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EFFECT OF SALICYLIC ACID ON GROWTH AND METABOLISM OF CHICKPEA (*CICER ARIETINUM* L.) UNDER DROUGHT STRESS

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SUMMARY

Four chickpea genotypes (Tyson, ICC 4958, JG 315 and DCP 92-3) were treated with 1.0 mM and 1.5 mM salicylic acid (SA) and subjected to pre- and post flowering drought stress to analyse its influence on nitrate reductase (NR) activity, relative water content (RWC), proline and antioxidant enzymes activity (superoxide dismutase and peroxidase). Leaf RWC significantly reduced during stress at both the growth stages and ranged between 71.67-74.43% (unstressed) and 67.96-71.67% (stressed), whereas in 1.5 mM SA treated plants leaf RWC increased comparable to the control (unstressed plant). NR activity significantly reduced under stress at the post anthesis stage of growth but was maintained higher in 1.5 mM SA treated plants in all the four genotypes studied. On the other hand, activities of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POX) were upregulated by drought stress and interestingly further enhanced by 1.5 mM SA treatment. The response of SA (1.5 mM) was relatively more in ICC 4958 and Tyson cultivars of chickpea. Hence, results signify the role of SA in protecting metabolic activity along with regulating the drought response of plants.

Key words: **Antioxidant enzymes, nitrate reductase, pre- and Post- anthesis, salicylic acid**

INTRODUCTION

Drought, or more generally, limited water availability is the main factor limiting crop production. Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata, and decrease in cell enlargement and growth. The effect of water deficit varies with the variety, degree and duration of stress and the growth stage of the plant (Adejare and Umebese 2007). 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).

Plants respond to water deficit and acclimate to drought stress through various physiological and biochemical changes (Farooq *et al*. 2009). It is well known that the antioxidant systems of plant act for tolerance mechanisms against drought stress that induces oxidative stress in various plants, in which reactive oxygen species, such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2) and alkoxyl radical ($RO^{\cdot}$), are produced (Munne´-Bosch and Penuelas 2003). Enhanced superoxide dismutase (SOD) and peroxidase (POX) activities have been associated with induced resistance of plants to drought stress (Barlets 2001). Activities of both are involved in superoxide radical and hydrogen peroxide scavenging.

Nitrate reductase (NR) activity is vital for the

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metabolic and physiological status of plants and can be used as a biomarker of plant stress including drought. NR activity decreases in plants exposed to water limitation because of a lower flux of nitrate from the soil to the root (Azco´n *et al.* 1996), therefore under the condition, the activity needs being protected by some means.

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 (Al-Hakimi 2006), 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.

Comparatively meagre work is on record regarding role of SA on drought tolerance in chickpea. However, precise mechanism by which SA mediates such processes has not been completely elucidated. The main objective of this work is to study the protective impact of SA on nitrate reductase activity in view of its vital position in plant metabolism from side to side strengthening the antioxidant system i.e. enzymatic (SOD and POX) in the regulation of growth and water content of chickpea genotypes.

MATERIALS AND METHODS

The seeds of *Cicer arietinum* L. genotypes (Tyson, ICC 4958, JG 315 and DCP 92-3) were obtained from the Indian Institute of Pulse Research (IIPR) Kanpur, India through material transfer agreement. Seeds 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). Thereafter, 10 seeds of each genotype for each treatment were soaked for 6 h in water (0 mM SA) as control (T_0) and in 1.0 mM SA (T_1) and 1.5 mM SA (T_2) before sowing in pots filled with farm soil having 12-14% moisture.

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

Relative water content (RWC) in the leaves was determined using the method of Weatherley (1950). Proline content was determined by the method of Bates *et al*. (1973). Nitrate reductase (NR) activity (EC 1.6.6.1) of green leaves was determined by the method of Srivastava *et al*. (1974). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Dhindsa *et al*. 1981). 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).

Samples for biochemical estimations were collected in 3 replicates and each replicate/sample was assayed twice. The design of the experiment was CRD, and 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 following Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The results indicate that RWC was low at pre-anthesis drought stress compared to post- anthesis in all the genotypes. Genotypes JG 315 and DCP 92-3 showed lower RWC compared with Tyson and ICC 4958 at pre-anthesis drought which maintained higher RWC at both the stages. RWC was in the range of 71.6-74.4 (unstressed) and 67.9-71.6 (stressed), whereas in SA treated plants (1.5 mM) leaf RWC increased closer to control (unstressed plant). Lowest and maximum RWC was maintained under stress in genotype JG 315 and Tyson, respectively (Table 1). It shows that SA potentially generates a wide array of metabolic responses in plants and also effective for plant water relations (Hayat *et al*. 2010). Exogenous application of SA may

help to reduce adverse effects of drought in chickpea. Change in RWC with alternate decrease and increase during the period of water stress suggests alteration in internal mechanisms for osmotic adjustments that helped to maintain RWC closer to normal pre stress value and thereby preventing RWC to fall below a critical level especially in Tyson genotype.

