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Original Article

Role of salicylic acid and ascorbic acid in alleviating the harmful effects of water stress in Maize (*Zea mays* L.)

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Abstract

Water scarcity is threatening productivity of crops throughout the globe. Different osmoprotectants such as salicylic acid and ascorbic acid have potential to mitigate the harmful effects of water deficit. Current study was conducted to examine the possible role of salicylic acid and ascorbic acid individually and in combination in improving the yield and yield components of maize (*Zea mays* L.) exposed to drought at different growth stages. Drought was imposed by skipping irrigation at two water sensitive crop growth stages i.e. 6 fully expanded leaves (V_6 as per Feekes scale) and initiation of silking (R_1 as per Feekes scale). Water deficit significantly decreased cob diameter (19.2%), cob weight (13.8%), grain rows per cob (14.4%), cob length (12.8%), 1000-grain weight (6.2%), stover yield (17.7%), grain yield (10.6%), biological yield (8.2) as compared to normal irrigation. Drought imposed at R_1 (as per Feekes scale) was more lethal in terms of reduction in different yield and yield components including 1000-grain weight, grain yield and biological yield by 0.2, 6.9 and 0.9 %, respectively. Exogenous application of combination of salicylic acid + ascorbic acid @ 0.5mM each + tween 20 (0.1%) applied at V_6 stage (as per Feekes scale) was more effective in mitigating the harmful effects of water deficit by improving cob diameter (6%) biological yield (1%) and grain yield of maize (6.9%) as compared to drought imposed at R_1(as per Feekes scale).

Keywords: Ascorbic acid, Drought, Maize, Salicylic acid, Yield

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Introduction

Food security is global concern including African and Asian continents (Aziz et al., 2015). Different abiotic stresses are hampering crop productivity (Zhu, 2001). Depleting water resources is indicative of the fact that water scarcity is one of the most importance factor limiting growth and yield of different crops (Golbashy et al., 2010). Harmful effects of drought are visible at any stage of crop growth (Farooq et al., 2009). Drought results in decline in leaf area, root proliferation, stem extension, leaf chlorophyll contents, chlorophyll fluorescence and water use efficiency due to disturbance in metabolism, ionic balance, solute accumulation and enzyme activity in plants (Guo-Hui, 2012). Nutrient uptake in plant is

more closely linked with water availability due to effect on transpiration rate and membrane permeability of plants (Farooq et al., 2009).
Maize (*Zea mays* L.) is the one of most important cereal crops which is more sensitive to water scarcity as compared to other cereal crops excluding barley (Bänziger and Araus, 2007). It is significant staple food globally (Cakmak, 2005). Different growth stages of maize are severely affected by drought leading to extension in anthesis-silking-duration, lower chlorophyll contents, less photosynthesis and thus lower yield (Aslam et al., 2013). Different attractive strategies are getting popular for alleviating the harmful effect of water deficit including crop breeding for drought tolerance, soil and crop management practices (Khaliq et al., 2004) and use of osmoprotectants (Hussain et al., 2008). Environment friendly and economic solution is very important for sustainable agriculture (Aziz et al., 2018a). Among different osmoprotectants kinetin, salicylic acid and glycine betaine are famous for induction of drought tolerance in different plants (Karlidag et al., 2009; Elwan and El-Hamahmy, 2009) though variability is reported in improving photosynthetic rates, plant dry matter production and leaf area of different crop plants exposed to water deficit (Khan et al., 2003). Salicylic acid (o-hydroxybenzoic acid) has ability to protect plants against abiotic stress (Gunes et al., 2007) by controlling multiple physiological and metabolic responses in plants. It helps to control plant resistance against drought stress (Ahmad et al., 2013). The effectiveness of foliar application of salicylic acid on various growth, physiological, biochemical and yield traits of different crops against different biotic and abiotic stresses have been reported by various researchers (Hayat et al., 2010; Qaiser et al., 2010) Ascorbic acid is a co-factor for numerous enzymes and control the phytohormones mediates signaling processes during transition from vegetative to reproductive phase, cell growth, senescence, mitosis in plants, final stage of development (Barth et al., 2006). Although there are lot of information regarding efficacy of salicylic acid (SA) and ascorbic acid (AsA) individually in mitigating the damaging effects of drought but limited reports are available to explore the possibility of combined application of salicylic acid and ascrobic acid in combating the harmful effect of water deficit in maize. Therefore, current study was carried out to investiate the efficacy of combined application of salicylic acid and ascorbic acid regarding alleviation of water deficit in maize.

