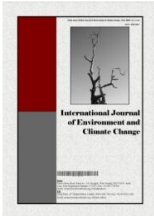

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Physio-Biochemical and Yield Response of Chickpea Genotypes under Salinity and High Temperature Stress

Trisha Sinha[1*], Shailesh Kumar[1] and Ajay Kumar Singh[1]

[1]Department of Botany, Plant Physiology and Biochemistry, Dr. Rajendra Prasad Central Agricultural University, Pusa- 848125, Samastipur, Bihar, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TS and SK and AKS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TS and SK managed the analyses of the study. Authors TS and AKS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A pot experiment was carried out with six chickpea genotypes *viz.* KPG-59, IPC-2013-74 and NDG-15-6 (tolerant group); and KWR-108, BG-3075 and BG-3076 (susceptible group) to study the responses of these genotypes under salinity stress (4.20 dSm^{-1}) with normal sowing, high temperature (HT) stress with late sowing and their combination (saline soil + late sowing), and compared with control (non-stress) condition based on several physio-biochemical traits such as malondialdehyde content (MDA), membrane stability index (MSI), relative water content (RWC) and proline in leaf at reproductive stage; and seed yield after harvesting. Both salinity and HT individually and in combination significantly affected the traits studied. Among the parameters, MDA increased under stress treatments over control, while MSI and RWC decreased for the same. However, combined stress exhibited hypo-additive effects for these parameters which might be due to developed cross-tolerance while facing salinity and HT stress in sequence. Increase in proline content under stress over control is an indication of osmotic adjustment in response to stress. These results might be good criteria in development of genotypes with improved response in terms of physio-biochemical traits and yield.

**Corresponding author: E-mail: trishasinhabckv@gmail.com*

Keywords: Chickpea; late sowing; combined stress; reproductive stage; cross-tolerance.

1. INTRODUCTION

Naturally, plants are often exposed to a number of adverse environmental conditions, known as stresses that influence plant growth negatively. Survival and growth of plants are highly based on their ambience both surrounding and within. Abiotic stresses are the most vital factors that influence crop establishment and overall growth. Crop yield is also influenced by the interaction of these stresses with the crop, individually or in combination.

Globally, chickpea is widely grown in the arid and semi-arid regions [1], where this crop faces both salinity stress [2] and high temperature stress [3] at terminal growth stage *viz*. reproductive stage. Plants are more inclined to get adversely affected under salinity stress at reproductive stage [4]. The late-sown chickpea crop is exposed to high temperatures (>35□) at its reproductive stage in the months of February and March [5]. Chickpea productivity is highly influenced by several abiotic stresses [6] for these affect the reproductive phase in a negative manner. Reduced yield in chickpea has been reported for its sensitivity towards the abiotic stress factors, specifically salinity [7,8] and high temperature [9,10] that lead to changes in various physiological and metabolic activities [11, 12] such as membrane damage, altered water status etc., which directly or indirectly affect yield. Ample studies previously conducted had expressed the physio-biochemical response of crop when exposed to salinity and high temperature stress individually. But limited studies have been available to reveal about the impact of combined stress of salinity and high temperature on crops and their counteracting mechanisms. The potential of salinity and high temperature to cause harmful effects in crop plant is different and complex. So there is an urgent need of investigation regarding their separate and combined effects on crops. So, this study was conducted with the aim of finding out the physio-biochemical response of chickpea genotypes under individual and combined salinity and high temperature stress at reproductive stage.

2. MATERIALS AND METHODS

2.1 Base of the Study

This study was conducted at the Department of Botany, Plant Physiology and Biochemistry in Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India. A previous study [13] was undergone with thirty genotypes at laboratory condition in order to screen the genotypes under individual and combined salinity and high temperature stress in terms of seedling growth parameters along with stress tolerance indices, based on which contrasting sets of genotypes were selected to further carry out the study.