Water deficit reduced NR activity significantly ($p = 0.05$) at both the stages (i.e. pre- and post-anthesis) and it was more pronounced at the post- anthesis of all four chickpea genotypes. Maximum reduction was noticed in DCP 92-3 (55.0%) over control. SA treatment @1.5 mM was more effective than 1.0 mM in improving NR activity (Table 1). Nitrate reductase (NR) activity inhibited at both the stages of development in chickpea stressed plants. Since water content of plants is higher at the pre-anthesis stage than the post anthesis stage, a further decrease in water content as a result of deficit treatment could have reduced NR activity markedly at the post - anthesis stage in control as well as stressed plants in chickpea genotypes (Forbes and Watson 1992). Results of this study clearly indicate that SA protected nitrate reductase activity coupled with high RWC under stress (Table 1) in all the four genotypes. Singh and Usha (2003) reported that the SA treatment, under water stress, protected nitrate reductase (NR) activity and maintained the protein and nitrogen content of leaves compared to water sufficient seedlings especially at 3 mM SA concentration. There is evidence that SA increases the activity of NR in the presence of NO_3, but water limitation creates the lower flux of nitrate from the soil to the root. Hence, under the situation SA helps to uphold water relation in plant to maintain nitrate flux from soil to root (Azco´n and Go´mez 1996) and protect NR enzyme against proteinase (Rane *et al*. 1995).

Drought stress at pre and post- flowering stages resulted in marked reduction in leaf area in all the genotypes and differed significantly by SA treatment. Genotypes JG315 and DCP92-3 attained more leaf area than ICC 4958 and Tyson in stressed and non stressed conditions (Table 2). Stress treatment also caused marked decrease in total dry weight of Tyson (51.6%) followed by ICC 4958 (49.4%) at the pre- flowering stage (Table 2). However, total dry weight of plant was improved by SA treatment. The results suggest that crops required to adjust their transpiring surface through reducing leaf size. Thus, drought tolerance in this study may be attributed to reduced transpiration and reducing leaf area expansion of genotypes under drought stress. Plasticity in leaf area has been suggested as an important characteristic in tolerant crops under drought stress (Blum 1996). In this study, SA application improved growth characteristics (leaf area of green leaves and total dry weight), which is in close conformity with Hussien *et al*. (2007).

Proline concentration increased at pre- and post-flowering stages under drought and higher accumulation was recorded at post- flowering stage. Among the genotypes, Tyson and ICC 4958 accumulated more proline as compared with JG 315 and DCP92-3 in response to SA over the control. Highest proline content was observed in plants treated with SA @1.5 mM under drought stress in Tyson and minimum in DCP 92-3 (Table 2).

Superoxide dismutase (SOD) and peroxidase (POX) are important antioxidant enzymes and protect the plants from oxidative damage (Erdal and Dumlupinar 2010a, b). SOD and POX activity increased significantly under 1.5 mM SA treatment at post- anthesis stress condition (Fig. 1). Tyson and ICC4958 showed higher activities than JG 315 and DCP 92-3 at this stage and under all the treatments. SA @1.0 and 1.5 mM also significantly increased antioxidant enzymatic activities and it was higher with 1.5 mM SA under drought condition in genotypes (Tyson and ICC4958) as compared to 1.0 mM SA. The increase in SOD activity was higher @1.5mM SA (3.3 fold) under stress over normal in genotype ICC4958 (Fig.1) 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 (2.8 fold) under stress over normal (Fig. 1).

The findings show that SA increases the activity of antioxidant enzymes such as SOD and POX, 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

Table 1. Effect of salicylic acid (SA) on relative water content (RWC) and nitrate reductase (NR) activity in four chickpea (*Cicer arietinum* L.) genotypes grown under drought stress imposed at pre- and post- flowering stage of development at 58 and 73 DAS, respectively.

