

FOLIAR APPLIED OF SALICYLIC ACID ALLEVIATED THE DETRIMENTAL EFFECTS OF DROUGHT ON VARIOUS GENOTYPES OF WHEAT (*TRITICUMA ESTIVIVUM* L.)

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Abstract

Drought is a global problem, limiting world crop production seriously and current global climate change has made this situation worst. This study was designed to assess the potential of salicylic acid (SA) as a growth promoter to counteract the detrimental impacts of drought on five wheat (*Triticum aestivum* L.) genotypes (NIA-Sarang, NIA-Sundar, NIA-Zarkhaiz, Khirman (check) and TD-1). Two concentrations of SA (0.7 and 1.44 mM) were used and the spray was done at vegetative stage. Water deficit was imposed as terminal drought i.e. no irrigation was applied except soaking dose. Generally wheat genotypes showed differential responses at two levels of SA. Most of the genotypes had enhanced proline accumulation at both levels of SA in comparison with the control treatment of no spray of SA. The genotype NIA-Sundar exhibited highest increase of 264.26 fold and 271.08 fold at 0.7 mM and 1.44 mM respectively, indicating the potential of the genotype to manage with the drought environment. Osmotic potential (MPa) was generally decreased under drought environment with both spray of SA, however higher decrease (more -ve values) in the O.P was observed at 0.7 mM SA as compared to 1.44 mM SA. Potassium (K) accumulation was reduced under drought stress. However less decrease was observed in the genotypes at the level of 0.7 mM, while more accumulation of Potassium was observed at 1.44 mM under drought stress. Grain weight (g plant⁻¹) was also seriously affected under drought. However the genotype NIA-Sarang (27.64 g plant⁻¹) and TD-1 (29.40 g plant⁻¹) at 0.7 mM and NIA-Sarang (23.11 g plant⁻¹) and Khirman (36.17 g plant⁻¹) at 1.44 mM exhibited comparatively low reduction. It was concluded from this study that spray of SA alleviated the detrimental impacts of drought with genotypic variations as recorded.

Key words: Wheat (*Triticum aestivum* L.), Salicylic acid, Physiological indices.

Introduction

Drought is a major limiting factor for agricultural output and in general hampers plant growth through reduced water and nutrient absorption (Reichstein *et al.*, 2013). Among the existing environmental stresses, it has now become one of the most serious threat worldwide (Mohammadi *et al.*, 2008). According to reports 99 m ha area in the developing countries and more than 60 m ha in the developed countries of the world are being affected by various degree of drought (Nabi *et al.*, 2019). Pakistan is among the countries where almost 15 m ha of land are influenced by this syndrome (Khosro *et al.*, 2015), while yield drop of 17 to 70% are reported in the country (Ganbalani & Hassanpanah, 2009).

Among the variety of plant types drought causes a range of physiological as well as biochemical restrictions and unfavorable effects (Kadam *et al.*, 2017). Drought stress raises the production of reactive oxygen species (ROS) like hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]), and superoxide (O₂^{•-}), which is the most common effect and earliest plant reply in plants exposed to abiotic stresses, especially drought (Ashraf & Akram, 2009). Enhanced accretion of ROS may direct to exert poisonous effects, e.g. protein degradation, lipid peroxidation and pigment bleaching. The effects of ROS are believed to be neutralized by the behavior of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Foyer & Noctor, 2000).

There are many ways to deal with this issue of drought stress which include screening of potential drought tolerant germplasm (genotypes) under drought, management practices that increase availability of stored moisture, use of chemicals that cause decline in

transpiration or closing of stomata and nutrient management like use of potassium which is highly reported to improve the detrimental effects of drought.

