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ORIGINAL ARTICLE

Growth and antioxidant system under drought stress in Chickpea (*Cicer arietinum* L.) as sustained by salicylic acid

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Key words: antioxidant / pre- and post- anthesis / proline / relative injury / salicylic acid

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Drought stress is a major abiotic stress limiting agricultural crop production worldwide. Plants respond to drought stress and acclimatize through various physiological and biochemical changes (Farooq et al., 2009a). Drought induces oxidative stress in plants, in which reactive oxygen species (ROS), such as superoxide radical ($O2^{\bullet-}$), hydroxy radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and alkoxy

radical (RO•) are produced (Munnne-Bosch and Penueals 2003). Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolism (Hasegava et al., 2000). These mechanisms include β-carotenes, ascorbic acid (AA), α-tocopherol (α-Toc), reduced glutathione (GSH) and enzymes including superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) (Prochazkova et al., 2001). Plants experience drought stress either when the water supply to roots is interrupted or when transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates. Drought stress tolerance has been seen in almost all plant species but its extent varies from species to species (Lin et al., 2006).

Salicylic acid (SA) is a naturally existing phenolic compound. Evidences put forward that externally applied SA increased plant's tolerance to several abiotic stresses, including osmotic stress (Wang *et al*. 2010), drought (Azooz and Youssef., 2010), salinity (Gunes et al., 2007), and heavy metal stress (Moussa and El-Gamel., 2010). Exogenous SA reduced transpiration and increased nitrate reductase activity, flower longevity as well as the yield of some plants (Raskin., 1992), which overall suggest that SA may enhance the multiple types of stress tolerance in plants through which interactive effects on several functional molecules or other signaling molecules participating in more complex stress responses.

Drought stress is a major concern affecting the agriculture production. Generally pulses are very susceptible to drought stress. Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world and first in the Mediterranean basin and South Asia that frequently experiences water stress during pod set and seed filling stage (terminal drought) in India and the Mediterranean basin, leading to a substantial yield loss (Turner et al., 2001). Drought stress during seed filling has been reported to be highly detrimental to yield in chickpea (Davis et al*.,* 1999).

Comparatively meagre work is on record regarding role of SA on drought tolerance in chickpea. SA appears to have innate potentiality for increasing antioxidants and influencing antioxidant enzyme activity in plants subjected to oxidative stress (Hayat et al., 2008). Therefore, the present investigation was conducted to evaluate the drought stress ameliorating ability of SA with special emphasis on growth and antioxidant system in drought stressed chickpea plants.

MATERIALS AND METHODS

Plant material and drought stress applications

Seeds of four *Cicer arietinum* L. genotypes viz. Tyson, ICC 4958, JG 315 and DCP 92-3 were surface sterilized with 0.2% $HgCl_2$ solution. Salicylic acid (SA) was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000 v/v) (Williams et al*.,* 2003). Thereafter 10 seeds of each genotype for each treatment were soaked in water (0 mM SA) taken as control (T_0), 1.0 mM SA (T_1) and 1.5 mM SA (T_2) for 6 h before sowing in pots containing farm soil having 12-14% moisture at the time of sowing.

Each genotype was grouped in three sets viz. irrigated, early drought stress and late drought stress. Water stress treatment was instigated at 50 DAS. Control plants (C) were given three irrigations (at 25, 50 and 65 DAS) from the date of sowing to maturity. Early drought stressed or pre- anthesis drought stressed plants (EDS) received two irrigations (25 and 65 DAS) whereas post- anthesis drought stressed plants (LDS) received two

irrigations at 25 and 50 DAS.

Physiological parameters

Relative water content (RWC) of the leaves was estimated according to the method of Weatherley (1950) and membrane permeability [MP or EC (%)] was measured by the method of Cekic et al., (2001).

Antioxidant and antioxidant enzymes estimation

Ascorbic acid (AA) content was determined by the method of Mukherjee and Choudhary (1983). Proline content was determined by the method of Bates et al. (1973). APX activity was measured according to Nakano and Asada (1980) as the decrease in absorbance at 290 nm due to ascorbate oxidation. CAT activity was measured as the decline in absorbance at 230 nm due to the decomposition of H_2O_2 (Aebi et al., 1983). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Dhindsa et al., 1981). H_2O_2 was determined by the method of Mukherjee and Choudhary (1983). The assay of POX activity was carried out by measuring the decrease in absorbance at 420 nm due to decomposition of H_2O_2 (Kar and Mishra., 1976). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation by the method of Hodges et al. (1999).

Statistical analysis

Samples for biochemical estimations were collected in 3 replicates (from 3 pots) and each replicate/sample was assayed twice. Experiments were conducted in CRD. Data was analyzed for analysis of variance (*ANOVA*) and means were compared by the least significant difference (LSD) test and those at $P < 0.05$. Standard error of mean was also calculated.