Material and Methods

The prescribed study was carried out at the research farm of Muhammad Nawaz Shareef University of Agriculture, Multan (30.1575^o N and 71.5249^o E), during autumn season 2017. Experimental soil was well drained, loam with pH 8.2. Maize hybrid " DK 6789" was sown in rows at 75 cm apart. Plant to plant distance was kept 20cm. Seed rate was kept 30kg ha^{-1}. Experiment was carried out in Randomized Complete Block Design with split-split plot arrangement having three replications. Different water regimes including normal irrigation and drought stress were kept in main plot while crop growth stages i.e. V_6 and R_1 (as per Feekes scale) in sub-plot and exogenous application of different osmoprotectants individually and in combination in sub-sub plot. Different osmoprotectants which were assessed for alleviating the harmful effects of water deficit included; C_0=control (distilled water spray) C_1=SA (1mM) + tween 20 (0.1 %), C_2=AsA (1mM) + tween 20 (0.1 %), C_3= SA (0.5mM) + AsA (0.5mM) + tween 20 (0.1%). Drought stress was imposed by skipping irrigation at different growth stages viz. 6 fully expended leaves (V_6 as per Feekes scale) and at initiation of silking (R_1 as per Feekes scale). The recommended dose of NPK (200:125:125 kg ha^{-1}) was applied. All P_2O_5 and K_2O was applied at sowing time by using diammonium phospate and sulphate of potash as sources. Nitrogen was applied in three equal splits ie. sowing, knee height and tasseling stage. All other agronomic paractices were kept uniform thorugh out the conduct of experiment. Net plot size was kept 5 m×3m. Different agronomic and yield traits were recorded throughout the conduct of experiment by using standard procedures.
As experiment was laid out in RCBD with split-split plot arrangement, data collected was subjected to ANOVA technique. Difference between the treatment means was tested using Tukey's Honest Significance Difference (HSD) test at 5% probability level (Steel et al., 1997).

Results and Discussion

Drought stress significantly decreased plant height at maturity (Table 1). Under drought conditions plant height at maturity was recorded less as compared to that achieved under normal irrigated condition.

Table 1: Effect of sole and combined application of salicylic and ascorbic acid on plant height at maturity and number of leaves per plant of maize exposed to normal irrigation and drought condition at vegetative and reproductive crop growth stages.

	Plant height (cm) at maturity					Number of leaves per plant				
	Normal irrigation		Drought			Normal irrigation		Drought		
Treatments	V_6	R_1	V_6	R_1	Means	V_6	R_1	V_6	R_1	Means
C_0	165.09 cdef	169.40 abc	158.90 f	162.33 def	163.9 B	12.33	13.00	11.33	11.33	12.00 B
C_1	175.83 a	174.33 a	159.20 ef	166.67 bcd	169.01A	15.00	14.00	12.33	13.33	13.67 A
C_2	173.33 ab	173.67 a	161.67 def	166.00 cde	168.67A	14.00	14.68	12.00	13.00	13.42 A
C_3	171.33 abc	174.67 a	165.00 cdef	166.67 bcd	169.42A	14.33	14.68	12.00	12.68	13.42 A
Means of water regimes	Normal irrigation = 172.21 A		Drought=163.30 B			Normal irrigation = 14.00 A		Drought= 12.25 B		
Means of crop growth stages	V_6= 166.30 B		R_1= 169.22 A			V_6=12.92		R_1=13.33		

C_0=Distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= Water deficit,V_6= (6 fully expanded leaves), R_1= Initiation of silking stage

Maximum plant height at maturity (172.21 cm) was recorded under normal irrigation condition which was 5.71% more than achieved under drought conditions. Moreover, drought imposed at V_6 stage resulted in reduced height (166.30 cm) as compared to plant height (169.22 cm) attained when drought was imposed at R_1 stage. Plant height (166.6 cm) was recorded more due to foliar application of combination of SA and AsA when drought was imposed at R_1 stage as compared to V_6 stage. These results are in agreement with finding reported by Cakir (2004) that vegetative stage was more effected by drought stress as compare to reproductive stage.

