 Goodbanand Stark (1957) with minor modification.

$$\text{Total mailcacid (mg g}^{-1}\text{fr.wt.)} = \frac{T \times 10 \times W_1}{W \times 5}$$

Where T = titre value of 0.0 1 N NaOH in ml

W_I = fresh weight taken for oven drying

W_2 = dry matter content after drying

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.

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 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}).

Comment [P27]: Is it the treatment?

Table 1. 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%**	
EC	0.004		0.010		0.004		0.012		0.002		0.006	
Genotypes	0.012		0.033		0.015		0.040		0.007		0.019	
Interaction (E*G)	0.037		0.098		0.045		0.120		0.022		0.058	

Comment [P28]: In the method was mention: The chlorophyll content was measured at 35 DAS and 45 DAS

Comment [P29]: Please consistent EC or dSm-1?

Comment [P30]: Do you use the replication? Mention the SD

Table 2. 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%**	
EC	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 (E*G)	0.014		0.039		0.015		0.040		0.010		0.027	

Table 3. 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%**	
EC	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	0.041		0.109		0.046		0.122		0.023		0.061	

(E*G)						

Table 4. 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%
EC	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 (E*G)	0.011	0.028	0.007	0.020	0.016	0.044

Comment [P31]: What it means? Significant? You can do it with add th* on significant number

Chlorophyll *b* content

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.

Comment [P32]: Please be consistent. In chlorophyll a and total chlorohyll the author only mention the general respons of each salt treatment, but in chlorohyll b you mention for each genotype

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 13). 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.

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

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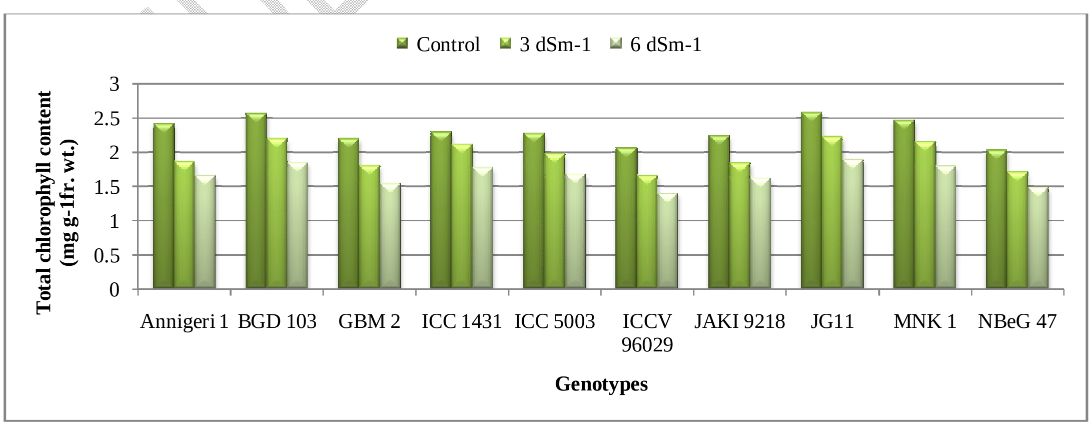


Figure 2. Effect of salinity on total chlorophyll content of chickpea genotypes at 60 days after sowing