One of the more feasible and economical approach is to use the growth regulators. Among the growth regulators, Salicylic acid (SA) has been widely reported to improve the detrimental impacts of drought stress. SA is a compound of phenolic nature that is concerned in the regulation of plants growth (Khan *et al.*, 2012). Foliar use of SA has been found to provoke plant stress tolerance. Various researches revealed encouraging impacts of SA on plants against salinity (Wang & Li, 2006), drought (Sharma *et al.*, 2017) and high temperatures (Hussain *et al.*, 2020). Foliar use of SA has also been found to amend the behavior of intracellular antioxidant enzymes SOD and POD and consequently enhance tolerance to environmental stresses (Parker *et al.*, 2016). SA is also known to have a dogmatic role in physiological processes, like, transpiration, photosynthetic activity, membrane permeability, mitochondrial respiration, nutrient acquisition and chlorophyll accumulation (Hasanuzzaman *et al.*, 2019). This growth regulator has already been known as a major signaling molecule that has effects on plant tolerance to drought stress. It has a great influence on the adaptation of metabolic as well as physiological processes throughout the complete lifecycle of plant, affecting bio-productivity and plant growth parameters (Reddy *et al.*, 2004).

Many reports have indicated the defensive function of SA under environmental stress conditions, however little attention has been focused on the genetic/genotypic variation of the wheat genotypes for the exogenous (Foliar) applications of SA. Therefore, the study was planned to assess the genetic/genotypic variation for the foliar applications of SA under drought conditions.

Materials and Methods

The study was conducted in the wire netted pot house in the cemented tanks measuring 2.44 m x 2.44 m x 1 m (depth) during Rabi season of 2017-18 and 2018-19 at Nuclear Institute of Agriculture (NIA), Tandojam. The results are presented as a grand mean of two years study. The soil was clay loam containing 1.08% O.M, 40 mg kg⁻¹ available nitrogen, 8.5 mg kg⁻¹ AB-DTPA extractable-P, 214 mg kg⁻¹ extractable-K. The treatment consisted of three factors **a**) Two water regimes 1) Control (Four irrigations of 75 mm each as per crop requirement) 2) Drought (No irrigation except soaking dose) **b**) Two levels of salicylic acid (0.7 m M and 1.44 m M) and **c**) five genotypes. Seeds of five wheat genotypes, viz. NIA-Sarang, NIA-Sundar, NIA-Zarkhaiz, Khirman (as a check variety) and T.D-1 were taken from plant breeding and genetics (PBG) division of NIA. Tando jam. The experiment was arranged in a completely randomized design (CRD) and each treatment was replicated thrice. The sowing was done by single coulter hand driven drill while plant to plant distance was at 10 cm and row to row at 20 cm. Foliar Application of salicylic acid (SA) was done manually with garden sprayer 1st at vegetative (60 days after sowing) and 2nd at anthesis (80 days after sowing). Recommended doses of fertilizers were applied @ 120-60-0 Kg N P K ha⁻¹ at the time of 1st irrigation. Three plants were randomly selected from each row or replicate at maturity for recording grain weight.

All the physiological analysis was carried out at vegetative stage. Relative water content (RWC) was estimated as described by Turner (1986) using the following formula:

$$\text{RWC} = \frac{(\text{FM} - \text{DM})}{(\text{TM} - \text{DM})} \times 100$$

where FM, DM and TM are fresh, dry and turgid material.

The proline contents ($\mu\text{mole g}^{-1}$ fresh weight) were estimated according to the method as described by Bates *et al.*, (1973), chlorophyll accumulation (mg g^{-1} F.wt) following the method of Lichtenthaler (1987).

For the determination of potassium in plant, next to flag leaf was sampled, dried uniformly and digested in di-acid (3:1) mixture of nitric (HNO₃) and perchloric acid (HClO₄) (Miller, 1998). Potassium concentration (mg g^{-1} dry wt.) in leaves was determined through flame photometer (Jenway PFP-7) by using standard curve for potassium. Leaf osmotic potential was measured by measuring osmolality of extracted leaf sap using a calibrated Osmomat 030 (Khan *et al.*, 1992).

Statistical analysis

The data were subjected to statistical analysis using 'statistix ver.8.1' and the means were compared through honestly significant test at $\alpha = 0.05$ (Tukey HSD_{0.05}).

