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IMPROVING OKRA PRODUCTIVITY BY MITIGATING DROUGHT THROUGH FOLIAR APPLICATION OF SALICYLIC ACID

Mariam Munir[1,*], Muhammad Amjad[2], Khurram Ziaf[2] and Ashfaq Ahmad[3]

[1]Sub-campus Burewala, University of Agriculture, Faisalabad, Pakistan;[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan; [3]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.
[*]Corresponding author's e-mail: mariammunir1534@gmail.com

Drought is major yield limiting factor in agriculture. Being perishable in nature vegetables are much more sensitive to drought stress compared to agronomic crops. Efficacy of two salicylic acid doses viz., 1mM and 2mM was evaluated on different growth stages of okra grown under 50% water stress. Study was conducted in Faisalabad during summer months of 2012-13. Data regarding morphological traits (plant height, root length, root shoot ratio and leaf area), physiological traits (cell membrane permeability, relative leaf water contents, photosynthetic rate and net assimilation rate) and yield related traits (total pods per plant, pod weight, fruit and seed yield per hectare) were recorded during both years. Results revealed that application of 1mM salicylic acid spray at 2+4 leaf stage as well as 2+4+flowering was equally effective in improving all morphological traits except root length. However, in other traits (physiological and yield) application of salicylic acid at all three growth stages (2+4+flowering stage) remained significantly superior than the application of salicylic acid at first two growth stages (2+4 leaf stage). Overall, application of 2mM spray at all growth stages of okra (2+4+flowering stage) was found to be most effective in improving all the traits under study consistently during both the years. From the results of this experiment we may conclude that foliar application of 2mM salicylic acid at 2 and 4 leaves, and flowering stage of okra can be effectively used for drought mitigation in okra.

Keywords: *Abelmoschus esculentus*, drought, morphological traits, physiological traits, yield.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is an important summer vegetable. However, Pakistan's okra productivity is lower than average productivity of India i.e. 11.6 t/ha (MNFSR, 2015). Water is one of the most limiting constraints for agricultural production in Pakistan. According to World Water Assessment Program, Pakistan is amongst one of the most water stressed countries in the world. Per capita water availability is on continuous decline since its independence. During 2012, water availability in Pakistan was 1000 cubic meter per person per annum, which was the critical limit according to UNESCO (FAO, 2013).

Vegetables are very sensitive to drought stress as compared to most of agronomic crops because of their high water requirements. Growth, yield and quality of vegetable crops are significantly affected by drought stress. Scientists reported significant reduction in morphological (Wullschleger *et al*., 2005; Sankar *et al*., 2007, 2008), physiological (Bhatt and Srinavasa, 2005; Altaf *et al*., 2015) and yield (Gunawardhana and de-Silva, 2011; Hussein *et al*., 2011) traits of okra grown under water stress.

Salicylic acid (SA) is a naturally occurring plant growth regulator (PGR) that influences several morpho-physiological and biochemical functions. It improves plant defense against various stresses through a mechanism known as systemic acquired resistance (Bideshki and Arvin, 2010; Fatima *et al*., 2014; Hussain *et al*., 2014). SA can be used to mitigate negative effects of drought stress in plants (Khodary, 2004; Kumar *et al*., 2014). It protects photosynthetic apparatus through increasing the ability of cell anti-oxidation and the synthesis of new proteins (Avancini *et al*., 2003; Daneshmand *et al*., 2010). Exogenous application of SA improved plant growth (Khodary, 2004), stomatal conductance, photosynthetic rate (Khan *et al*. 2010) transport and ion uptake (Gunes *et al*., 2005). The results of early studies revealed that foliar application of SA stimulated drought resistance in tomato (Hayat *et al*., 2008), beans (Sadeghipour and Aghaei, 2012; Nezhad *et al*., 2014) and wheat (Waseem *et al*., 2006; Kang *et al*., 2012). However, response of SA has yet to be tested in Okra crop grown under water stress.