		RWC [%]				NR activity (µmol NO_2 h^{-1} g^{-1} fw)			
		EDS		LDS		EDS		LDS	
G	T	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Tyson	T_0	73.33 (58.91)	70.33 (57.00)	73.67 (59.12)	69.33 (56.38)	0.433	0.350 (-19.1)	0.290	0.183 (-36.8)
	T_1	74.33 (59.68)	70.92 (57.21)	74.33 (59.55)	69.70 (56.59)	0.435	0.350 (-19.6)	0.290	0.187 (-35.6)
	T_2	74.43 (59.56)	71.17 (57.63)	75.00 (59.99)	71.00 (59.16)	0.422	0.377 (-10.7)	0.427	0.277 (-35.2)
ICC 4958	T_0	74.00 (59.34)	70.67 (57.21)	74.00 (59.34)	70.33 (57.00)	0.450	0.343 (-23.7)	0.370	0.253 (-31.5)
	T_1	74.00 (59.34)	71.17 (57.63)	73.67 (59.11)	71.17 (57.63)	0.467	0.357 (-23.6)	0.350	0.267 (-23.8)
	T_2	73.67 (59.13)	71.67 (60.47)	73.67 (59.13)	71.00 (57.42)	0.487	0.410 (-15.8)	0.433	0.320 (-26.2)
JG 315	T_0	72.67 (58.48)	67.96 (55.55)	72.67 (58.48)	69.00 (56.17)	0.391	0.197 (-49.7)	0.280	0.200 (-28.6)
	T_1	72.17 (58.39)	68.07 (55.55)	72.50 (58.48)	69.00 (56.17)	0.450	0.240 (-46.7)	0.273	0.206 (-24.6)
	T_2	72.42 (58.43)	68.33 (55.76)	72.75 (58.48)	69.33 (56.38)	0.430	0.260 (-39.5)	0.277	0.223 (-19.3)
DCP 92-3	T_0	72.33 (58.26)	68.33 (55.76)	72.33 (58.26)	69.00 (56.17)	0.407	0.183 (-54.9)	0.323	0.163 (-49.5)
	T_1	71.67 (57.84)	68.53 (55.78)	71.67 (57.84)	69.27 (56.38)	0.400	0.180 (-55.0)	0.323	0.183 (-43.3)
	T_2	72.00 (58.05)	68.77 (55.96)	72.67 (58.48)	70.00 (56.79)	0.430	0.207 (-51.9)	0.350	0.203 (-41.9)
	Mean	73.08	69.66	73.24	69.84	0.433	0.288	0.332	0.222
		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
G		0.18	0.51	0.19	0.54	0.007	0.019	0.007	0.020
C		0.13	0.36	0.13	0.38	0.005	0.014	0.005	0.014
T		0.15	0.44	0.16	0.47	0.006	0.017	0.006	0.018
G X C		0.25	0.72	0.27	NS	0.010	0.027	0.010	0.029
G X T		0.31	NS	0.33	NS	0.012	NS	0.012	0.035
C X T		0.22	0.62	0.23	NS	0.008	NS	0.009	NS
G X C X T		0.44	1.24	0.47	NS	0.017	NS	0.018	0.050

G= Genotype, C= Condition (normal and stress), T= SA treatments, EDS= early drought stress or pre- flowering drought stress, LDS= late drought stress or post- flowering drought stress. DAS= days after sowing, T_0 (0.0 mM SA), T_1 (1.0 mM SA), T_2 (1.5 mM SA). For RWC values expressed in parenthesis are angular transformed, for NRA values in parenthesis indicate % decrease (-) under drought stress over normal.

Table 2. Effect of salicylic acid (SA) on leaf area, total dry weight and proline in four chickpea (*Cicer arietinum* L.) genotypes grown under drought stress imposed at pre- and post- flowering stage of development at 58 and 73 DAS, respectively.