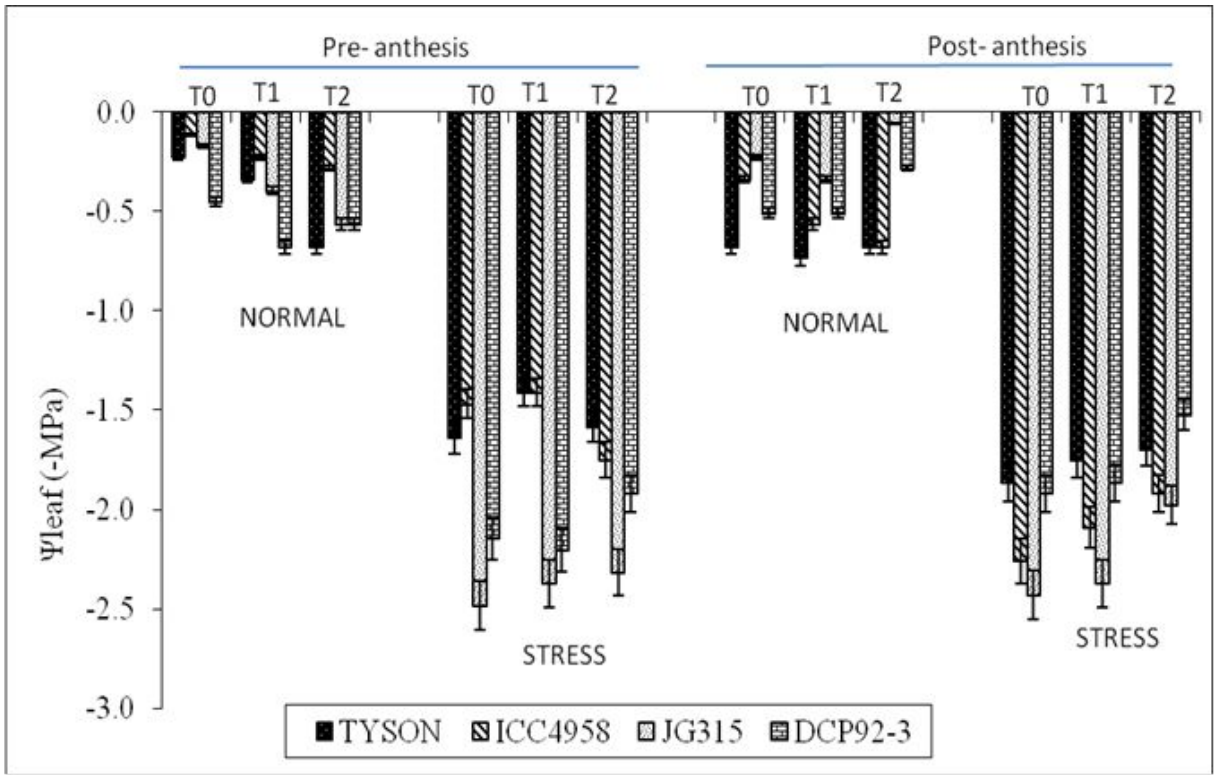

Figure 1. Effect of salicylic acid (SA) on leaf water potential [Ψ_{leaf} (-MPa)] in four chickpea (*Cicer arietinum* L.) genotypes.

Table 1 Effect of salicylic acid (SA) on relative water content (RWC) in chickpea (*Cicer arietinum* L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	81.33	73.00 (-10.2)	78.67	71.67 (-8.9)
	T_1 (1.0 mM SA)	80.67	74.33 (-7.9)	78.33	72.33 (-7.7)
	T_2 (1.5 mM SA)	78.67	73.33 (-6.8)	78.67	72.67 (-7.6)
ICC 4958	T_0 (Control)	83.00	74.00 (-10.8)	80.67	69.33 (-14.0)
	T_1 (1.0 mM SA)	81.33	74.33 (-8.6)	79.33	70.33 (-11.3)
	T_2 (1.5 mM SA)	81.00	72.33 (-10.7)	78.67	71.33 (-9.3)
JG 315	T_0 (Control)	81.67	68.00 (-16.7)	81.33	68.33 (-16.0)
	T_1 (1.0 mM SA)	80.33	68.67 (-14.5)	80.67	68.67 (-14.9)
	T_2 (1.5 mM SA)	79.33	69.00 (-13.0)	82.33	71.00 (-13.8)
DCP 92-3	T_0 (Control)	80.00	70.00 (-12.5)	79.67	71.33 (-10.5)
	T_1 (1.0 mM SA)	78.67	69.67 (-11.4)	79.67	71.67 (-10.0)
	T_2 (1.5 mM SA)	79.33	71.33 (-10.1)	81.00	73.67 (-9.1)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		0.49	1.38	0.36	1.03
Treatments (T)		0.42	1.20	0.31	0.89
G X T		0.84	2.40	0.63	1.78

Values in parenthesis indicate % increase (+), or decrease (-) under drought stress condition over normal.