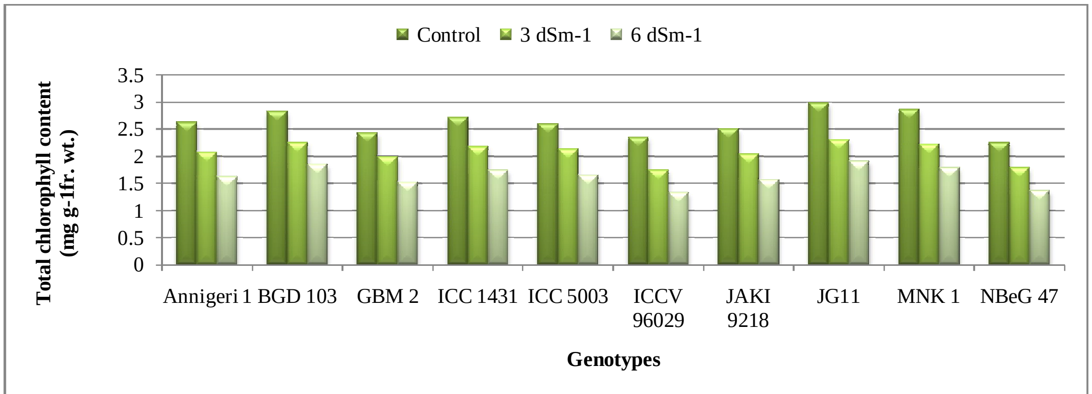
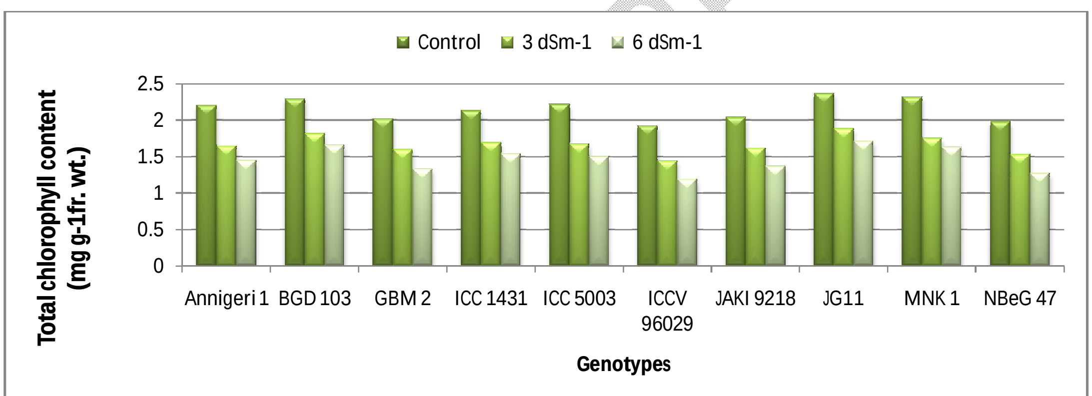


Figure 3. Effect of salinity on total chlorophyll content of chickpea genotypes at 90 days after sowing



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.

Table 5. 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**	

	30 days SEm±	30 days LSD @5%	60 days SEm±	60 days LSD @5%	90 days SEm±	90 days 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 6. 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	

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 6dSm^{-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.

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

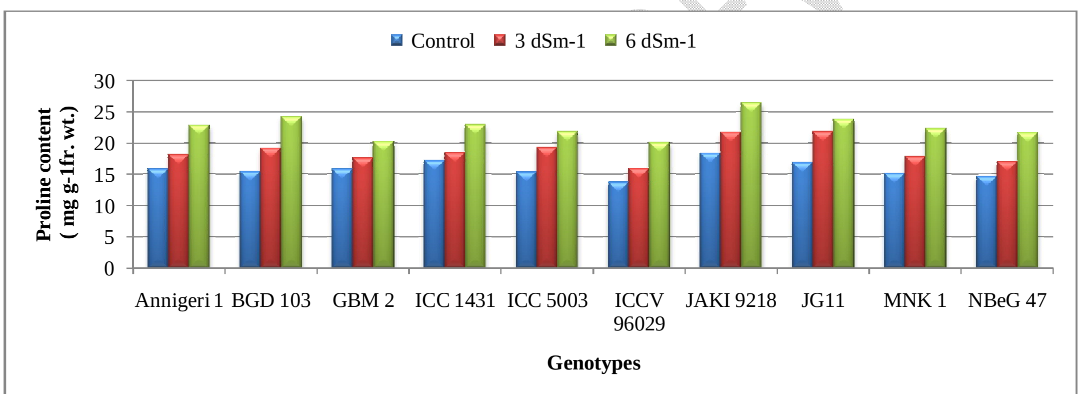


Figure 5. Effect of salinity on proline content of chickpea genotypes at 60 days after sowing