Both water regimes influenced significantly number of leaves per plant (LP) (Table 1). LP (14) were maximum under normal irrigation condition while under drought condition these were 12.25. Both crop growth stages were recorded equally sensitive for effecting LP. Exogenous application of different osmoprotectants significantly influenced LP. Although maximum LP (13.67) were recorded with foliar application of SA @ 0.1mM +Tween 20 (0.1%) but statistically same number of leaves per plant were observed with foliar application of ascorbic acid (sole application) and combination of SA and AsA. Minimum LP (12) was recorded in case of C_0 (distilled water spray). These results are in harmony with reports of Anjum et al. (2011) as they also recorded less LP exposed to drought as compared to normal irrigation. Interaction between osmoprotectants and crop growth stages was significant regarding number of cobs per plant (CP) (Table 2). CP were maximum (2) when AsA (1mM) + Tween 20 (0.1%) was applied at initiation of silking stage (R_1 as per Feekes scale) as compared to application at vegetative stage (V_6 as per Feekes scale) where CP (1.16) were lowest. Statistically same CP (1.16) were recorded when distilled water were applied at 6 fully expanded leaves stage. Moreover, CP achieved under normal irrigated condition were 11.11% greater than under drought stress condition.

The results are different from results reported by Cakir (2004) as they found that CP were reduced when drought stress was imposed at any water sensitive growth stage of maize crop.

Statistically different cob diameter (CD) was noticed under different water regimes (Table 2). Maximum CD (14.00 cm) was recorded under normal irrigation regime while under drought stress cobs diameter was 16% less. Initiation of silking stage of maize was recorded more water sensitive regarding decrease in cobs diameter. Cob diameter was significantly affected by foliar application of different osmoprotectants. Maximum CD (13.50 cm) was recorded in case of foliar application of combination of salicylic and ascorbic acid while it was at par with sole application of both osmoprotectants. Minimum CD (11.67 cm) was recorded with distilled water spray. These results are in accordance with the finding reported by Khodarahmpour and Hamidi (2012) as they reported that water deficit stress significantly affected CD. They also reported silking stage as more water sensitive regarding reduction in CD as compared to vegetative stage.

Table 2: Effect of sole and combined application of salicylic and ascorbic acid on number of cobs per plant and cob diameter of maize exposed to normal irrigation and drought condition at vegetative and reproductive growth crop stages.

	Cob diameter (cm)					**Number of cobs per plant**		**Means**
	Normal irrigation		**Drought**		**Means**			
Treatments	V_6	R_1	V_6	R_1		V_6	R_1	
C_0	12.00	13.00	11.00	10.67	11.67 B	1.16 c	1.67 abc	1.41
C_1	14.67	14.00	12.67	11.33	13.17 A	1.83 ab	1.33 bc	1.58
C_2	14.67	13.67	12.33	12.00	13.17A	1.16 c	2.00 a	1.58
C_3	16.00	14.00	12.67	11.33	13.50 A	1.33 bc	1.33 bc	1.33
Means of water regimes	Normal irrigation= 14.00 A		Drought = 11.75 B			Normal irrigation= 1.80 A	Drought = 1.16 B	
Means of crop growth stage	V_6= 13.25 A		R_1= 12.50 B			V_6 =1.37	R_1 =1.58	

C_0= distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= Water deficit, V_6= (6 fully expanded leaves), R_1= Initiation of silking stage.

Cob length (CL) was significantly affected by both water regimes (Table 3). CL was 11.32% more under normal irrigation condition as compared to drought stress. Both crop growth stages were recorded equally water sensitive as CL was statistically same in both crop growth stages. Exogenous application of different osmoprotectants significantly affected CL. Moreover, CL (15.16 cm) was maximum where combination of salicylic acid and ascorbic acid was exogenously applied while it was at par with sole application of both osmoprotectants and minimum CL (13.22 cm) was recorded with distilled water spray. These results portrayed that combined effect of SA and AsA improved CL more as compared to individual effect of each osmoprotectant. Khodarahmpour and Hamidi (2012) also stated that combined application of SA and AsA increased CL in maize as compared to sole application of both.

Both the water regimes significantly influenced number of grain rows per cob (GR) (Table 3). Maximum GR (15.59) were noticed under normal irrigation condition as compared to drought stress condition where it was (13.63). GR were statistically similar under both water sensitive crop growth stages. GR were influenced significantly by different osmoprotectants.

Maximum GR (15.09) were observed with foliar application of salicylic acid + ascorbic acid @ 0.5 mM each + Tween 20 (0.1%) and minimum GR (13.50) were recorded with distilled water spray.

Table 3: Effect of sole and combined application of salicylic and ascorbic acid on cob length and number of grain rows per cob of maize exposed to normal irrigation and drought condition at different vegetative and reproductive crop growth stages.