2.2 Experimental Treatments and Programme

Now, this current study was carried out with these six genotypes to find out several physiological and biochemical changes under saline and high temperature stress in pot culture. Seeds were surface sterilized using 1.0% sodium hypochlorite solution for ten minutes and then washed thoroughly with distilled water before sowing. Normal and saline soils were collected from Mohammadpur (Samastipur) and Motipur (Muzaffarpur) respectively. Electrical conductivity of saturation paste (EC_e) of both soils *viz*. normal soil and saline soil measured at Department of Soil Science, RPCAU, Pusa, Samastipur, Bihar was found to be 0.40 dSm^{-1} and 4.20 dSm^{-1} respectively. After that, plastic pots were filled with 15.00 kg normal soil (0.40 dSm^{-1}) for control, and 15.00 kg of saline soil (4.20 dSm^{-1}) for salinity stress. Seed sowing programme with ten seeds per pot was done in 15th of November, 2019 for control (T_0) and individual salinity stress (T_1) in normal (0.40 dSm^{-1}) and saline soil (4.20 dSm^{-1}) respectively. For exposure to individual high temperature stress (T_2), late sowing of seeds on 15th of December, 2019 in normal soil (0.40 dSm^{-1}) was done with the purpose of coinciding with high temperature at reproductive stage (March-April, 2020). Seeds were imposed to salinity and high temperature stress combination (T_3) through late sowing (15th of December, 2019) in saline soil (4.20 dSm^{-1}). Air temperature data of the whole cropping season is listed too (Fig. 1). Plants were thinned to five plants per pot after 15 days of sowing (DAS). Irrigations were given as per requirement. Table 1 describes different treatments used in the present experiment. After attaining the reproductive stage, physio-biochemical changes of chickpea genotypes on the basis of the parameters *viz*. malondialdehyde content [14], membrane stability index [15], relative water content [16] and proline content [17] were determined using chickpea leaves at reproductive stage. Seed yield was also calculated in terms of seed weight per plant (g $plant^{-1}$).

2.3 Statistical Analysis

The observations were replicated three times and the data were subjected to analysis of variance (ANOVA) and CRD at 5% with OPSTAT software.

3. RESULTS AND DISCUSSION

3.1 Malondialdehyde Content (MDA)

Salinity as well as high temperature significantly affected chickpea genotypes in terms of malondialdehyde (MDA) content in leaves as presented in Table 2. Increase in MDA contents was observed in response to individual and combined salinity and high temperature stress for all the genotypes, however, the rate of increase was relatively lower in the tolerant genotypes than that of the susceptible genotypes. It was observed from the experimental findings that genotype KPG-59 (G_1) recorded the lowest percentage increase of MDA contents in each stress treatment with the mean MDA content of 18.00 µmol g^{-1} fresh weight. In the contrast, BG-3076 (G_6) recorded the highest mean MDA content of 22.28 µmol g^{-1} fresh weight. Individual high temperature stress (T_2) expressed least detrimental effects for the genotypes followed by salinity stress and combined stress. Percentage increase in MDA content under T_2 for the genotypes G_1, G_2, G_3, G_4, G_5 and G_6 recorded was 10.32, 12.05, 13.37, 15.43, 16.48 and 19.55 respectively over control. Salinity (T_1) induced percentage increase in MDA content over control for all the genotypes varied from its lowest of 18.06 in G_1 to its highest of 24.58 in G_6. Combined stress (T_3) led to huge increase in MDA content by 36.13%, 38.55%, 42.44%, 45.71%, 50.00% and 53.63% respectively for G_1, G_2, G_3, G_4, G_5 and G_6 over control.

Table 1. Details of the genotypes used in the experiment

Sl. No.	Code	Treatment name	Treatment details
1.	T_0	Control	0.40 dSm^{-1}
2.	T_1	Salinity	4.20 dSm^{-1}
3.	T_2	High temperature	Late sowing
4.	T_3	Salinity + high temperature	4.20 dSm^{-1} + late sowing