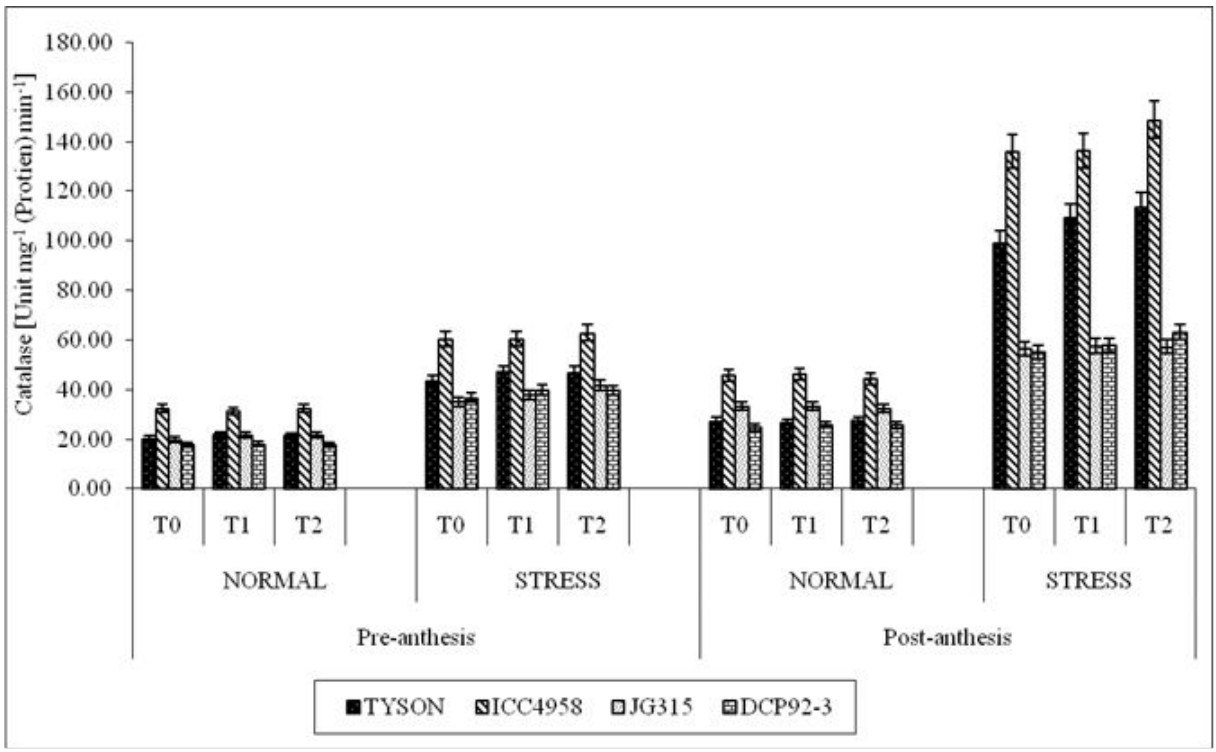

Figure 2. Effect of salicylic acid (SA) on catalase (CAT) activity in four chickpea (*Cicer arietinum* L.) genotypes.

Table 2 Effect of salicylic acid (SA) on proline contents [µg g $^{-1}$ (f.w.)] in chickpea (Cicer arietinum L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	136.00	229.67 (+68.9)	142.33	280.67 (+97.2)
	T_1 (1.0 mM SA)	144.67	262.67 (+81.6)	152.00	285.00 (+87.5)
	T_2 (1.5 mM SA)	134.67	283.33 (+110.4)	149.00	310.67 (+108.5)
ICC 4958	T_0 (Control)	133.00	211.67 (+59.1)	153.33	241.67 (+57.6)
	T_1 (1.0 mM SA)	132.67	214.67 (+61.8)	143.67	235.67 (+64.0)
	T_2 (1.5 mM SA)	137.67	231.67 (+68.3)	152.67	250.67 (+64.2)
JG 315	T_0 (Control)	148.67	165.33 (+11.2)	164.67	199.00 (+20.9)
	T_1 (1.0 mM SA)	164.33	189.33 (+15.2)	175.67	213.33 (+21.4)
	T_2 (1.5 mM SA)	174.33	215.00 (+23.3)	174.33	216.33 (+24.1)
DCP 92-3	T_0 (Control)	127.67	152.33 (+19.3)	138.67	188.00 (+35.6)
	T_1 (1.0 mM SA)	125.67	166.67 (+32.6)	138.00	189.33 (+37.2)
	T_2 (1.5 mM SA)	124.67	184.67 (+48.1)	135.00	193.00 (+43.0)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		1.57	4.46	1.31	3.73
Treatments (T)		1.36	3.86	1.14	3.23
G X T		2.72	7.72	2.27	6.46

Values in parenthesis indicate % increase (+), or decrease (-) under drought stress condition over normal.