		Leaf area (cm^2 $plant^{-1}$)				Total dry weight (g $plant^{-1}$)				Proline (µg g^{-1} fw)			
		EDS		LDS		EDS		LDS		EDS		LDS	
G	T	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Tyson	T_0	171.60	97.76 (-43.0)	194.48	107.61 (-44.7)	3.82	2.06 (-46.1)	6.29	3.82 (-39.3)	136.00	229.67 (+68.9)	142.33	280.67 (+97.2)
	T_1	159.28	90.73 (-43.0)	190.96	113.24 (-40.7)	3.55	1.91 (-46.1)	6.27	4.01 (-36.0)	144.67	262.67 (+81.6)	152.00	285.00 (+87.5)
	T_2	159.28	80.88 (-49.2)	205.92	106.91 (-48.1)	3.55	1.72 (-51.6)	6.69	3.93 (-41.3)	134.67	283.33 (+110.4)	149.00	310.67 (+108.5)
ICC 4958	T_0	147.52	83.57 (-43.4)	204.05	100.28 (-50.9)	2.37	1.26 (-46.9)	5.24	3.15 (-39.8)	133.00	211.67 (+59.1)	153.33	241.67 (+57.6)
	T_1	145.75	83.57 (-42.7)	207.58	98.83 (-52.4)	2.35	1.26 (-46.2)	5.32	3.15 (-40.7)	132.67	214.67 (+61.8)	143.67	235.67 (+64.0)
	T_2	161.65	87.20 (-46.1)	207.58	98.10 (-52.7)	2.59	1.31 (-49.4)	5.32	3.17 (-40.3)	137.67	231.67 (+68.3)	152.67	250.67 (+64.2)
JG 315	T_0	155.17	122.43 (-21.1)	213.03	154.77 (-27.3)	2.54	1.93 (-24.0)	4.97	4.26 (-14.3)	148.67	165.33 (+11.2)	164.67	199.00 (+20.9)
	T_1	155.17	113.96 (-26.6)	217.41	159.39 (-26.7)	2.55	2.27 (-10.8)	5.07	4.36 (-14.0)	164.33	189.33 (+15.2)	175.67	213.33 (+21.4)
	T_2	164.38	119.35 (-27.4)	216.98	156.31 (-28.0)	2.69	2.37 (-11.9)	5.05	4.33 (-14.3)	174.33	215.00 (+23.3)	174.33	216.33 (+24.1)
DCP 92-3	T_0	170.24	130.53 (-23.3)	234.08	164.12 (-29.9)	3.13	2.02 (-35.6)	6.38	4.09 (-36.0)	127.67	152.33 (+19.3)	138.67	188.00 (+35.6)
	T_1	161.37	118.32 (-26.7)	233.19	155.72 (-33.2)	2.98	1.84 (-38.1)	6.38	3.95 (-38.1)	125.67	166.67 (+32.6)	138.00	189.33 (+37.2)
	T_2	164.03	120.61 (-26.5)	234.97	154.19 (-34.4)	3.02	1.88 (-37.9)	6.45	3.97 (-38.4)	124.67	184.67 (+48.1)	135.00	193.00 (+43.0)
	Mean	159.62	104.08	213.35	130.79	2.93	1.82	5.79	3.85	140.33	208.92	151.61	233.61
		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%
G		4.05	11.52	4.09	11.62	0.07	0.20	0.09	0.24	1.57	4.46	1.31	3.73
C		2.87	8.15	2.89	8.22	0.05	0.14	0.06	0.17	1.11	3.15	0.93	2.64
T		3.51	NS	3.54	NS	0.06	NS	0.07	NS	1.36	3.86	1.14	3.23
G X C		5.73	16.29	5.78	16.44	0.10	0.28	0.12	0.34	2.22	6.30	1.86	5.27
G X T		7.02	NS	7.08	NS	0.12	NS	0.15	NS	2.72	7.72	2.27	6.46
C X T		4.96	NS	5.01	NS	0.09	NS	0.10	NS	1.92	5.46	1.61	4.57
G X C X T		9.93	NS	10.01	NS	0.17	NS	0.21	NS	3.84	10.92	3.21	9.14

G= Genotype, C= Condition (normal and stress), T= SA treatments, EDS= early drought stress or pre- flowering drought stress, LDS= late drought stress or post-flowering drought stress. DAS= days after sowing, T_0 (0.0 mM SA), T_1 (1.0 mM SA), T_2 (1.5 mM SA). Values expressed in parenthesis indicate % increase (+) and decrease (-) under drought stress over normal.