	Cob length					**Number of grain rows per cob**				
	Normal irrigation		**Drought**		**Means**	**Normal irrigation**		**Drought**		**Means**
Treatments	V_6	R_1	V_6	R_1		V_6	R_1	V_6	R_1	
C_0	14.23	13.63	12.56	12.433	13.22 B	14.68	13.68	13.00	12.68	13.50 B
C_1	15.96	14.80	14.20	13.467	14.61 A	16.68	15.33	14.00	13.33	14.83 A
C_2	16.00	16.100	14.40	13.63	15.03 A	16.00	16.00	14.00	14.00	15.00 A
C_3	16.53	15.73	14.23	14.13	15.16 A	16.00	16.33	14.33	13.68	15.09 A
Means of water regimes	Normal irrigation= 15.37 A		Drought= 13.63 B			Normal irrigation= 15.59 A		Drought =13.63 B		
Means of crop growth stages	V_6= 14.76		R_1= 14.24			V_6=14.83		R_1=14.38		

C_0= Distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= water deficit, V_6= (6 fully expanded leaves), R_1= Initiation of silking stage.

These results are inconformity with the results reported by Rivera-Hernández et al. (2010) as they found reduction in GR under drought condition.

Interaction between water regimes and crop growth stages was significant regarding cob weight (CW) (Table 4). Statistically similar CW was noticed when drought was exhibited at both water sensitive stages of crop growth. Results depicted that both crop growth stages were equally sensitive regarding cob weight. Under drought stress CW was 141.21 g while under normal irrigation it was 160.71 g. Maximum CW (164.17 g) was recorded under normal irrigation at V_6 (six fully expended leaves) and minimum cob weight (140.08 g) was recorded when drought was imposed at 6 fully expended leaves. These finding are same as reported by Khanzada et al. (2001) as they stated that drought stress reduced CW in maize.

Stover yield (SY) was influenced significantly by interaction between water regimes and crop growth stages (Table 4). SY was maximum (12.23 t ha^{-1}) when normal irrigation was applied at 6 fully expanded leaves while lowest SY (9.77 t ha^{-1}) was recorded when drought was imposed at initiation of silking stage of maize. Initiation of silking stage was noticed more water sensitive regarding reduction in SY as compared to vegetative stage. Maximum SY (12 .14 t ha^{-1}) were recorded under normal irrigation condition while under drought condition SY was 9.99 t ha^{-1}. These results are similar to the finding reported by Yadav et al. (2002) as they noticed reduction in SY in maize under drought condition.

Thousand grain weight was significantly affected by both water regimes (Table 5). Maximum 1000-grain weight (308.92 g) was recorded under normal irrigation condition while it was decreased (290.96 g) under drought stress. These results are same as reported by Zinselmeier et al. (1995) that 1000-grain weight. Thousand grain weight was not affected significantly by both water sensitive crop growth stages. Different osmoprotectants significantly influenced 1000-grain weight.

Table 4: Interaction between water regimes and crop growth stages influencing Cob weight and stover yield of maize exposed to normal irrigation and drought conditions.

Crop growth stages	Cob weight (g)			Stover yield (t ha^{-1})		
	Normal irrigation	Drought	Means	Normal irrigation	Drought	Means
V_6	164.17 a	140.08 c	152.12	12.23 a	10.21 b	11.17
R_1	157.25 b	142.33 c	149.79	12.15 a	9.77 c	10.96
Means of water regimes	160.71 A	141.21B		12.14 A	9.99 B	

C_0= Distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= Water deficit, V_6= (6 fully expanded leaves), R_1= Initiation of silking stage.

Table 5: Effect of sole and combined application of salicylic and ascorbic acid on 1000 grain weight and grain yield of maize exposed to normal irrigation and drought condition at different vegetative and reproductive crop growth stages.

	1000-grain weight					Grain yield (t ha^{-1})				
	Normal irrigation		Drought		Means	Normal irrigation		Drought		Means
Treatments	V_6	R_1	V_6	R_1		V_6	R_1	V_6	R_1	
C_0	299.67	302.00	280.67	286.00	292.08 B	9.43 abcd	9.31 abcd	8.9 def	7.9 ef	8.95 C
C_1	315.67	311.67	293.33	291.67	303.08 A	10 .33 abc	8.97 de	9.37 bcd	8.87 def	9.38 B
C_2	311.00	312.00	294.33	294.33	302.92 A	10.20 abc	9.4 abcd	9.33 cd	8.17 f	9.20 B
C_3	311.33	308.00	296.00	291.33	301.67 A	10.36 ab	10.4 a	9.63 abcd	9.33 cd	9.93 A
Means of water regimes	Normal irrigation = 308.92 A		Drought = 290.96 B			Normal irrigation= 9.89 A		Drought= 8.94 B		
Means of crop growth stages	V_6=300.25		R_1=299.62			V_6= 9.73 A		R_1= 9.10 B		

C_0= Distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= Water deficit, V_6= (6 fully expanded leaves), R_1= Initiation of silking stage

Maximum 1000- grain weight (303.08 g) was observed with SA (1mM) applied alone while minimum was recorded with distilled water spray. It was observed that effect of different treatments of osmoprotectants either alone or in combination was statistically similar.