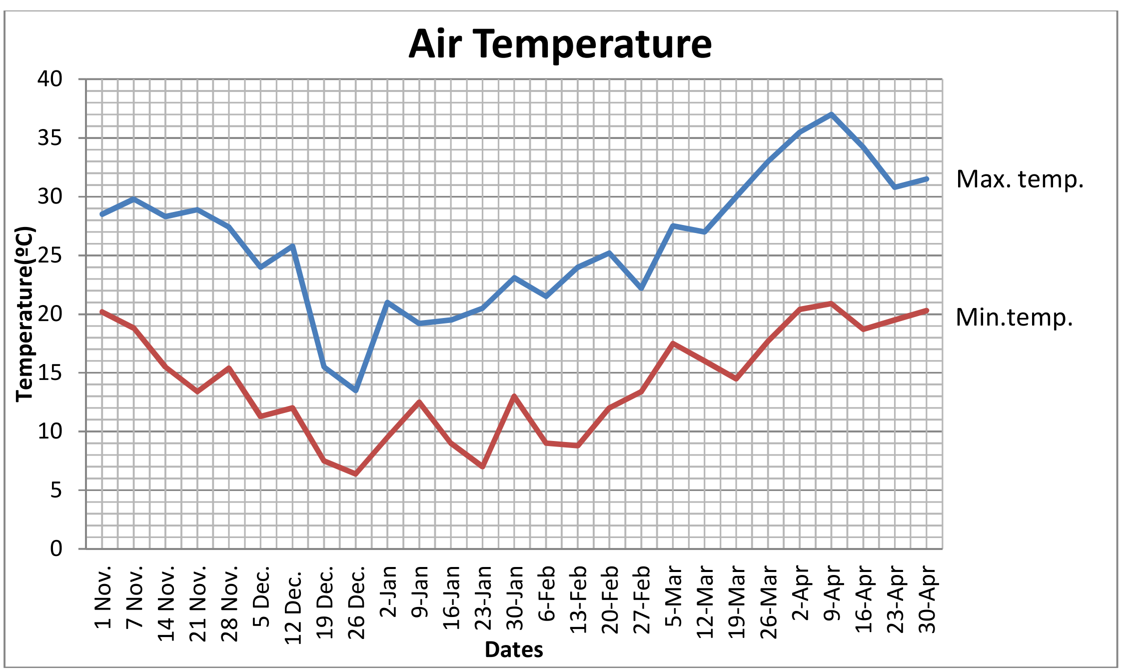

Fig. 1. Air temperature data of whole cropping season (November 2019-April 2020) of chickpea

Malondialdehyde (MDA) is considered as the potential biomarker of membrane lipid peroxidation in the cellular environment [18]. Kaur et al. [19] also reported marked increase in MDA contents in salinity stress which may be due to inadequate induction of antioxidant system [20]. Similar results of salinity induced increase in MDA content with relatively more increase in susceptible genotypes than the tolerant genotypes in chickpea were obtained by Kalefetoglu and Ekmekci [21]. Almeselmani et al. [22] reported higher MDA content in susceptible genotypes than the tolerant ones in wheat under high temperature stress, similar as our findings.

3.2 Membrane Stability Index (MSI)

Effect of salinity and high temperature stress on MSI of chickpea genotypes was described in Table 3. Decreasing pattern of MSI was observed under all the stress treatments over control in this present study. Membrane stability index recorded from a range of 75.20 in KPG-59 to 73.90 in BG-3076 in control (T_0) condition. Gradually it decreased in high temperature followed by salinity stress (T_1) and combined stress (T_3) with an average of 62.70, 57.88 and 49.50 respectively from an average of 74.53 at control (T_0). In case of MSI also, T_2 caused minimum reduction in MSI over control for all the genotypes with the highest reduction percentage of 22.06 in BG-3076 and the lowest reduction percentage of 10.64 in KPG-59. At T_1, percentage decrease of 17.55, 19.28, 18.42, 25.81, 23.62 and 29.50 in MSI over control was recorded respectively by G_1, G_2, G_3, G_4, G_5 and G_6. At combined stress (T_3), percentage decrease in MSI recorded by the susceptible genotypes *viz.* G_4, G_5 and G_6 was respectively 43.11, 40.94 and 45.06 which were in contrast, quite higher than the percentage decrease of 23.14, 25.70 and 23.90 in case of tolerant genotypes G_1, G_2, G_3 respectively. This simply relates the said tolerant genotypes with their better resistance due to developed cross-tolerance while facing two stresses in sequence.

Membrane stability index declined gradually with increasing salt concentration as reported by Noreen and Ashraf [23]. Our results matched with those of Shahid et al. [24] who recorded more decline in MSI in the susceptible genotypes than tolerant genotypes of pea. Higher value of MSI is also an indicator of high temperature tolerance in several crops [25]. Decrease in MSI in chickpea genotypes as found in our study was also supported by Kumar et al. [26].