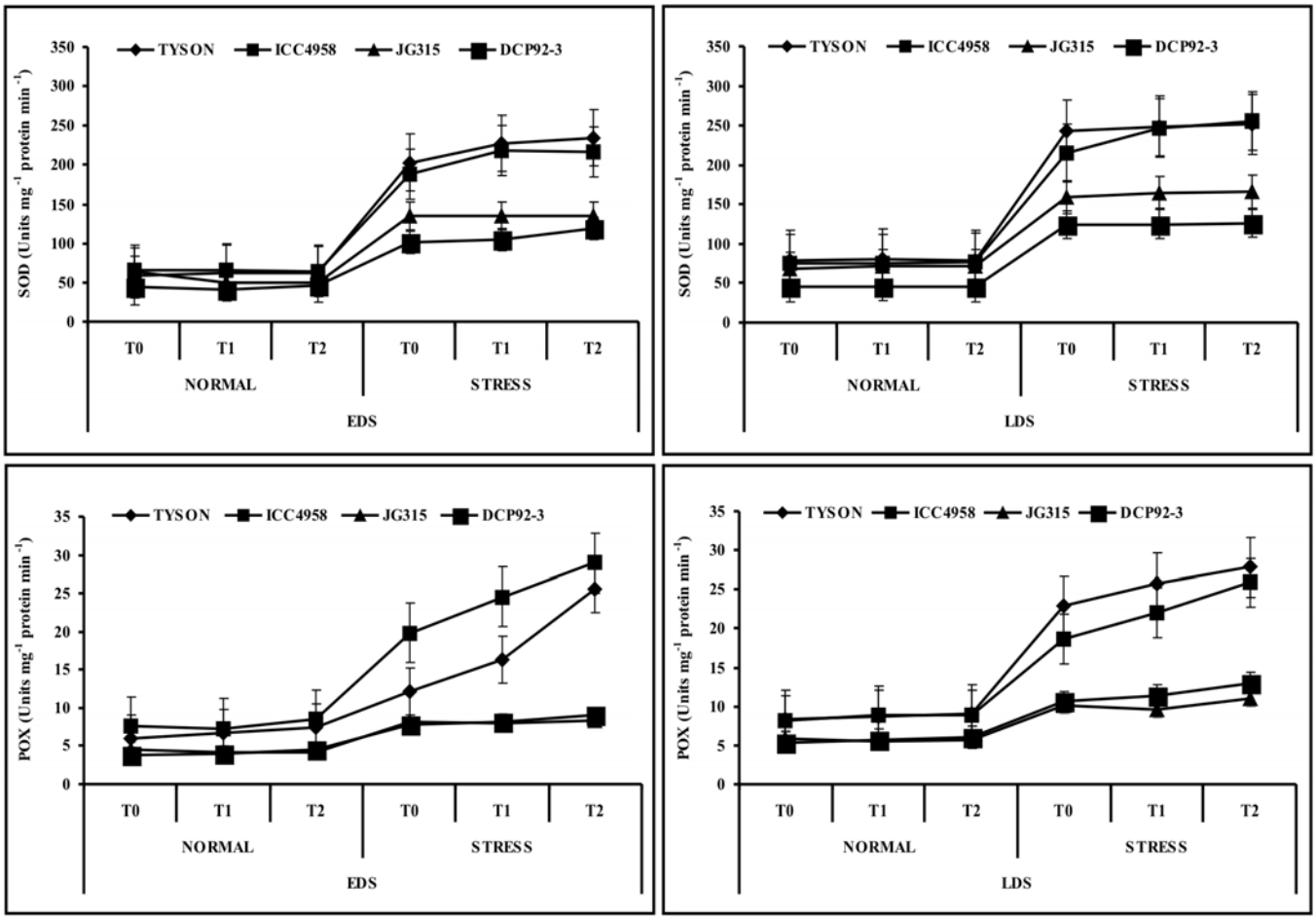

Fig. 1. Effect of salicylic acid (SA) on superoxide dismutase (SOD) activity and peroxidase (POX) activity in four chickpea (*Cicer arietinum* L.) genotypes under drought stress imposed at pre (i.e. EDS) and post - flowering (i.e. LDS) stage of development at 58 and 73 DAS, respectively.

stress. Besides these, SA might have helped in regulating the stomatal functioning and water status of plant under water stress, which in turn maintained various physiological processes needed for increased growth and yield. Genotype Tyson and ICC 4958 were found more tolerant to drought than DCP 92-3 and JG 315. However, the response of seed pre- treatment with SA was more pronounced in Tyson and ICC4958 than others. Therefore, seed soaking treatment of SA is beneficial to protect NR activity by improved antioxidant system in organizing overall physiology under drought.

CONCLUSION

This study concludes that salicylic acid can improve the drought tolerance in chickpea by improving RWC and nitrate reductase activity and growth under drought stress environment. Based on the performance of chickpea genotypes at different SA treatments under pre- and post- flowering drought, it is suggested that pre-flowering stage was most sensitive to drought and SA @ 1.5 mM can play an important role in maintaining high osmotic adjustment capacity under drought stress.

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