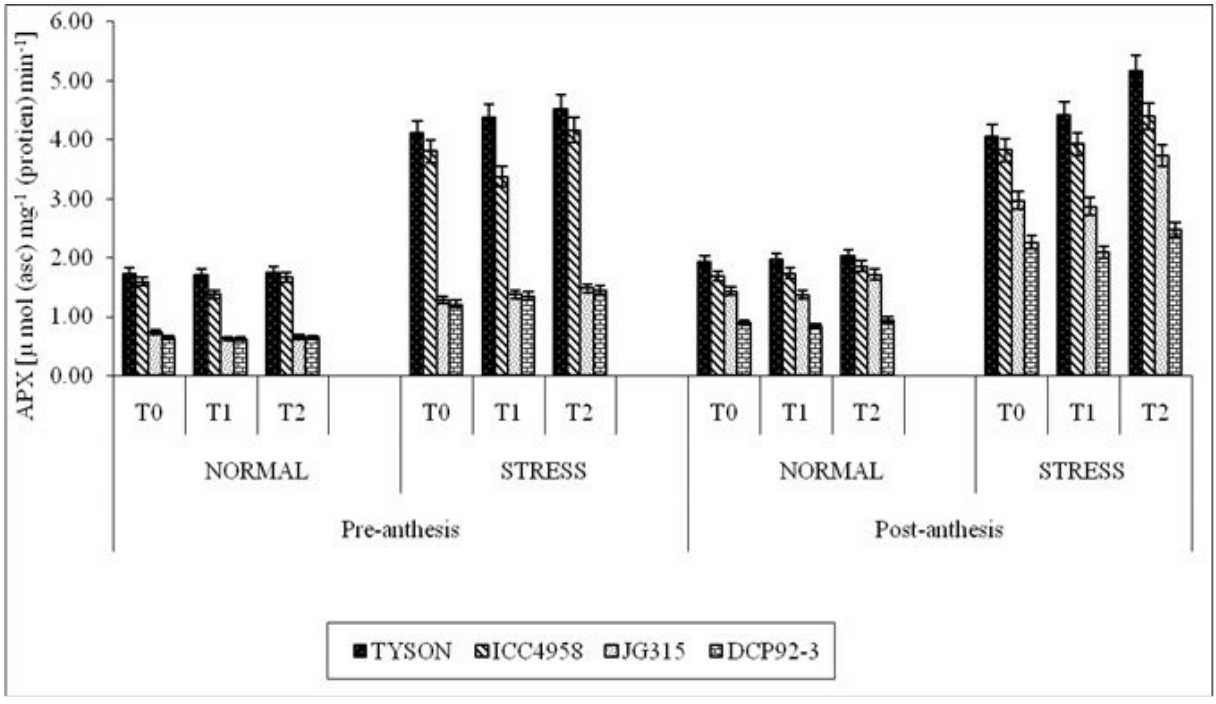

Figure 3. Effect of salicylic acid (SA) on ascorbate peroxidase (APX) activity in four chickpea (*Cicer arietinum* L.) genotypes.

Table 3. Effect of salicylic acid (SA) on malondialdehyde (MDA) contents [nmol g $^{-1}$ (f.w.)] in chickpea (Cicer arietinum L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	1.08	1.54 (+42.6)	1.37	2.28 (+66.3)
	T_1 (1.0 mM SA)	1.08	1.53 (+41.6)	1.39	2.15 (+54.0)
	T_2 (1.5 mM SA)	1.07	1.48 (+39.1)	1.71	2.41 (+41.1)
ICC 4958	T_0 (Control)	1.14	1.51 (+32.7)	1.65	2.49 (+50.4)
	T_1 (1.0 mM SA)	1.24	1.55 (+24.7)	1.68	2.24 (+33.6)
	T_2 (1.5 mM SA)	1.17	1.42 (+21.1)	1.70	2.52 (+48.0)
JG 315	T_0 (Control)	1.19	3.23 (+170.9)	1.08	2.55 (+135.4)
	T_1 (1.0 mM SA)	1.25	3.18 (+154.0)	1.39	3.10 (+123.8)
	T_2 (1.5 mM SA)	1.23	2.73 (+123.1)	1.35	2.94 (+117.8)
DCP 92-3	T_0 (Control)	1.20	3.11 (+158.4)	1.63	2.73 (+66.9)
	T_1 (1.0 mM SA)	1.23	3.04 (+148.1)	1.63	2.72 (+66.7)
	T_2 (1.5 mM SA)	1.19	2.84 (+138.9)	1.60	2.51 (+56.9)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		0.05	0.13	0.32	0.24
Treatments (T)		0.04	0.11	0.28	0.21
G X T		0.08	0.22	0.55	0.41

RESULT AND DISCUSSION

Water relation parameters (Ψ_{leaf} and RWC):