Keeping in view the importance of okra as a vegetable with respect to its nutritional value, area and production compounded with alarming condition of drought occurrence in Pakistan, a comprehensive study was conducted. The objectives of this study were to check the efficacy of different doses of SA in okra and to identify the time and combination of different growth stages for foliar application of SA to get more economic gains under water scarcity scenario.

MATERIALS AND METHODS

Seeds of okra variety "Sabz Pari" were obtained from Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Experiment was conducted at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, during summer season (i.e. March-April) for two consecutive years (2013 and 2014). Okra seeds were sowed on ridges keeping plant to plant and row to row distance constant at 20 cm and 75 cm, respectively. Area of each experimental unit was 75 ft^2 with 45 plants. Deficit irrigation (50% drought) was applied using cut throat flume that was obtained from Faculty of Agricultural, Engineering and Technology, University of Agriculture, Faisalabad. Recommended cultural practices were carried out throughout the growing season in all experimental units.

Two different concentrations of salicylic acid (1mM and 2mM) were sprayed on okra at different growth stages (2-leaf stage, 4-leaf stage, flowering stage, alone and in all possible combinations) under 50% drought level (Table 1). The experiment was laid out in RCBD under factorial arrangements with 15 treatments and three replications.

Data regarding morphological traits (plant height, root length, root shoot ratio and leaf area) and physiological traits (cell membrane permeability, relative leaf water contents, photosynthetic rate and net assimilation rate) were recorded 70 days after sowing while, yield related traits (total pods per plant, pod weight, fruit and seed yield per hectare) were recorded at the end of growing season.

Recorded data was analyzed statistically by employing Fisher's analysis of variance technique. Differences among treatments means were compared by using Duncan's Multiple Range test at 5% as well as 1% probability level (Steel *et al*., 1997).

Table 1. Detailed layout of experimental treatments

Treatments	Stage of foliar application	Dose of salicylic acid
T1	2-leaf stage	1mM
T2	2-leaf stage	2mM
T3	4-leaf stage	1mM
T4	4-leaf stage	2mM
T5	Flowering stage	1mM
T6	Flowering stage	2mM
T7	2 + 4 leaf stage	1mM
T8	2 + 4 leaf stage	2mM
T9	2 + flowering stage	1mM
T10	2 + flowering stage	2mM
T11	4 + flowering stage	1mM
T12	4 + flowering stage	2mM
T13	2 + 4 + flowering stage	1mM
T14	2 + 4 + flowering stage	2mM
T15	Control (drought without spray)	

RESULTS

Response of foliar application of SA regarding different physiological, morphological and yield related traits with respect to different growth stages was found to be significantly similar across the study years. Therefore, the data for both the years were pooled for further statistical analysis.

***Morphological traits*:** Results revealed significant effect of salicylic acid on morphological traits of okra plant (Table 2). SA spray increased plant height, root length and leaf area of okra grown under water stress. Among various treatments,

Table 2. Okra morphological traits as affected by SA spray at different growth stages