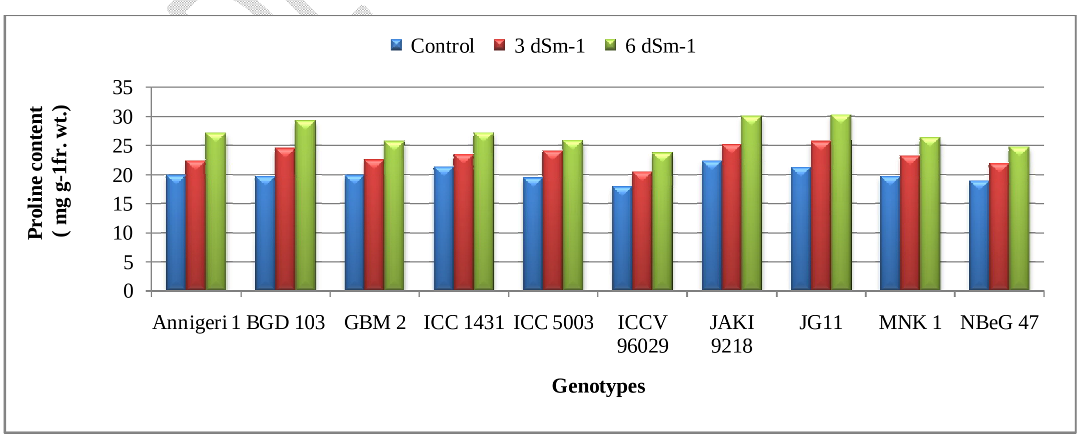
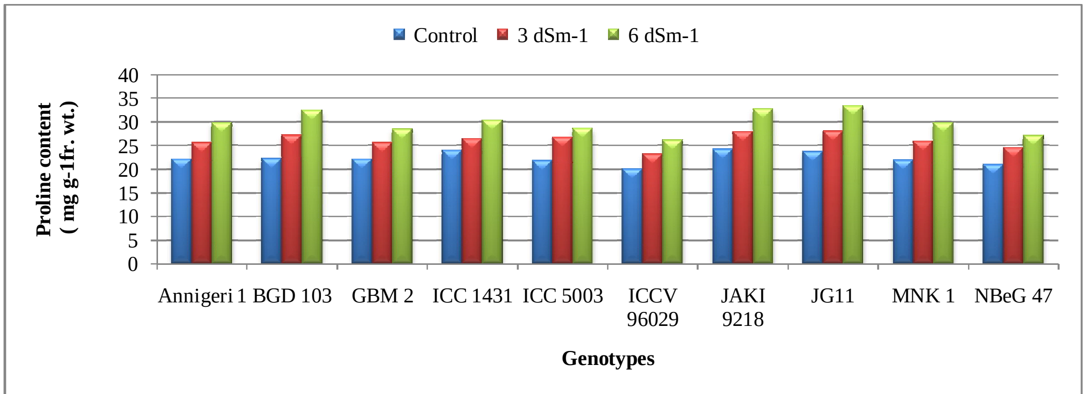


Figure 6. Effect of salinity on proline content of chickpea genotypes at 90 days after sowing



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 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. 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 3dSm^{-1} and 6 dSm^{-1}.

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

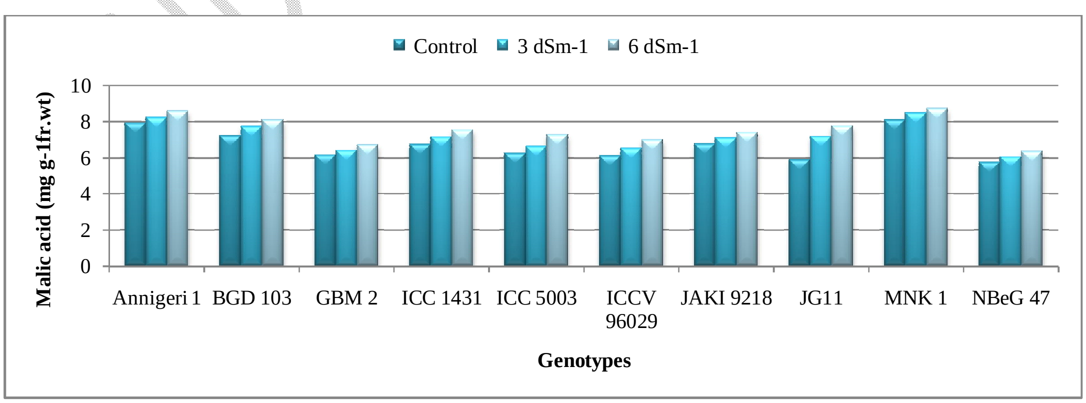


Figure 8. Effect of salinity on malic acid content of chickpea genotypes at 60 days after sowing