In the present study, RWC and Ψ_{leaf} were low at pre-anthesis drought stress compared to post-anthesis in all the genotypes. Genotypes JG 315 and DCP 92-3 showed lower RWC as compared to Tyson and ICC4958 at pre- anthesis drought, which indicates that drought resistant genotypes, i.e. Tyson and ICC4958 had maintained higher RWC at both the stages. Leaf water potential (Ψ_{leaf}) decreased with declining RWC when drought stress was imposed at pre- and post- anthesis. However, mean Ψ_{leaf} did not fall below (- 2.5 MPa) even at high stress (RWC 68%) (Table1). As the drought progressed, Ψ_{leaf} repeatedly declined to lowest mean value of about -2.5 MPa (fig.2). The results are in close conformity of the fact that SA potentially generates a wide array of metabolic responses in plants and also affects plant water relations (Hayat et al., 2010). The present investigation suggests that exogenous application of SA may help reduce adverse effects of drought in chickpea. Relative water content (RWC) and leaf water potential (Ψ_{leaf}) is useful variables to evaluate the physiological water status of plants (Gonzales and Gonzales-Vilar., 2001). In this experiment the leaf water potential (Ψ_{leaf}) was calculated through regression line, which was formulated between Ψ_{leaf} and RWC in chickpea genotypes ($Y= 0.0951X + 0.8266$; $R^2 = 0.3536$, Y is the RWC and X is the Ψ_{leaf}) (Basu et al., 2007). Leaf water potential decreased sharply at pre-anthesis drought as compared to the post- anthesis drought in all genotypes and all the treatments including control plants. The decrease in Ψ_{leaf} was significantly higher with value falling to -2.4 to -2.5 MPa in JG 315 which was -1.3 to -1.65 MPa in Tyson and ICC 4958 (fig.2). A significant genotypic variation in leaf water potential (Ψ_{leaf}) from -1.3 to -2.5 MPa in Tyson and ICC 4958 (fig.1). Results showed lowest RWC value (68%) in JG 315 and maximum in Tyson (75%) (Table1). Change in RWC with alternate decrease and increase during the period of water stress suggests alteration in internal mechanisms for osmotic adjustment that

help to maintain RWC closer to normal pre stress value and thereby preventing RWC to fall below a critical level especially in Tyson genotype. Therefore, this experiment at post- anthesis imposition of drought stress in genotype Tyson performed better at threshold level of SA @ 1.5 mM by retaining high Ψ_{leaf} and RWC under drought stress. Earlier, SA treatment was found to increase RWC of wheat also (Agarwal et al., 2005).

Antioxidants and antioxidant enzymes

Results show that proline accumulation increases at pre- and post- anthesis stages under drought with higher accumulation at post- anthesis stage. As far as genotypes are concerned, Tyson and ICC 4958 accumulated more proline as compared to JG 315 and DCP92-3 in response to SA over the control. Proline content in SA (@1.5 mM) treated plants under drought stress was maximum [310.67 µg g $^{-1}$(f.w.)] in Tyson and minimum in DCP 92-3 [188. µg g $^{-1}$(f.w.)] (Table 2). Farooq et al., (2009 b) indicated that there was a strong correlation between plant water content and accumulation of compatible solutes (proline and glycine betaine) under drought stress. In the present investigation, SA treatment obviously improved RWC and Ψ_{leaf} with simultaneous and significant increase in proline resulted in osmotic adjustment to a great extent. The critical role of osmolytes accumulation under drought stress is well documented that explicates plant tolerance for dehydration. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells (Anjum et al., 2011).

Both MDA and MP decreased in SA treated plants as compared to control plants and the reduction was highest @1.5 mM SA at both the critical stages of drought induction. Genotypes Tyson and ICC 4958 showed higher response with SA @1.5 mM compared to JG 315 and DCP 92-3. Lipid peroxidation was significantly higher in JG 315 (MDA, 3.23 and MP, 79 %) at pre- anthesis drought stage over control (1.19, 50 %) respectively (Table 3, 4), as increasing ROS level causes oxidative damage to biomolecules such as lipids, proteins and nucleic acids. Reactive oxygen species

Table 4 Effect of salicylic acid (SA) on membrane permeability (MP) in chickpea (*Cicer arietinum* L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	59.33	68.00 (+14.6)	53.33	62.33 (+16.9)
	T_1 (1.0 mM SA)	62.67	70.33 (+12.2)	57.33	66.67 (+16.3)
	T_2 (1.5 mM SA)	66.00	73.33 (+11.1)	59.67	68.67 (+15.1)
ICC 4958	T_0 (Control)	62.67	72.00 (+14.9)	64.00	74.00 (+15.6)
	T_1 (1.0 mM SA)	62.67	71.00 (+13.3)	64.00	73.67 (+15.1)
	T_2 (1.5 mM SA)	62.00	70.33 (+13.4)	62.67	69.33 (+10.6)
JG 315	T_0 (Control)	50.00	79.00 (+58.0)	47.67	67.67 (+42.0)
	T_1 (1.0 mM SA)	46.33	72.33 (+56.1)	45.67	64.00 (+40.1)
	T_2 (1.5 mM SA)	48.50	72.67 (+49.8)	51.00	65.00 (+27.5)
DCP 92-3	T_0 (Control)	48.33	73.00 (+51.0)	49.67	65.67 (+32.2)
	T_1 (1.0 mM SA)	47.00	71.00 (+51.1)	53.00	65.00 (+22.6)
	T_2 (1.5 mM SA)	49.00	71.33 (+45.6)	51.67	61.67 (+19.4)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		0.65	1.84	0.75	2.13
Treatments (T)		0.56	1.60	0.65	1.84
G X T		1.12	3.19	1.30	3.69

Values in parenthesis indicate % increase (+), or decrease (-) under drought stress condition over normal.