Treatments	Dose	Plant height (cm)			Root length (cm)			Root shoot ratio			Leaf area (cm^2)		
		2013	2014	Avg	2013	2014	Avg	2013	2014	Avg	2013	2014	Avg
2 LS	1mM	62.9cd	64.7b-e	63.8cd	29.93g	31.68e	30.81g	0.47g	0.49f	0.48h	907.02fg	1031.7a-c	969.36 cd
	2mM	51.44f	62.46c-e	56.95f	34.96e	33.76d	34.36ef	0.67a	0.54ef	0.61bc	868.59fg	1045a-c	956.79 cd
4 LS	1mM	58.37e	53.11f	55.74e	32.9f	34.25d	33.58f	0.56de	0.64ab	0.6b-d	930.09fg	841.32d	885.70 d
	2mM	65.77c	55.6e	60.69c	35.94d	34.18d	35.06de	0.54ef	0.57c-e	0.5ef	1076.1c-e	991.86bc	1033.98bc
FS	1mM	43.66gh	41.23g	42.45gh	25.35h	26.92g	26.14i	0.58c-e	0.65ab	0.61bc	707.16h	615.5e	671.91 ef
	2mM	46.25g	44.16g	45.21g	30.49g	29.17f	29.83h	0.66ab	0.61a-d	0.63a	799.41gh	628.78e	714.09 ef
2 + 4 LS	1mM	70.27b	65.74ab	68.01b	35.86de	35.98c	35.92d	0.5fg	0.53ef	0.51gh	1245.2ab	1045a-c	1145.1 a
	2mM	71.38a	66.56ab	68.97a	38.78b	38.52b	38.65b	0.5fg	0.56c-e	0.53fg	1349a	1005.1a-c	1177.05 a
2 + FS	1mM	58.02de	63.98a-e	61de	35.32de	35.17cd	35.25de	0.59c-e	0.55d-f	0.57c-f	968.52ef	1000.7a-c	984.61 c
	2mM	60.27cd	63.21b-e	61.74cde	37.42c	38.86b	38.14bc	0.6cd	0.61a-d	0.6bc	1006.9d-f	1071.6ab	1039.25bc
4+FS	1mM	61.64c	61.28de	61.46cd	35.42de	34.07d	34.75e	0.56de	0.55d-f	0.55fg	1133.7bc	885.6cd	1009.65 c
	2mM	63.32c	67.1a-d	65.21c	38.64b	36.61c	37.63c	0.58c-e	0.55ef	0.56d-f	1179.9bc	1129.1ab	1154.5 a
2+4+FS	1mM	70b	67.88a	68.94ab	37.63c	38.8b	38.22bc	0.51fg	0.57c-e	0.54fg	1145.3b-d	1084.8ab	1115.05ab
	2mM	71.81a	65.42a-d	68.62b	40.03a	42.26a	41.15a	0.55ef	0.65ab	0.59b-e	1118.4b-d	1142.4a	1130.4 a
Control (no spray)		40.9 h	40.2g	40.55h	25.1h	24.94h	25.02j	0.7a	0.69a	0.69a	668.73h	602.21e	635.47f
DMR value		**4.30****	**5.53***	**4.3****	**0.97***	**1.47****	**0.48****	**0.05***	**0.06***	**0.05***	**100.2****	**152.1****	**89.04****

*Significant at $p<0.05$ and ** significant at $p<0.01$. 2LS = 2-leaf stage, 4LS = 4-leaf stage, FS = Flowering stage, 2+4 LS = 2+4 leaf stage, 2+FS = 2 leaf stage + flowering stage, 4+FS = 4 leaf stage + flowering stage, 2+4+FS = 2 leaf stage + 4 leaf stages + flowering stage and control = untreated plants; n = 3 replicates.

SA spray at all three growth stages i.e. 2+4+flowering stage was found most effective in improving okra morphological traits under drought stress. Interestingly, SA spray at 2+4 leaf stage was found equally effective as was 2+4+flowering for most of the morphological parameters except root length. Overall, 1mM SA spray at all growth stages significantly improved plant height (68.94cm) and normalized root shoot ratio (0.54) while, 2mM SA spray at all growth stages was found more effective in increasing root length (41.15 cm) and leaf area (1130.4 cm^2). Similarly, application of 2mM SA spray at 2+4 leaf stage showed more plant height (68.97 cm) as compared to 1mM SA spray while, 1mM SA spray at 2+4 leaf stage was found superior in normalizing root shoot ratio (0.51) of okra plant. Control plants showed minimum height (40.55 cm), root length (25.02 cm), leaf area (635.47 cm^2) and maximum root shoot ratio (0.69) (Table 2).

***Physiological traits*:** Results revealed minimum relative leaf water contents in control plants (58.86%) while, application of 2mM SA spray at all growth stages (2+4+FS) significantly increased relative leaf water contents (89.92%) in okra grown under drought (Table 3). Maximum cell membrane permeability was observed in control (69.23%) while, application of 1mM as well as 2mM SA spray at all growth stages (2+4+FS) were found equally effective in reducing cell membrane permeability during both years. The results revealed maximum photosynthetic rate (39.49 $\mu mol.CO_2.m^{-2}.s^{-1}$) as well as net assimilation rate (2.95 $mg.m^{-2}.day^{-1}$) when okra plants were sprayed with 2mM SA at all three growth stages (2+4+FS) while, control plants showed minimum photosynthetic rate (20.86

Table 3. Okra physiological traits as affected by SA spray at different growth stages.