3.3 Relative Water Content (RWC)

In our experiment, RWC declined in every stress treatment over control for all the genotypes (Table 4). Individual high temperature (T_2) was the least affecting factor with causing the minimum decline, recording an average of 71.80, followed by individual salinity stress (T_1) with its average of 61.14; while the combined stress (T_3) causing maximum decline recorded the average of 52.15 over control (T_0) which had the average of 87.87 for all the genotypes. In this study, percentage decrease of 14.96, 16.53 and 16.16 over control (T_0) in RWC was noticed respectively in the tolerant genotypes *viz.* KPG-59, IPC-2013-74 and NDG-15-6 at T_2. Susceptible genotypes KWR-108, BG-3075 and BG-3076 recorded its percentage decline of 20.64, 19.23 and 22.34 respectively at T_2 and 34.25, 32.36 and 36.11 respectively at T_1 over T_0. RWC further decreased by 47.71%, 45.73% and 48.84% at T_3 for the genotypes KWR-108, BG-3075 and BG-3076 respectively over T_0. Tolerant genotypes G_1, G_2 and G_3 managed to maintain relatively lower percentage decline of RWC at T_1 (24.63, 28.88 and 26.10 respectively) over control than the susceptible genotypes. These genotypes *viz.* KPG-59, IPC-2013-74 and NDG-15-6 exhibited improved tolerance in terms of RWC at combined stress also, where the percentage reduction of 31.38, 37.49 and 33.11 was observed respectively, which was not that high as the susceptible genotypes.

Relative water content (RWC) is considered to induce osmotic adjustment [27], which is the process that delays dehydration as a response to stress condition in plants. Simply, higher value of it under stress denotes greater tolerance exhibited by genotypes. Therefore, determining the effects of salinity and high temperature on RWC is very important and physiologically relevant. Gradual decrease in RWC of chickpea genotypes with increase in salt concentration was reported by Garg and Bhandari [28]. However, it was observed in our experiment that tolerant genotypes were able to maintain lower reduction in RWC than the susceptible ones. These results were similar with the findings of Sairam et al. [29] in wheat. Study by Sita et al. [30] revealed that RWC reduced significantly under high temperature in both tolerant and susceptible genotypes of lentil, while the reduction was higher in the susceptible genotypes than the tolerant genotypes.

Table 2. Effect of salinity and high temperature stress on MDA content (μmol g^{-1} fresh weight) of chickpea genotypes at reproductive stage

Genotypes (G)	Treatments (T)				
	Control	Salinity (4.20 dSm^{-1})	High Temperature (Late sowing)	Salinity (4.20 dSm^{-1}) + High Temperature (Late sowing)	Mean
KPG-59 (G_1)	15.50	18.30 (+18.06)	17.10 (+10.32)	21.10 (+36.13)	18.00
IPC-2013-74 (G_2)	16.60	19.80 (+19.28)	18.60 (+12.05)	23.00 (+38.55)	19.50
NDG 15-6 (G_3)	17.20	20.70 (+20.35)	19.50 (+13.37)	24.50 (+42.44)	20.48
KWR-108 (G_4)	17.50	21.20 (+21.14)	20.20 (+15.43)	25.50 (+45.71)	21.10
BG-3075 (G_5)	17.60	21.70 (+23.30)	20.50 (+16.48)	26.40 (+50.00)	21.55
BG-3076 (G_6)	17.90	22.30 (+24.58)	21.40 (+19.55)	27.50 (+53.63)	22.28
Mean	17.05	20.67	19.55	24.67	
Factors	C.D. at 5%	SE(d)	SE(m)	G_1, G_2 and G_3 - Tolerant group	
Factor (G)	0.341	0.169	0.120	G_4, G_5 and G_6 - Susceptible group	
Factor (T)	0.279	0.138	0.098	Figures in the parentheses indicate the per cent	
Interaction (G X T)	0.682	0.338	0.239	increase (+) over control	

Table 3. Effect of salinity and high temperature stress on membrane stability index (%) of chickpea genotypes at reproductive stage