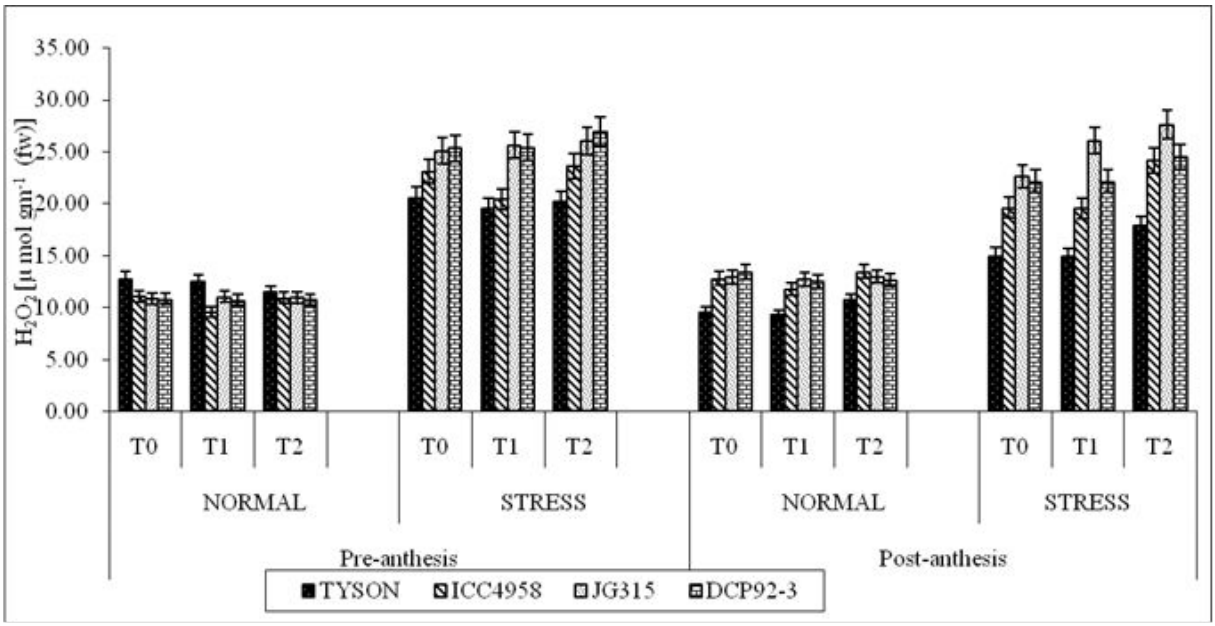

Figure 4. Effect of salicylic acid (SA) on H_2O_2 content of four chickpea (*Cicer arietinum* L.) genotypes.

e.g., peroxides of polyunsaturated fatty acids generate malondialdehyde (MDA) on decomposition, and in many cases MDA is the most abundant individual aldehydic lipid breakdown product (Esterbauer and Cheeseman., 1990) and strikingly malondialdehyde (MDA) is a widely used marker of oxidative lipid injury whose concentration varies in response to biotic and abiotic stress (Davey et al., 2005). Present findings support that the prevention of membrane damage may be related to the induction of antioxidant responses by SA, which protects the plant from oxidative damage

Antioxidant defense system plays vital role in plant's tolerance to stressful conditions. It is obvious from the results (fig. 5) that SA has induced antioxidant concentration particularly ascorbic acid (AA) and antioxidant enzymes activities more than the control plants during the drought period. Results showed that increase in AA contents, SOD, CAT, APX and POX activities may be related to the induction of antioxidant responses that protect the plant from oxidative damage. Superoxide dismutase (SOD) showed maximum response to SA. An increase in activity of APX was observed, though not as marked as for SOD. In plants treated with SA @ 1.5 mM ascorbic acid (AA) content, SOD and POX activity increased significantly at post-anthesis stress condition as compared to pre-anthesis drought stress as well as control plants (fig.5) and (Table 5, 6). This well elucidates that POX should play a more significant role than CAT in detoxifying the produced H_2O_2 since the activity of POX increased, in contrast to that of CAT (Dey et al., 2007). It is well documented that CAT is less efficient than POX in scavenging H_2O_2 because of its low substrate affinity (Erdal and Dumlupinar., 2010).Tyson and ICC4958 showed higher activities than JG 315 and DCP 92-3 at this stage under all the treatments. However, activity was higher with SA @ 1.5 mM under drought condition and genotypes (Tyson and ICC4958) as compared to SA @ 1.0 mM. The increase in SOD activity was higher in

plants treated with SA @1.5mM [255.07 units mg $^{-1}$(protein) min^{-1}] than plants treated with 1.0 mM SA [247.35 units mg $^{-1}$(protein) min^{-1}] in genotype ICC4958 (Table 5) whereas, POX activity was similar with respect to SA treatment but differed in genotypes. Highest values of POX activity were observed at 1.5 mM SA in ICC 4958 [28.97 unit mg $^{-1}$(protein) min^{-1}] (Table 6). Rao et al., (1997) also reported that even though SA was required in hypersensitive cell death, there was no inhibition of CAT or APX, suggesting that SA induced hypersensitive reaction or enhanced H_2O_2 production leading to cell death was not associated with the inhibition of these H_2O_2 scavenging enzymes. Similarly, there was no inhibition of APX activity by SA, thus confirming the results reported by Durner and Klessig., (1995). It is apparent that SA induced redox signal (H_2O_2) leading to increase in antioxidant activity is not linked to inhibition of CAT and APX. The redox signal required for antioxidant enzyme induction is most probably generated by some other process such as plasma membrane linked NADPH oxidase (Kauss and Jeblick., 1996), which may be the cause of H_2O_2 required for redox signaling for antioxidant enzyme induction.