Treatments	Dose	Cell Membrane Permeability (%)			Relative leaf water contents (%)			Photosynthetic rate ($\mu mol\ CO_2\ m^{-2}s^{-1}$)			Net assimilation rate ($mg\ m^{-2}day^{-1}$)		
		2013	2014	Avg	2013	2014	Avg	2013	2014	Avg	2013	2014	Avg
2 LS	1mM	55.52c	58.28b	56.9b	63gh	62.31g	62.66hi	23.39gh	20.56hi	21.98hi	1.93b-d	1.5de	1.72 b-d
	2mM	62.53b	59.5b	61.02b	65.4fg	63.27g	64.34h	22.36gh	20.48hi	21.42hi	1.46d-g	1.9b-d	1.68 b-d
4 LS	1mM	45.45ef	44.19c-e	44.82de	71.29de	74.01de	72.65f	25.55fg	24.81e-g	25.18fg	1.97b-d	1.83b-d	1.9 bc
	2mM	48.58de	49.15cd	48.87cd	67.66ef	68.94f	68.3g	28.51ef	23.68gh	26.1ef	1.6c-g	1.7b-e	1.65 cd
FS	1mM	41.89fg	44.05de	42.97ef	73.9cd	70.68ef	72.29f	30.32de	26.78c-g	28.55cd	1.7c-f	1.5de	1.6 cd
	2mM	47.21ef	49.7c	48.46cd	77.15bc	75.87cd	76.51de	29.22de	24.42fg	26.82d-f	1.5d-g	1.87b-d	1.69 b-d
2 + 4 LS	1mM	46.93ef	42.48e	44.71de	79.68b	79.66c	79.67c	29.1d-f	27.72c-f	28.41c-e	1.77b-e	2.37b	2.07 bc
	2mM	49.3de	44.85c-e	47.08cde	79.01b	79.62c	79.32cd	28.42ef	25.5d-g	26.96d-f	2.23b	1.8b-d	2.02 bc
2 + FS	1mM	43.54e-g	43.1e	43.32ef	74.41cd	74.2de	74.31ef	31.66de	28.13cd	29.9c	1.33e-g	2.03b-d	1.68 b-d
	2mM	53.51cd	49.16cd	51.34c	78.81b	79.35c	79.08cd	31.32de	27.75c-e	29.54c	2.07bc	1.9b-d	1.99 bc
4+FS	1mM	39.17gh	40.65e	39.91f	87.44a	84.77b	86.11b	32.31cd	28.98c	30.65c	1.8b-e	2.23bc	2.02 bc
	2mM	45.08ef	45.99c-e	45.54de	87.43a	87.96ab	87.7ab	35.39bc	33.07b	34.23b	1.46d-g	1.9b-d	1.68 cd
2+4+FS	1mM	38.71i	31.93f	35.32g	89.35a	88.04ab	88.7ab	36.56b	33.28b	34.92b	2.1bc	2.23bc	2.17 b
	2mM	39hi	33.71f	36.36g	90.09a	89.74a	89.92a	41.85a	37.12a	39.49a	2.77a	3.13a	2.95 a
Control (No Spray)		68.72 a	69.74a	69.23a	58.98i	58.74g	58.86j	21.59h	20.12i	20.86i	1.13g	1.03e	1.08e
DMR value		**5.86****	**5.64****	**4.29****	**3.88****	**4.96****	**3.15****	**3.58****	**3.30****	**2.32****	**5.18***	**7.03***	**0.46***

*Significant at $p<0.05$ and ** significant at $p<0.01$. 2LS = 2-leaf stage, 4LS = 4-leaf stage, FS = Flowering stage, 2+4 LS = 2+4 leaf stage, 2+FS = 2 leaf stage + flowering stage, 4+FS = 4 leaf stage + flowering stage, 2+4+FS = 2 leaf stage + 4 leaf stages + flowering stage and control = untreated plants; n = 3 replicates.