Interaction between water regimes, crop growth stages and osmoprotectants was significant regarding grain yield (GY) (Table 5). Grin yield was maximum (10.4 t ha^{-1}) with foliar application of combination of salicylic and ascorbic acid @ 0.5 mM each under normal irrigation condition applied at initiation of silking stage. Minimum GY (7.9 t ha^{-1}) was recorded under drought stress where distilled water was sprayed at initiation of silking. Maximum GY (9.89 t ha^{-1}) was recorded under normal irrigation while it was 9.60 % greater than achieved under drought condition. Initiation of silking stage was more water sensitive regarding reduction in grain yield. Grain yield was recorded 9.73 t ha^{-1} at 6 fully expanded leaves as compared to grain yield (9.10 t ha^{-1}) recorded at initiation of silking. These results are similar to findings reported by Abdelmula et al. (2007) as they found that reproductive stage of maize was more sensitive. Results suggested that combination of SA and AsA alleviated the damaging effects of water deficit stress by improving grain yield as compared to application of osmoprotectant alone. These findings match with findings reported by Anjum et al. (2011a) that brassinolide application under different irrigation conditions significantly affected maize grain yield. Aziz et al. (2018b) reported that combined application of SA and AsA in equal quantity improved yield traits of cotton under water deficit conditions.

Table 6: Interaction between water regimes and osmoprotectants influencing biological yield (t ha^{-1}) of maize exposed to normal irrigation and drought condition

Biological yield (t ha^{-1})			
Treatments	Normal irrigation	Drought	Means
C_0	20.03 c	18.73 e	19.38 C
C_1	21.65 a	19.55 cd	20.60 A
C_2	20.88 b	19.16 de	20.02 B
C_3	21.82 a	19.50 c	20.88 A
Means of water regimes	21.09 A	19.35 B	
Means of crop growth stages	V_6= 20.33 A	R_1 = 20.13 B	

C_0= Distilled water spray, C_1= Salicylic acid (1mM) + Tween 20 (0.1 %), C_2= Ascorbic acid (1mM) + Tween 20 (0.1 %), C_3= Salicylic acid (0.5mM) + Ascorbic acid (0.5mM) + Tween 20 (0.1%), W_0= Normal irrigation, W_1= Water deficit, V_6= (6 fully expanded leaves), R_1= Initiation of silking stage.

Interaction between water regimes and different osmoprotectants was found significant regarding biological yield (BY) (Table 6). Maximum BY (21.82 t ha^{-1}) was recorded with foliar application of combination of SA and AsA @ 0.5 mM each under normal irrigation. Minimum BY (18.73 t ha^{-1}) was recorded under drought condition due to distilled water spray. Under drought stress, BY was 8.25 % greater under normal irrigation as compared to drought stress condition. These results are in conformity with findings reported by Kalamian et al. (2006) as they also reported that drought stress reduced the biological yield in maize. Different osmoprotectant treatments significantly affected biological yield. Maximum BY (20.88 t ha^{-1}) was observed in case of exogenous application of combination of SA and AsA @ 0.5 mM each while minimum biological yield (19.38 t ha^{-1}) was recorded with distilled water pray. It was also reported by Darvishan et al. (2013) that foliar application of AsA increased yield of maize.

Conclusion

Initiation of silking stage in maize was more water sensitive as compared to six fully expanded leaves in terms of more reduction in grain yield, biological yield and cob diameter. However, exogenous application of salicylic acid + ascorbic acid @ 0.5mM each + tween 20 (0.1%) at six fully expanded leaves stage was more effective in mitigating the harmful effects of water deficit by improving cob diameter, biological yield and grain yield of maize as compared to drought imposed at initiation of silking stage.

Contribution of Authors

Qasim M: Performed experiment, recoded data and wrote the full article,
Aziz M: Conceived idea, designed the study, supervised whole research, performed data analysis and proofread the article
Nawaz F: Assisted in study design and proofreading of article

Arif M: Assisted in study design

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