Genotypes (G)	Treatments (T)				
	Control	**Salinity (4.20 dSm^{-1})**	**High Temperature (Late sowing)**	**Salinity (4.20 dSm^{-1}) + High Temperature (Late sowing)**	**Mean**
KPG-59 (G_1)	75.20	62.00 (-17.55)	67.20 (-10.64)	57.80 (-23.14)	65.55
IPC-2013-74 (G_2)	74.70	60.30 (-19.28)	65.90 (-11.78)	55.50 (-25.70)	64.10
NDG 15-6 (G_3)	74.90	61.10 (-18.42)	66.80 (-10.81)	57.00 (-23.90)	64.95
KWR-108 (G_4)	74.00	54.90 (-25.81)	58.30 (-21.22)	42.10 (-43.11)	57.33
BG-3075 (G_5)	74.50	56.90 (-23.62)	60.40 (-18.93)	44.00 (-40.94)	58.95
BG-3076 (G_6)	73.90	52.10 (-29.50)	57.60 (-22.06)	40.60 (-45.06)	56.05
Mean	74.53	57.88	62.70	49.50	
Factors	C.D. at 5%	SE(d)	SE(m)	G_1, G_2 and G_3 - Tolerant group	
Factor (G)	1.276	0.633	0.447	G_4, G_5 and G_6 - Susceptible group	
Factor (T)	1.042	0.517	0.365	Figures in the parentheses indicate the per cent	
Interaction (G X T)	2.552	1.265	0.895	decrease (-) over control	

Table 4. Effect of salinity and high temperature stress on relative water content (RWC) of chickpea genotypes at reproductive stage

Genotypes (G)	Treatments (T)				
	Control	**Salinity (4.20 dSm^{-1})**	**High Temperature (Late sowing)**	**Salinity (4.20 dSm^{-1}) + High Temperature (Late sowing)**	**Mean**
KPG-59 (G_1)	88.90	67.00 (-24.63)	75.60 (-14.96)	61.00 (-31.38)	73.13
IPC-2013-74 (G_2)	88.30	62.80 (-28.88)	73.70 (-16.53)	55.20 (-37.49)	83.00
NDG 15-6 (G_3)	88.50	65.40 (-26.10)	74.20 (-16.16)	59.20 (-33.11)	83.58
KWR-108 (G_4)	87.20	57.33 (-34.25)	69.20 (-20.64)	45.60 (-47.71)	80.08
BG-3075 (G_5)	87.90	59.10 (-32.76)	71.00 (-19.23)	47.70 (-45.73)	81.45
BG-3076 (G_6)	86.40	55.20 (-36.11)	67.10 (-22.34)	44.20 (-48.84)	79.07
Mean	87.87	61.14	71.80	52.15	
Factors	C.D. at 5%	SE(d)	SE(m)	G_1, G_2 and G_3 - Tolerant group	
Factor (G)	1.434	0.711	0.503	G_4, G_5 and G_6 - Susceptible group	
Factor (T)	1.171	0.580	0.410	Figures in the parentheses indicate the per cent	
Interaction(G X T)	2.867	1.422	1.005	decrease (-) over control	

Table 5. Effect of salinity and high temperature stress leaf proline content (μmol g^{-1} fresh weight) of chickpea genotypes at reproductive stage

Genotypes (G)	Treatments (T)				
	Control	Salinity (4.20 dSm^{-1})	High Temperature (Late sowing)	Salinity (4.20 dSm^{-1}) + High Temperature (Late sowing)	Mean
KPG-59 (G_1)	3.68	6.58 (+78.80)	5.10 (+38.59)	8.04 (+118.48)	5.85
IPC-2013-74 (G_2)	3.46	6.00 (+73.41)	4.53 (+30.92)	7.20 (+108.09)	5.30
NDG 15-6 (G_3)	3.37	5.75 (+70.62)	4.23 (+25.52)	6.90 (+104.75)	5.06
KWR-108 (G_4)	3.31	5.35 (+61.63)	4.11 (+24.17)	6.50 (+96.37)	4.82
BG-3075 (G_5)	3.18	4.85 (+52.52)	3.85 (+21.07)	6.00 (+88.68)	4.47
BG-3076 (G_6)	3.25	5.05 (+55.38)	3.95 (+21.54)	6.30 (+93.85)	4.64
Mean	3.38	5.60	4.30	6.82	
Factors	C.D. at 5%	SE(d)	SE(m)	G_1, G_2 and G_3 - Tolerant group	
Factor (G)	0.107	0.053	0.038	G_4, G_5 and G_6 - Susceptible group	
Factor (T)	0.088	0.043	0.031	Figures in the parentheses indicate the per cent	
Interaction (G X T)	0.215	0.106	0.075	increase (+) over control	

