

SALICYLIC ACID MEDIATED PHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS TO CONFER DROUGHT STRESS TOLERANCE IN *ZINNIA* (*Z. ELEGANS*)

ALTERACIONES FISIOLÓGICAS Y BIOQUÍMICAS MEDIADAS POR ÁCIDO SALICÍLICO PARA CONFERIR TOLERANCIA AL ESTRÉS POR SEQUÍA EN *ZINNIA* (*Z. ELEGANS*)

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Abstract

Background: Protective role of salicylic acid against drought has been widely reported, but its effects on *Zinnia elegans* remain unknown.

Hypotheses: Foliar salicylic acid (SA) improves water status, pigments, and antioxidant systems of *Zinnia* plants under drought stress.

Studied species: *Z. elegans* (cv. Dreamland) were purchased from Chanan Seeds Store Lahore, Pakistan.

Study site and dates: MNS University of Agriculture, Multan, Punjab, Pakistan; March-May, 2018.

Methods: After two weeks of transplanting, one set of seedlings was exposed to drought stress (60 % field capacity, FC) while other control set was maintained at 100 % FC. Foliar SA (100 mg L⁻¹) was applied to plants at drought initiation.

Results: Foliar SA treatment to water stressed plants (60 % FC) increased the leaf area (46.89 cm²), shoot and root lengths (25 cm and 26.67 cm, respectively), leaf relative water content (75.98 %); chlorophyll *a* (0.68 mg g⁻¹), chlorophyll *b* (0.38 mg g⁻¹) and carotenoid concentrations (1.10 mg g⁻¹); and catalase (116.67 U min⁻¹ mg⁻¹ protein), guaiacol peroxide (72 U min⁻¹ mg⁻¹ protein) and superoxide dismutase (93 U min⁻¹ mg⁻¹ protein) activities.

Conclusions: Foliar SA could be used to minimize impacts of water stress in *Zinnia* plants.

Keywords: Drought tolerance, Salicylic acid, Pigments, Antioxidant machinery, *Zinnia*.

Resumen

Antecedentes: Se ha informado ampliamente sobre el papel protector del ácido salicílico (AS) contra la sequía, pero aún se desconocen sus efectos sobre *Zinnia elegans*.

Hipótesis: El AS foliar mejora el estado del agua, los pigmentos y los sistemas antioxidantes de las plantas de *Zinnia* bajo estrés por sequía.

Especies estudiadas: *Z. elegans* (cv. Dreamland) se adquirió en Chanan Seeds Store Lahore, Pakistán.

Lugar de estudio y fechas: Universidad de Agricultura MNS, Multan, Punjab, Pakistán; marzo-mayo, 2018.

Métodos: Después de dos semanas del trasplante, un grupo de plántulas se expuso a estrés por sequía (60 % de la capacidad de campo, CC) mientras que el otro grupo de control se mantuvo al 100 % de CC. Se aplicó AS foliar (100 mg L⁻¹) a las plantas al inicio de la sequía.

Resultados: El tratamiento foliar con AS a plantas con estrés hídrico (60 % CC) incrementó el área foliar (46.89 cm²), la longitud de tallo y de raíz (25 cm y 26.67 cm, respectivamente), contenido relativo de agua de la hoja (75.98 %); concentraciones de clorofila *a* (0,68 mg g⁻¹), clorofila *b* (0,38 mg g⁻¹) y carotenoides (1,10 mg g⁻¹) y la actividad de la catalasa (116,67 U min⁻¹ mg⁻¹ de proteína), el peróxido de guayacol (72 U min⁻¹ mg⁻¹ de proteína) y el superóxido dismutasa (93 U min⁻¹ mg⁻¹ de proteína).

Conclusiones: AS foliar podría usarse para minimizar los impactos del estrés hídrico en las plantas de *Zinnia*.

Palabras clave: Ácido salicílico, Maquinaria antioxidante, Pigmentos, Tolerancia a la sequía, *Zinnia*.

Z*innia* (*Z. elegans*) is one of the most popular cut flowers and bedded plant of the summer season in Pakistan, partly because of its diverse color range and potential to survive drought periods in arid regions (Riaz *et al.* 2013). Most of the tall varieties are used as cut flowers and dwarf varieties as potted plants. The long stem (15-100 cm) and reasonable shelf life have resulted in increased demand for tall cultivars popular as cut flowers (Ahmad & Dole 2014).