Data support that SA increases the activity of antioxidant enzymes such as SOD, POX and CAT which in turn protect plants against ROS generation and membrane injury or may affect synthesis of other substances having a protective effect on plants under stress. Present findings indicate that exogenous SA elevated the levels of AA under drought stress. SA may regulate the synthesis of AsA under senescence and stress conditions (Huang et al., 2008). A high level of endogenous AsA is essential to maintain the antioxidant capacity that protects plants from oxidative stresses (Zhou et al., 2009). Interestingly, increase in AA content play an important role in preserving APX activity. Exogenous SA might have alleviated the damaging effects of drought stress and provided tolerance to stress in the plant by inducing the antioxidant system.

Table 5 Effect of salicylic acid (SA) on superoxide dismutase (SOD) activity [units mg -1 (protein) min-1] in chickpea (*Cicer arietinum* L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	57.81	203.32 (+251.7)	79.51	243.59 (+206.4)
	T_1 (1.0 mM SA)	62.52	227.57 (+264.0)	81.31	249.13 (+206.4)
	T_2 (1.5 mM SA)	61.96	234.50 (+278.5)	79.59	251.91 (+216.5)
ICC 4958	T_0 (Control)	66.33	187.66 (+182.9)	75.57	215.62 (+185.3)
	T_1 (1.0 mM SA)	65.26	218.59 (+234.9)	75.76	247.35 (+226.5)
	T_2 (1.5 mM SA)	63.33	216.11 (+241.3)	77.13	255.07 (+230.7)
JG 315	T_0 (Control)	64.59	135.23 (+109.4)	68.91	159.22 (+131.1)
	T_1 (1.0 mM SA)	50.05	135.05 (+169.8)	72.37	165.28 (+128.4)
	T_2 (1.5 mM SA)	49.33	135.27 (+174.2)	71.41	165.68 (+132.0)
DCP 92-3	T_0 (Control)	45.21	100.85 (+123.1)	44.84	124.05 (+176.7)
	T_1 (1.0 mM SA)	41.59	104.34 (+150.9)	45.19	124.86 (+176.3)
	T_2 (1.5 mM SA)	45.97	118.94 (+158.7)	45.03	126.30 (+180.5)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		1.02	2.90	0.59	1.67
Treatments (T)		0.88	2.51	0.51	1.45
G X T		1.77	5.02	1.02	2.89

Values in parenthesis indicate % increase (+), or decrease (-) under drought stress condition over normal.

Table 6 Effect of salicylic acid (SA) on peroxidase (POX) activity [units mg $^{-1}$ (protein) min^{-1}] in chickpea (*Cicer aritinum* L.) genotypes.

		Pre-anthesis drought stress		Post- anthesis drought stress	
Genotypes	Treatments	Normal	Stress	Normal	Stress
Tyson	T_0 (Control)	5.97	12.11 (+102.7)	8.32	22.88 (+174.9)
	T_1 (1.0 mM SA)	6.67	16.25 (+143.7)	8.71	25.83 (+196.6)
	T_2 (1.5 mM SA)	7.38	25.52 (+245.8)	9.02	27.88 (+209.2)
ICC 4958	T_0 (Control)	7.56	19.79 (+161.8)	8.19	18.66 (+127.9)
	T_1 (1.0 mM SA)	7.28	24.54 (+237.1)	8.90	22.04 (+147.7)
	T_2 (1.5 mM SA)	8.45	28.97 (+242.9)	8.97	25.88 (+188.7)
JG 315	T_0 (Control)	4.50	8.12 (+80.6)	5.86	10.04 (+71.5)
	T_1 (1.0 mM SA)	4.23	8.04 (+90.2)	5.46	9.53 (+74.5)
	T_2 (1.5 mM SA)	4.14	8.28 (+100.2)	5.70	10.94 (+91.9)
DCP 92-3	T_0 (Control)	3.88	7.73 (+99.6)	5.40	10.58 (+95.9)
	T_1 (1.0 mM SA)	3.95	8.20 (+107.6)	5.76	11.41 (+98.3)
	T_2 (1.5 mM SA)	4.46	9.07 (+103.4)	6.06	13.03 (+115.1)
		SEm±	CD at 5%	SEm±	CD at 5%
Genotypes (G)		0.15	0.44	0.96	0.72
Treatments (T)		0.13	0.38	0.83	0.62
G X T		0.27	0.76	1.67	1.25

Values in parenthesis indicate % increase (+), or decrease (-) under drought stress condition over normal.