Table 6. Effect of salinity and high temperature stress on seed yield (g plant^{-1}) of chickpea genotypes after harvesting

Genotypes (G)	Treatments (T)				
	Control	Salinity (4.20 dSm^{-1})	High Temperature (Late sowing)	Salinity (4.20 dSm^{-1}) + High Temperature (Late sowing)	Mean
KPG-59 (G_1)	6.11	4.12 (-32.57)	5.13 (-16.04)	3.13 (-48.77)	4.62
IPC 2013-74 (G_2)	6.05	3.99 (-34.05)	5.00 (-17.36)	3.01 (-50.25)	4.51
NDG 15-6 (G_3)	5.99	3.89 (-35.06)	4.88 (-18.53)	2.87 (-52.09)	4.41
KWR-108 (G_4)	5.78	3.37 (-41.70)	4.38 (-24.22)	2.36 (-59.17)	3.97
BG-3075 (G_5)	5.93	3.73 (-37.10)	4.75 (-19.90)	2.74 (-53.79)	4.29
BG-3076 (G_6)	5.86	3.55 (-39.42)	4.56 (-22.18)	2.56 (-56.31)	4.13
Mean	5.95	3.77	4.78	2.77	
Factors	C.D. at 5%	SE(d)	SE(m)	G_1, G_2 and G_3 - Tolerant group	
Factor (G)	0.074	0.037	0.026	G_4, G_5 and G_6 - Susceptible group	
Factor (T)	0.060	0.030	0.021	Figures in the parentheses indicate the per cent	
Interaction (G X T)	0.148	0.073	0.052	decrease (-) over control	

3.4 Proline

Response of chickpea genotypes to salinity and high temperature stress in terms of proline content was shown in Table 5. Increase in proline content at stress treatments was observed over control in all the genotypes with the more increase in tolerant genotypes than that of the susceptible genotypes. In this experiment, average proline content for genotypes KPG-59 (G_1), IPC-2013-74 (G_2) and NDG-15-6 (G_3) was 5.85, 5.30 and 5.06 µmol g^{-1} fresh weight; whereas it was 4.82, 4.47 and 4.64 µmol g^{-1} fresh weight for the genotypes KWR-108 (G_4), BG-3075 (G_5) and BG-3076 (G_6) respectively. Salinity treatment (T_1) resulted in much higher percentage increase in proline content for all the genotypes from 78.80 in KPG-59 to 55.52 in BG-3075 compared to their respective high temperature treatment (T_2) at which their observed percentage increase varied from 38.59 (KPG-59) to 21.07 (BG-3075) over control (T_0). Increase in proline content was also recorded under T_3, but the rate of increase was not similar for all the genotypes as observed in T_1 and T_2 over control. A remarkable increase in proline content was observed at T_3 for all the genotypes with the more increase in tolerant genotypes G_1, G_2 and G_3 (118.48, 108.09 and 104.75 respectively) and relatively lower in susceptible genotypes G_4, G_5 and G_6 (96.37, 88.68 and 93.85 respectively).

Salt induced proline synthesis and accumulation was reported by El-Bassiouny and Bekheta [31] in wheat. Najaphy et al. [32] also reported more increase of proline content in salinity tolerant genotypes of chickpea under salinity stress. Leaf proline content also increased five-fold under high temperature treatment in French bean as reported by Babu and Devaraj [33]. Kumar et al. [5] also reported gradual increase in proline content with increasing temperature up to a range of 40/35□. Proline is best known for its role in osmotic adjustment, scavenging free radicals and protection of macromolecules from denaturation as a key antioxidant [34]. The higher percentage increase in case of tolerant genotypes when shifted from individual stresses to combined stress indicates the ability of these tolerant genotypes to develop better tolerance against combined stress. It may be said that proline which is known to reduce the magnitude of stress by inducing osmotic adjustment in plants accumulated more for genotypes while facing two stresses one after another.