Results and Discussion

The (Table1) revealed chlorophyll accumulation (mg g^{-1} F.wt) of wheat genotypes as affected by foliar spray of SA (0.7 m M and 1.44 m M) under drought environment. Under control treatment (no spray) four genotypes

exhibited large reduction (20.25-36.46%) in the chlorophyll contents under drought, however surprisingly the genotype Khirman (local check) accumulated significantly ($p \leq 0.05$) higher chlorophyll contents (0.201 mg g^{-1} F.wt), showing 2.86% increased chlorophyll accumulation than control. The remedial effects of SA @ 0.7 mM were quite evident under drought conditions where the genotypes namely NIA-Sundar (10.81%), Khirman (21.39%) and TD-1 (21.09%) showed comparatively less reduction, while bit more reduction was observed in NIA-Sarang (29.99%) and NIA-Zarkhaiz (33.05%). At 1.44 mM, the genotype NIA-Sundar (16.18%), NIA-Zarkhaiz (15.68%) and TD-1 (13.35%) depicted comparatively low reduction, indicating the potential of these genotypes to cope with the drought conditions with foliar spray of 1.44 m M. The decrease in chlorophyll contents under water stress has already been well documented, however Nikolaeva *et al.*, (2010) reported that decrease in chlorophyll contents under drought may be attributed more to elevated rate of degradation of chlorophyll than a decrease in chlorophyll biosynthesis in wheat.

It was found that usually drought decreased the chlorophyll accumulation irrespective of the treatments (No spray, SA (0.7 mM) and SA (1.44 m M) however exogenous use of SA led to relatively small decrease in some genotypes. These results are in line with Kalaji *et al.*, (2011) who also found development in chlorophyll contents under drought by exogenous use of SA in many crops.

Osmotic potential (-MPa) was generally decreased (more -ve values) under drought environment in all the genotypes and in all the three SA treatments i.e. No spray, SA (0.7 m M) and SA (1.44 m M) (Table 2). Among the genotype no significant ($p \leq 0.05$) differences in the osmotic potential were observed under drought and control with no spray. At no spray maximum decrease (180.35%) was observed in the genotype NIA-Sarang. At 0.7 m M SA two genotypes i.e Khirman and TD-1 showed significantly lower osmotic potential of 1.87 and 1.95 MPa respectively corresponding to 164.14 and 134.54% decrease under drought stress. Similarly at 1.44 m M statistically ($p \leq 0.05$) lower values of osmotic potential (1.64 MPa) was found in the genotype NIA-Sarang corresponding to 107.5% decrease under drought stress. The decrease (more -ve values) in leaf osmotic potential under drought stress has now become the general phenomenon in various crops including wheat and reported by many workers (Khan *et al.*, 2010; Khan *et al.*, 2017). The greatest reduction in OP by the Khirman (164.14%) and TD-1 (134.54%) at 0.7 m M SA and NIA-Sarang (107.5%) at 1.44 mM under drought stress might be due to higher adaptation of these genotypes to drought through effective osmoregulation caused by exogenous use of SA (Hura *et al.*, 2010). However, behavior of the genotype NIA-Sarang at no spray was not understood and needs deep insight.

Compared to control, the proline accumulation significantly ($p \leq 0.05$) increased by drought and SA treatment (Table 3). The accumulation generally increased many fold in all three SA treatments (No spray, SA (0.7 m M) and SA (1.44 m M) under water stress in comparison with control (Normal irrigation). However more increase