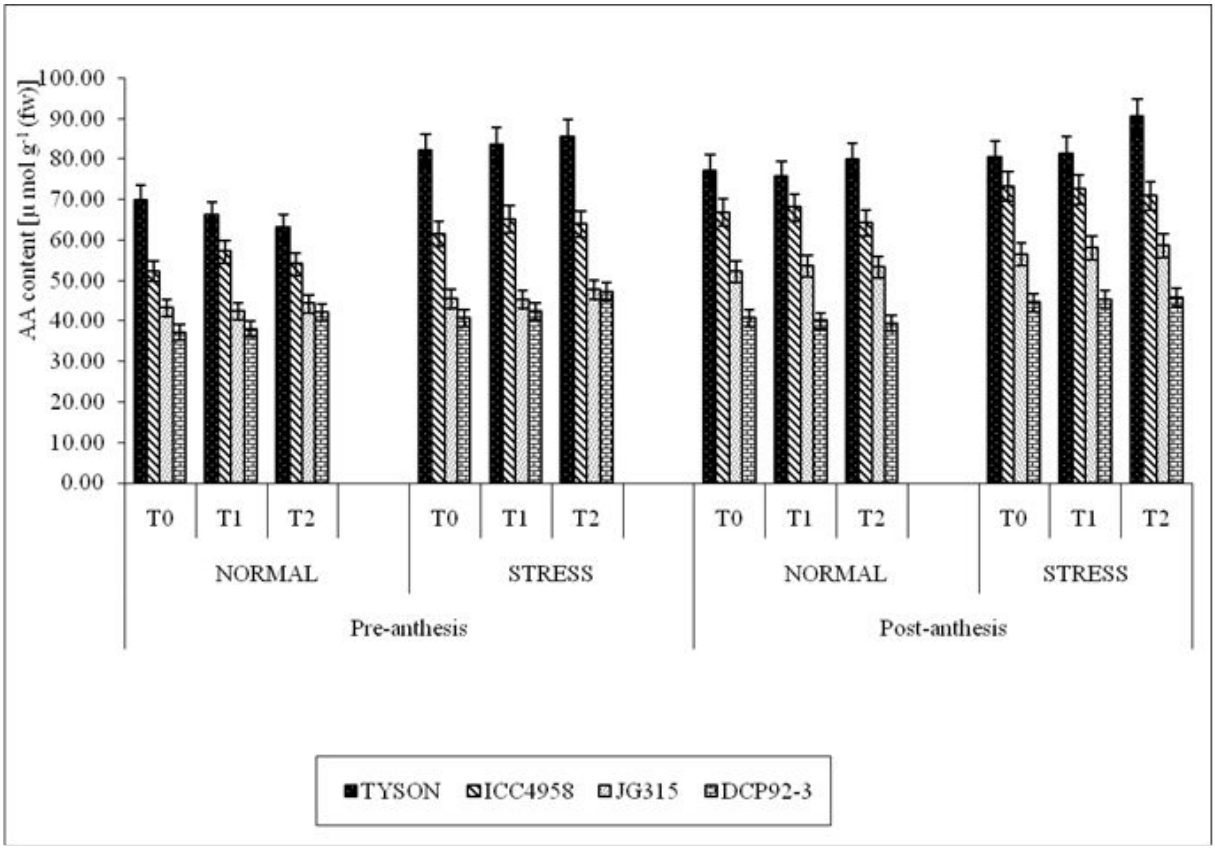

Figure 5. Effect of salicylic acid (SA) on ascorbic acid (AA) content in four chickpea (*Cicer aritinum* L.) genotypes.

Besides these, SA helps in regulating the stomatal functioning and maintains the water status of plant under water stress, which in turn maintains various physiological processes needed for increased growth and yield. Genotype Tyson and ICC 4958 substantiated relatively more tolerant to drought than DCP 92-3 and JG 315. However, the response of exogenously applied SA was more pronounced in Tyson and ICC4958 than its counterpart.

Overall observations point out that accumulation of osmolytes allows additional water to be taken up from the environment reducing the immediate effect of water shortage within the plant so as to help stabilizing protein tertiary structure and cells. The results of present study are in agreement with Umebese et al., (2009) who reported that during water deficit SA enhanced proline production in stressed tomato and amaranthus plant. Therefore, it is evident that seed soaking treatment of SA increased antioxidant activity which decreased oxidative stress and increased proline and ascorbic acid (AsA) content in order to maintain RWC in leaves.

CONCLUSION

Based on analyses of four chickpea genotypes and two salicylic acid treatments, there were substantial variation in tolerance to drought within chickpea genotypes. A perusal of the results shows that drought tolerance genotypes have higher RWC, ascorbic acid content, proline accumulation, enzymatic activities such as SOD, APX, CAT, POX and lower level of MDA, MP, and H_2O_2 in comparison to drought susceptible genotypes. These parameters showed a considerable variability and heritability under drought stress conditions. The performance of chickpea genotypes at different levels of SA treatments at two different drought situations (pre- and post- anthesis stages of development), it is concluded that pre- anthesis stage was most sensitive. The level of SA @ 1.5 mM in Tyson had high osmotic adjustment capacity under drought stress. The relationship amongst water relation, antioxidant and antioxidant enzymes and other related parameters could be instrumental in predicting the drought tolerance of chickpea genotypes.

Meagre information is available regarding genotypic variation for drought tolerance in chickpea. Henceforth, this study could help to understand some adaptive mechanisms developed by chickpea genotypes along with role of salicylic acid in contributing useful identified traits for chickpea breeding programme.

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