3.5 Seed Yield

Data regarding seed yield (g $plant^{-1}$) obtained by each genotype at each treatment were presented in Table 6. Genotypes varied in terms of seed yield too in response to individual and combined salinity and high temperature stress. At control (T_0), the seed yield of six genotypes ranged from the highest in G_1 *viz.* KPG-59 (6.11 g $plant^{-1}$) to the lowest in G_4 *viz.* KWR-108 (5.78 g $plant^{-1}$) with the overall mean of 5.95 g $plant^{-1}$. Reduced seed yield in susceptible genotypes *viz.* KWR-108, BG-3075 and BG-3076 was 24.22%, 19.90% and 22.18% respectively at high temperature stress (T_2) over control. It further recorded reduction by 41.70%, 37.10% and 39.42% respectively when exposed to salinity stress (T_1). Tolerant genotypes *viz.* KPG-59, IPC-2013-74 and NDG-15-6 obtained seed yield of 5.13, 5.00 and 4.88 g $plant^{-1}$ respectively at T_2 with the respective percentage decrease of 16.04, 17.36 and 18.53 with respect to control. Percentage reduction in seed yield by the genotypes KPG-59, IPC-2013-74 and NDG-15-6 at T_1 recorded was 32.57, 34.05 and 35.06 respectively over control. On the other hand, T_3 resulted in percentage decrease of 59.17, 53.79 and 56.31 in seed yield for the susceptible genotypes KWR-108, BG-3075 and BG-3076. The tolerant genotypes KPG-59, IPC-2013-74 and NDG-15-6 recorded relatively lesser percentage decrease of 48.77, 50.25 and 52.09 in seed yield than the susceptible genotypes at T_3 over control.

Salinity induced reduction in seed yield of chickpea was also reported by Singla and Garg [35], and Sohrabi et al. [36]. The reduction in seed yield for all the genotypes under high temperature found in this experiment is to be attributed to the failure of pod set [5].

4. CONCLUSION

The effect of combined stress of salinity and high temperature on the physio-biochemical parameters such as MSI, RWC and yield of chickpea genotypes studied in this experiment has been found hypo-additive *i.e.* effect of combined stress was more than each individual stress but less than their sum. Increase in MDA content was found additive under combined stress but lesser percentage increase was observed in the tolerant genotypes than the susceptible ones. Remarkable proline accumulation was recorded for all the stress treatment over control irrespective of the genotype. Developing cross-tolerance with stress and comparatively improved responses in terms of physio-biochemical parameters at combined stress studied above could be related with their better yield performance for the tolerant genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar J, Abbo S. Genetics of flowering time in chickpea and its bearing on productivity in semi-arid environments. Advances in Agronomy. 2001;72:107-138.
2. Flowers TJ, Gaur PM, Gowda CLL, Krishnamurthy L, Samineni S. et al. Salt sensitivity in chickpea. Plant, Cell and Environment. 2010;33:490-509.
3. Singh MK. Protective role of antioxidant enzymes in chickpea (*Cicer arietinum* L.) genotypes under high temperature stress. Current Life Sciences. 2018;4(1):1-9.
4. Vadez V. et al. Large number of flowers and tertiary branches, and higher reproductive success increase yields under salt stress in chickpea. European Journal of Agronomy. 2012;41:42-51.
5. Kumar S, Thakur P, Kaushal N, Malik JA, Gaur P. et al. Effect of varying high temperatures during reproductive growth on reproductive function, oxidative stress and seed yield in chickpea genotypes differing in heat sensitivity. Archives of Agronomy and Soil Science. 2013;59(6):823-843.
6. Hussain N, Aslam M, Ghaffar A, Irshad V, Naeem-ud-Din. Chickpea genotypes evaluation for morpho-yield traits under water stress conditions. The Journal of Animal & Plant Sciences. 2015;25(1):206-211.
7. Jha UC, Chaturvedi SK, Bohra A, Basu PS, Khan MS. et al. Abiotic stresses, constraints and improvement strategies in chickpea. Plant Breeding. 2014;133(2):163-178.
8. Flowers TJ, Gaur PM, Gowda CLL, Krishnamurthy L, Samineni S. et al. Salt sensitivity in chickpea. Plant, Cell and Environment. 2010;33:490-509.
9. Wang J, Gan YT, Clarke F, Mc-Donald CL. Response of chickpea yield to high temperature stress during reproductive development. Crop Science. 2006;46(5):2171-2178.
10. Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Physiological, biochemical and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences. 2013;14(5):9643-9684.
11. Basu PS, Ali M, Chaturvedi SK. Terminal heat stress adversely affects chickpea productivity in Northern India – Strategies to improve thermotolerance in the crop under climate change. In: ISPRS Archives XXXVIII-8/W3 Workshop Proceedings: Impact of Climate Change on Agriculture, 23-25 February, New Delhi, India, 2009;189-193.
12. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 2008;59:651-681.
13. Sinha T, Singh AK, Kumar S. Cross-tolerance physiology of chickpea (*Cicer arietinum* L.) genotypes under combined salinity and high temperature stress condition. doi. 10.18805/LR-4390
14. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968;125(1):189-198.
15. Premachandra G, Saneoka H, Ogata S. Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soybean. Journal of Agricultural Science. 1990;115:63-66.
16. Barrs HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Australian Journal of Biological Sciences. 1962;15:413-428.
17. Bates L, Waldren R, Teare I. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39:205-207.
18. Faried HN, Ayyub CM, Amjad M, Ahmed R. Salinity impairs ionic, physiological and biochemical attributes in potato. Pakistan Journal of Agricultural Science. 2016;53(1):17-25.
19. Kaur P, Kaur J, Kaur S, Singh S, Singh I. Salinity induced physiological and biochemical changes in chickpea (*Cicer arietinum* L.) genotypes. Journal of Applied and Natural Science. 2014;6(2):578-588.