(more fold) was observed in the treatment where SA (0.7 & 1.44 mM) was applied in comparison with the treatment where SA was not sprayed (No spray) (Table 3). The genotype NIA-Sundar exhibited significantly ($p \leq 0.05$) higher proline accumulation of $88.0 \mu \text{mol g}^{-1}$. F.wt at 0.7 mM and $59.64 \mu \text{mol g}^{-1}$. F.wt at 1.44 mM corresponding to 264.26 and 271.08 fold increase respectively under drought stress in comparison with control. The highest enhancement of proline accumulation was observed in the genotype NIA-Sundar (264.26 fold at 0.7 mM & 271.08 fold at 1.44 mM) (Table 3) while other four genotypes i.e., NIA-Sarang, NIA-Zarkhaiz, Khirman and TD-1 exhibited lower increase at both levels. These findings are in agreement with the findings of Kordi *et al.*, (2013), who also observed genotypic variation for the application of SA in Sweet Basil (*Ocimum basilicum* L). Bose *et al.*, (2014) found that proline acts as an antioxidant suggestive of its task as ROS scavenger and singlet oxygen quencher. The improvement in proline accumulation and its defensive role as well as its contribution in osmotic adjustment under water stress has now been extensively reported (Cha-um & Kirdmanee, 2008; Teixeira & Pereira, 2007). Therefore, proline accumulation may be documented to be one of the significant factors involved in SA induced defensive mechanism in field crops including wheat in response to

water stress. This study showed that proline accumulation was more at 0.7 mM as compared to 1.44 mM, indicating that 0.7 mM SA was more useful particularly in terms of proline accumulation.

The table 4 showed the potassium accumulation (mg g^{-1} . dry wt.) in leaf tissue as affected by foliar application of SA (0.7 and 1.44 mM) under drought stress. At no spray, four genotypes, namely NIA-Sarang (25.50%), NIA-Sundar (16.22%), Khirman (8.70%) and TD-1(4.22%) exhibited more decrease in potassium accumulation under drought as compared to 0.7 and 1.44 mM. Among the genotypes at 0.7 mM, Khirman (Check) maintained K accumulation (18.58 mg g^{-1} . dry wt) and exhibited low reduction of 4.75% under drought stress, while the genotype TD-1(23.18 mg g^{-1} . dry wt) depicted significantly ($p \leq 0.05$) higher K accumulation corresponding to 5.11% more K accumulation under drought stress. Similarly at 1.44 mM the genotype TD-1 accumulated significantly higher K (24.92 mg g^{-1} . dry wt.) under drought, showing minimum reduction of 0.82%, while three genotypes namely NIA-Sarang (16.85 mg g^{-1} . dry wt.), NIA-Sundar (21.60 mg g^{-1} . dry wt) and Khirmam (check) (21.68 mg g^{-1} . dry wt.) accumulated 4.46, 1.97 and 18.84% more K, respectively under water stress.

Table 1. Chlorophyll accumulation (mg g^{-1} . F.wt) of wheat genotypes as influenced by foliar use of SA (0.7 & 1.44 mM) under drought stress.

Genotypes	No spray		R.D/ R.I (%)	SA (0.7 mM)		R.D/ R.I (%)	SA (1.44 mM)		R.D/ R.I (%)
	Control	Drought		Control	Drought		Control	Drought	
	NIA-Sarang	0.158 ab	0.126 ab	20.25	0.166 abc	0.116 de	29.99	0.178 abc	0.141 f
NIA-Sundar	0.194 a	0.144 ab	26.04	0.160 abc	0.143 cd	10.81	0.190 ab	0.159 cdef	16.18
NIA-Zarkhaiz	0.192 ab	0.144 ab	24.84	0.167 abc	0.112 e	33.05	0.177 bc	0.149 def	15.68
TD-1	0.181 ab	0.115 b	36.46	0.174 ab	0.137 cde	21.09	0.170 bcd	0.147 ef	13.35
Khirman Check)	0.196 a	0.201 a	(2.86)	0.187 a	0.147 bc	21.39	0.200 a	0.164 cde	18.03
Mean	0.184	0.146		0.171	0.131		0.183	0.152	
HSD _{0.05} for Treat X Geno.	0.0778			0.0303			0.0221		

Means Means followed by similar alphabets in the column do not differ significantly at $p \leq 0.05$

Values Values in the parenthesis denote percentage increase over control

R.D/R.I = Relative dec./ Relative Inc

Table 2. Osmotic potential (-MPa) of wheat genotypes as influenced by foliar use of SA (0.7 & 1.44 mM) under drought stress.