Table 4. Okra yield traits as affected by SA spray at different growth stages.

Treatments	Dose	Pods per plant			Pod weight (g)			Fruit yield (t/ha)			Seed yield (kg/ha)		
		2013	2014	Avg	2013	2014	Avg	2013	2014	Avg	2013	2014	Avg
2 LS	1mM	7.40g	6.80fg	7.10hi	6.40g	6.73fg	6.57f	3.16h	3.17i	3.17h	592.0f	593.5f	592.7i
	2mM	7.46g	7.06fg	7.26h	6.96fg	6.89e-g	6.93ef	3.50h	3.38i	3.44h	628.3ef	631.2f	629.7hi
4 LS	1mM	10.06e	8.86de	9.46fg	7.71ef	6.88e-g	7.30de	5.18f	4.25gh	4.72f	972.9d	764.3ef	868.6fg
	2mM	10.06e	9.66d	9.86f	8.00de	7.93cd	7.97c	5.22f	5.23f	5.23f	950.5d	959.5d	955.0f
FS	1mM	13.46cd	14.13ab	13.80cd	8.49c-e	7.51de	8.00c	7.42de	7.41d	7.42de	1329.2bc	1362.1b	1345.6d
	2mM	13.80c	14.26ab	14.03b-d	8.94bc	8.39bc	8.67b	8.11cd	8.26bc	8.19cd	1448.9b	1443b	1445.9b-d
2 + 4 LS	1mM	9.93e	7.66ef	8.80g	6.49g	6.59g	6.54f	4.35g	3.61hi	3.98g	845.8d	631.9f	738.8gh
	2mM	9.00f	9.46d	9.23fg	7.70ef	7.26ef	7.48c-e	4.60fg	4.72fg	4.66fg	812.9de	840.9de	826.9fg
2 + FS	1mM	12.93d	12.46c	12.70e	7.87e	7.34d-f	7.61cd	6.71e	6.34e	6.53e	1242.7c	1151.9c	1197.3e
	2mM	13.73c	14.73ab	14.23b-d	8.75b-d	8.58bc	8.67b	7.73cd	8.66b	8.20cd	1383.2bc	1528.7b	1455.9b-d
4+FS	1mM	13.26cd	13.46bc	13.36de	8.83bc	8.32bc	8.58b	7.59d	7.79cd	7.69d	1359.5bc	1435.8b	1397.6cd
	2mM	15.06ab	13.8bc	14.43bc	9.53ab	8.77b	9.15b	9.02b	8.37bc	8.70b	1677.9a	1459.9b	1568.9b
2+4+FS	1mM	14.60b	14.93ab	14.77ab	8.78b-d	8.35bc	8.57b	8.36bc	8.50bc	8.43bc	1466.8b	1531.7b	1499.2bc
	2mM	15.46a	15.60a	15.53a	9.87a	9.68a	9.78a	9.97a	10.20a	10.09a	1517.4a	1874.2a	1695.8a
Control (No Spray)		5.80h	6.06g	5.93j	4.72i	4.32h	4.52g	1.52i	1.68j	1.60i	334.4g	376.7g	355.5j
DMR value		**0.71****	**1.53****	**0.89****	**0.80****	**0.64****	**0.5****	**0.76****	**0.82****	**0.76****	**186.56****	**191.96****	**142.64****

*Significant at $p<0.05$ and ** significant at $p<0.01$. 2LS = 2-leaf stage, 4LS = 4-leaf stage, FS = Flowering stage, 2+4 LS = 2+4 leaf stage, 2+FS = 2 leaf stage + flowering stage, 4+FS = 4 leaf stage + flowering stage, 2+4+FS = 2 leaf stage + 4 leaf stages + flowering stage and control = untreated plants; n = 3 replicates.

μmol.CO_2.m^{-2}.s^{-1}) and net assimilation rate (i.e. 1.08 mg.m^{-2}.day^{-1}) (Table 3).