20. Hossain MA, Mostofa MG, Fujita M. Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. Molecular Plant Breeding. 2013;4:50-70.
21. Kalefetoglu MT, Ekmekci Y. Alterations in photochemical and physiological activities of chickpea (*Cicer arietinum* L.) cultivars under drought stress. Journal of Agronomy and Crop Science. 2009;195(5):335-346.
22. Almeselmani M, Deshmukh PS, Sairam RK. High temperature stress tolerance in wheat genotypes: role of antioxidant defence enzymes. Acta Agronomica Hungarica. 2009;57:1-14.
23. Noreen Z, Ashraf M. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. Journal of Plant Physiology. 2009;166:1764-1774.
24. Shahid MA, Balal RM, Pervez MA, Abbas T, Ashfaq M. et al. Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Australian Journal of Crop Science. 2012;6(5):828-838.
25. Wahid A, Shabbir A. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. Plant Growth Regulation. 2005;46:133-141.
26. Kumar N, Nandwal AS, Yadav R, Bhasker P, Kumar S. et al. Assessment of chickpea genotypes for high temperature tolerance. Indian Journal of Plant Physiology. 2012;17(3&4):224-232.
27. Singh TP, Deshmukh PS, Tyagi RK, Dutta M. Morpho-physiological evaluation of chickpea (*Cicer arietinum* L.) cultivars under restricted soil moisture conditions. Indian Journal of Plant Genetic Resources. 2013;26:162-165.
28. Garg N, Bhandari P. Silicon nutrition and mycorrhizal inoculations improve growth, nutrient status, K^+/Na^+ ratio and yield of *Cicer arietinum* L. genotypes under salinity stress. Plant Growth Regulation. 2016;78:371-387.
29. Sairam RK, Veerabhadra Rao K, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 2002;163:1037-1046.
30. Sita K, Sehgal A, Kumar J, Kumar S, Singh S. et al. Identification of high-temperature tolerant lentil (*Lens culinaris* Medik.) genotypes through leaf and pollen traits. Frontiers in Plant Science, 2017;8: 744.
31. El-Bassiouny HMS, Bekheta MA. Effect of salt stress on relative water content, lipid peroxidation, polyamines, amino acids and ethylene of two wheat cultivars. International Journal of Agriculture and Biology. 2005;7(3):363-368.
32. Najaphy A, Khamssi NN, Mostafaie A, Mirzaee H. Effect of progressive water deficit stress on proline accumulation and protein profiles of leaves in chickpea. African Journal of Biotechnology. 2010;9:7033-7036.
33. Babu NR, Devaraj VR. High temperature and salt stress response in French bean (*Phaseolus vulgaris*). Australian Journal of Crop Science. 2008;2:40-48.
34. Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR. et al. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Current Science. 2005;88:424-438.
35. Singla R, Garg N. Influence of salinity on growth and yield attributes in chickpea cultivars. Turkish Journal of Agricultural Forestry. 2005;29:231-235.
36. Sohrabi Y, Heidari G, Esmailpoor B. Effect of salinity on growth and yield of *desi* and *kabuli* chickpea cultivars. Pakistan Journal of Biological Sciences. 2008;11(4):664-667.

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