Genotypes	No spray		R.D (%)	SA (0.7 mM)		R.D (%)	SA (1.44 mM)		R.D (%)
	Control	Drought		Control	Drought		Control	Drought	
	NIA-Sarang	0.59 b	1.66 a	180.35	0.85 c	1.70 ab	99.23	0.79 d	1.64a
NIA-Sundar	0.78 b	1.51 a	94.41	0.84 c	1.49 b	77.08	0.77 d	1.33 ab	71.56
NIA-Zarkhaiz	0.78 b	1.43 a	83.33	0.80 c	1.59 b	97.93	0.86 cd	1.33 ab	54.65
TD-1	0.82 b	1.48 a	81.62	0.83 c	1.95 a	134.54	0.80 d	1.23 bc	53.34
Khirman Check)	0.69 b	1.35 a	95.65	0.71 c	1.87 a	164.14	0.75 d	1.48 ab	97.33
Mean	0.73	1.49		0.81	1.72		0.79	1.40	
HSD _{0.05} for Treat X Geno.	0.3933			0.2714			0.3853		

Means followed by similar alphabets in the column do not differ significantly at $p \leq 0.05$ R.D = Relative Inc.

Table 3. Proline accumulation ($\mu \text{mol g}^{-1}$.F.wt) of wheat genotypes as influenced by foliar use of SA (0.7 & 1.44 mM) under drought stress.

Genotypes	No spray		Fold	SA (0.7 mM)		Fold	SA (1.44 mM)		Fold
	Control	Drought		Control	Drought		Control	Drought	
	NIA-Sarang	3.40 c	70.02 a	20.58	2.41 c	65.97 ab	27.37	4.39 d	74.34 a
NIA-Sundar	0.82 c	73.64 a	89.80	0.33 c	88.00 a	264.26	0.22 d	59.64ab	271.08
NIA-Zarkhaiz	2.38 c	67.28 a	28.27	1.37 c	81.75 ab	59.67	0.74 d	50.03 b	67.61
TD-1	4.45 c	45.94 b	10.32	4.98 c	52.95 b	10.63	3.08 d	73.44 a	23.82
Khirman Check)	1.34 c	70.20 a	52.51	0.98 c	84.23 ab	85.68	1.60 d	28.85 c	18.03
Mean	2.48	65.42		2.01	74.58		2.01	57.26	
HSD _{0.05} for Treat X Geno.	20.78		32.267	19.155					

Means followed by similar alphabets in the column do not differ significantly at $p \leq 0.05$

Fold indicates increase in the proline accumulation in the drought over control

Table 4. Potassium accumulation (mg g⁻¹, dry wt) of wheat genotypes as influenced by foliar use of SA (0.7 & 1.44 mM) under drought stress.

Genotypes	No spray		R.D/ R.I (%)	SA (0.7 mM)		R.D/ R.I (%)	SA (1.44 mM)		R.D/ R.I (%)
	Control	Drought		Control	Drought		Control	Drought	
NIA-Sarang	21.34 ab	15.90 c	25.50	17.86cde	15.77e	11.70	16.13d	16.85 d	(4.46)
NIA-Sundar	21.93 ab	18.37bc	16.22	19.22bcd	17.49de	9.00	21.18 bc	21.60 b	(1.97)
NIA-Zarkhaiz	20.51 ab	20.04ab	2.28	20.70abc	18.29cde	11.63	20.23 bc	19.97 bc	1.30
TD-1	23.71 a	22.71 a	4.22	22.05ab	23.18 a	(5.11)	25.12 a	24.92 a	0.82
Khirman Check)	20.00 ab	18.26 bc	8.70	19.51abc	18.58cde	4.75	18.24 cd	21.68 b	(18.84)
Mean	21.498	19.056		19.868	18.662		20.18	21.004	
HSD _{0.05} for Treat X Geno.	3.8681		3.0256	3.0256					

Means followed by similar alphabets in the column do not differ significantly at $p \leq 0.05$

Values in the parenthesis denote percentage increase over control

R.D/R.I = Relative dec./ Relative Inc.