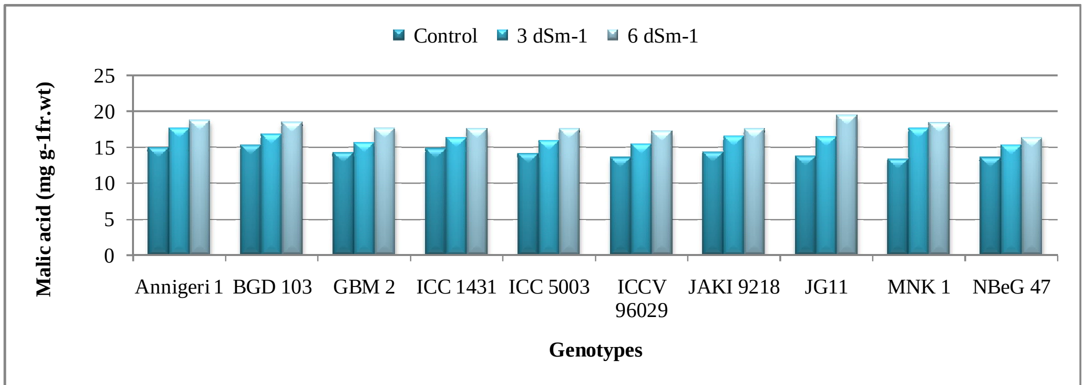
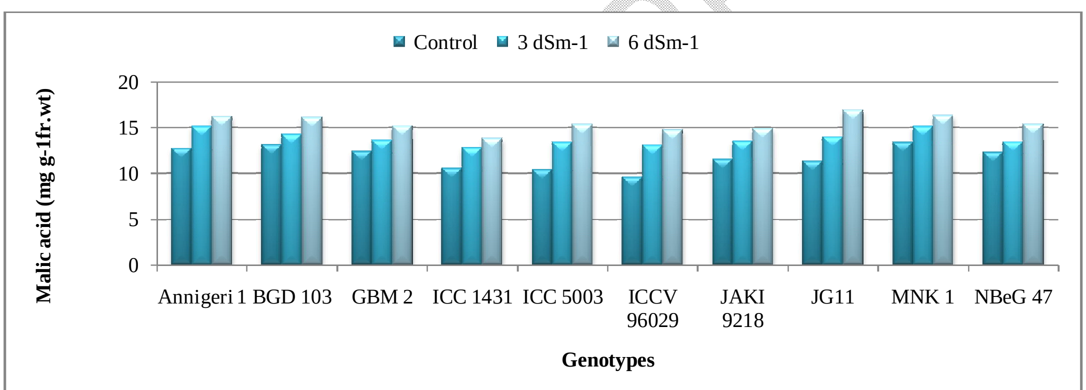


Figure 9. Effect of salinity on malic acid content of chickpea genotypes at 90 days after sowing



Discussion:

In photosynthetic activity the chlorophyll content plays an important role. The higher chlorophyll content increases photosynthetic rate. The data on chlorophyll a, chlorophyll b and total chlorophyll content 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.The content of chlorophyll a (chl a), chl b, carotenoids, chla+b and total pigments gradually decreased with increase in salinity concentration and highest reduction of photosynthetic pigments were recorded at 200mM NaCl level (Hassanein*et al.*, 2012). Among the genotypes significantly maximum total chlorophyll content was recorded at 60 days after sowing by the genotype JG 11and BGD 103.Similar findings were noticed by Taibi*et al.* (2016) reported that the lipid peroxidation of chloroplast during salt stress decrease the chlorophyll pigments and in all genotypes the increa levels reducd

Comment [P34]: 1.You must mention the table and figure in the text
2.please add a discussion that links all the variables you observed into a story, why then conclude genotypes a, b, c, as tolerant, while the others are sensitive.
3.Has any other article ever reported the JG11 genotype as tolerant to salt?

Comment [P35]: Reference?

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the drysed salinity mass and chlorophyll pigments and increased malondialdehydecontent. Kaur*et al.* (2014) 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 (Giri*et al*., 2011). 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 (Sivashankaramoorthy, S., 2013).

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 6dSm^{-1} followed by 3dSm^{-1} and 0dSm^{-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 (Flowers *et al*., 2010). 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*., 2017, 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 (Kotula*et al*., 2019). 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. (2019) 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.

Comment [P39]: Please end the discussion by mentioning the conclusion of the study

Reference:

Comment [P40]: 18 of 27 references are less than the last 10 years, please use references at least the last 10 years

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