***Yield traits*:** When 2mM SA was sprayed at all three growth stages (2+4+FS) of okra grown under water stress maximum pods per plant (15.53), pod weight (9.78 g), fruit yield (10.09 t/ha) and seed yield (1695.8 kg/ha) was observed. While on contrary, control plants showed minimum pods per plant (5.93), pod weight (4.52 g), fruit yield (1.6 t/ha) as well as seed yield (355.5 kg/ha) (Table 4).

DISCUSSION

Under water stress, growth process is slowed down due to limited cell elongation and differentiation (Tardieu *et al.*, 2000) which ultimately resulted in lesser plant height as well as root length. Reduced plant height has already been reported by scientists in okra grown under water stress (Sankar *et al.*, 2008; Kusvuran *et al.*, 2008). Similarly, reduced root length at higher levels of water stress was observed by Fraser *et al*. (1990) and Lum *et al*. (2014) in maize and rice plants, respectively. Despite the fact of reduced root and shoot length, maximum root shoot ratio was observed in control plants. According to Pearsall (1923) and White (1937) root growth mainly depends upon availability of assimilates after the shoot had used its requirement. Later it was proposed that under limited availability of water, a large portion was retained in the roots thus improved root to shoot ratio (Shank, 1945). Thus, under limited water availability the retained nutrients in the root significantly increased root shoot ratio as was also depicted by Wilson (1988). In addition to above facts, increased root length compared to shoot length might be associated with the fact that roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000). SA is an important phenolic phyto-hormone reported to have antioxidant properties (Kang *et al*., 2012) and involved in protective mechanisms during water stress (Miura and Tada, 2014). In okra no work has been reported yet on mitigation of such higher levels of drought. However, Sadeghipour and Aghaei (2012) and Nezhad *et al*. (2014) reported significant improvement in vegetative parameters of common bean and mung bean under drought stress respectively, due to application of SA.

Despite the fact that 98% water transported from roots to leaves is transpired, the remaining 2% water is very essential for normal functioning of all physiological processes of a plant (Starr, 2013). Relative leaf water contents provide an excellent tool to determine water status in plants. Under drought stress due to lower soil water potential, uptake of water by the roots is reduced (Steudle, 2000; Aroca and Ruiz-Lozano, 2012) which ultimately reduced cellular leaf water potential as was observed in control plants in our experiment. Relative leaf water potential has direct influence on cell membrane permeability as was also reported by Li-Ping *et al*. (2006) in maize plant. Scientists reported that mild stress levels did not cause significant alteration in membrane permeability (Douglas and Paleg, 1981; Simonds and Orcutt, 1988); however, higher levels of water stress as were applied in our experiment (i.e. 50% water stress throughout growing season) did increase membrane permeability as was also reported by Navari-Izzo *et al*. (1989) and Liljenberg *et al*. (1985). Major reason of increased membrane permeability was solute leakage as a result of membrane damage under low water potential (Pessarakli, 1999). Rate of photosynthesis was also reduced in control plants. Under water stress, activity of ATP-synthase is reduced which ultimately affects activity of ribulose-bisphosphate, resulting in reduced photosynthetic rates (Tezara *et al*., 1999). Exogenous application of SA under stress condition has been already reported by several scientists to have alleviating effect on cell membrane permeability (Gunes *et al*., 2007; Hosseini *et al*., 2015), relative leaf water contents (Hayat *et al*., 2008) and photosynthesis (Singh and Usha, 2003: Syeed *et al*., 2011; Janda *et al*., 2014). However, net assimilation rate has not yet been reported.

It is well known fact that vegetative traits like plant height and leaf area have very strong positive correlation on okra yield traits like number of pods per plant and average pod size (Ariyo, 1992; Akinyele and Osekita, 2006; Falusi *et al*., 2012). Furthermore, improvement in physiological traits of okra plant due to exogenous application of SA even under water stress significantly increased average pod weight, fruit and seed yield per hectare.

***Conclusion*:** Present results revealed that even under severe drought (50% water stress) morpho-physiological and yield related traits of okra plant were significantly improved by the foliar application of 2mM SA spray at three growth stages (2+4+flowering stage).

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