Table 5. Grain yield (g plant⁻¹) of wheat genotypes as influenced by foliar use of SA (0.7 & 1.44 mM) under drought stress.

Genotypes	No spray		R.D (%)	SA (0.7 mM)		R.D (%)	SA (1.44 mM)		R.D (%)
	Control	Drought		Control	Drought		Control	Drought	
NIA-Sarang	10.85 a	3.06 d	71.83	6.34 bcd	4.59 cd	27.64	6.23 bcd	4.79 cd	23.11
NIA-Sundar	9.99 a	4.53 cd	54.67	14.96 a	3.40 d	77.28	11.81 a	3.41 d	71.13
NIA-Zarkhaiz	9.04 ab	4.11 cd	54.57	8.86 b	2.71 d	69.38	8.52 abc	3.36 d	60.56
TD-1	8.50 abc	2.94 d	65.40	7.74 bc	5.47 bcd	29.40	10.30 a	3.13 d	69.59
Khirman Check)	12.14 a	4.68 bcd	61.46	15.45 a	3.04 d	80.32	8.93 ab	5.70 bcd	36.17
Mean	10.10 a	3.86 b	61.58	10.67 a	3.84 b	56.80	9.16 a	4.08 b	52.11
HSD _{0.05} for Treat X Geno.	4.47			3.66			3.80		

Means followed by similar alphabets in the column do not differ significantly at $p \leq 0.05$

R.D = Relative dec.

Hasanuzzaman *et al.*, (2014) augmented that occurrence of water stress distressed the ionic equilibrium due to which physiological process of the plant was affected. Our findings for low decrease/increase of K accumulation at the foliar spray of SA (0.7 mM and 1.44 mM) under water stress are in line with the findings of Noreen *et al.*, (2017), who also found increased uptake of K⁺ ion at exogenous application of SA. Similar findings were also observed by Hussain *et al.*, (2010) in maize and barley. Noreen & Ashraf (2008) augmented that ameliorative impacts of SA (low decrease at 0.7 & 1.44 mM and/or higher accumulation as compared to no spray) on the growth and physiology of plants under abiotic stress might be due to its role in nutrient uptake. The increase in drought tolerance in the crop plant due to K accumulation might be due to the role of the potassium as an in-organic osmoticum. Wang *et al.*, (2013) have already documented a close relationship between K-nutritional status and plant drought tolerance.

The table 5 showed the grain yield (g plant⁻¹) as affected by foliar use of SA (0.7 and 1.44 mM) under drought. Large reduction (54.57-71.83%) in grain yield was observed at no spray under water stress. At the foliar application of SA with 0.7 mM, two genotypes namely, NIA-Sarang (27.64%) and TD-1 (29.40%) revealed relatively less reduction as compared to other genotypes. Similarly at 1.44 mM, two genotypes NIA-Sarang (23.11%) and Khirman (36.17%) depicted low reduction under drought stress. In case of grain yield at 0.7 and 1.44 mM SA large genotypic variation was observed. Improvement in grain yield (comparatively low reduction under drought in comparison with control) at the foliar use of SA under drought has been reported by many workers (Kareem *et al.*, 2017; Maghsoudi *et al.*, 2019). Leeuwen *et al.*, (2007) reported that in hyper-responsive genotypes, more genes would be expected to change their expression levels in response to SA treatment compared with hypo-responsive genotypes. The relatively less reduction of two

genotypes (NIA-Sarang and TD-1) at 0.7 mM and (NIA-Sarang and Khirman) at 1.44 mM might be attributable due to the activation of hyper-responsive gene to the SA treatment in these genotypes.

The low reduction (higher yield under drought stress) may also be attributable due to increased behavior of antioxidant enzymes SOD and POD (data not presented) under foliar use of SA (Tasgin *et al.*, 2003).

Conclusion

Generally differential responses and wide genetic/genotypic variation for the foliar spray of SA were observed. However at 0.7 mM NIA-Sarang and TD-1 and at 1.44 mM NIA-Sarang and Khirman performed well under water stress conditions.

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