Plants for flower production are mostly grown in pots where irrigation management is more critical due to limited soil volume (Grant *et al.* 2012). The drought stress exposure restricts the water supply to plant rhizosphere that results in reduced root growth and prompted flower abortion. Nutrient uptake and net photosynthesis declines (Praba *et al.* 2009, Anjum *et al.* 2011). The production of reactive oxygen species (ROS) increases, resulting in increased damage to soluble proteins, lipids, ATP synthesis, and the photo-stabilizing efficiency of chlorophyll (Shehzad *et al.* 2020). Plants reduce impacts of drought by developing extensive root systems, leaf rolling to reduce stomatal water loss, and lowering tissue water potential through the accumulation of organic osmolytes and phytohormones (Forni *et al.* 2017). Furthermore, supplemental use of nutrients and growth regulators is considered essential for alleviating the drought effects, particularly, in flowering plants (Prerostova *et al.* 2018, Waqas *et al.* 2019).

Salicylic acid (SA) is an important phytohormone that serves as signaling molecule in drought mediated resistance mechanisms of plants (Pieterse *et al.* 2012). Exogenous SA is an effective non-enzymatic antioxidant that relieves the plants from drastic effects of drought through its involvement in vital physiological processes (Belkadhi *et al.* 2014). The up-regulation of secondary metabolites and stomatal closure, and reduction in transpiration rate is also simulated by SA (Khan *et al.* 2015). Ghasemi *et al.* (2016) and Shahmoradi & Naderi (2018) showed positive effects of supplemental SA in improving growth rate in jasmine (*Jasminum nudiflorum* L.), amaranth (*Amaranthus tricolor* L.), starflower (*Borago officinalis* L.) and chamomile (*Matricaria chamomilla* L.) under drought conditions. In fact, SA application directly influences the cellular contents and volume of chloroplast to overcome chlorophyll degradation and fragmentation in drought-prone plants (Uzunova & Popova 2000, Cheng *et al.* 2016). Positive effects of SA in reducing the impact of stress conditions may vary with the application rate and timing, and environmental growth conditions. SA supplementation directly influences the expansion of grana, coagulation of stroma, and increases the chloroplast volume to overcome chlorophyll degradation and fragmentation under drought stress (Uzunova & Popova 2000, Cheng *et al.* 2016).

The up-regulation of expression of stress-responsive genes involved in signal transduction, photosynthesis and protein metabolism under drought stress also attributed to SA supplementation (Kang *et al.* 2012). Recent study involving various petunia varieties also showed that SA application helped to maintain photosynthetic pigments by decreasing electrolyte leakage in drought stressed seedlings. Habibi (2012) also proposed that SA contributes to photoprotection and maintains photosynthetic pigments in plants under drought stress. Similarly, Aldesuquy *et al.* (2012) suggested that enhanced drought tolerance in drought-prone plants treated with SA was correlated with improved photosynthetic activity.

The positive effects of exogenous SA on growth and regulatory mechanisms in plants have been well reported, but flowering plants like *Zinnia* still are scant. The present study focused on the major physiological and biochemical processes that are considered vital for improved drought tolerance characteristics in flowering plants. A better understanding of such physio-biochemical stress regulatory mechanisms would contribute to reducing drought-induced losses in ornamental *Zinnia*.

Materials and methods

Experimental setup. Healthy, uniform seeds of *Z. elegans* var. 'Dreamland' was purchased at Chanan Seeds Store Lahore, Pakistan and sown in 24 cm diameter, 30 cm high earthen pots at MNS-University of Agriculture, Multan, Punjab, Pakistan. The earthen pots were filled with 3 kg air-dried and sieved (2 mm mesh) mixture of silt (sweet soil) and slug (70:30 w/w), with pH 8.0, organic matter 0.39 %, electrical conductivity (ECe) 10.91 mS cm⁻¹, saturation 18 %, total available N 0.021 %, available P 5.20 mg kg⁻¹ and available K 110 mg kg⁻¹. The pots were placed under natural conditions from March 20th to May 20th, 2018. The environmental variables (air temperature, and relative humidity) are presented in [Figure 1](#). Seedlings

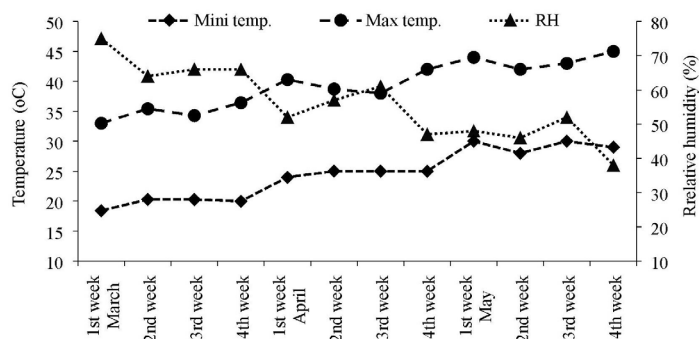


Figure 1. The maximum and minimum air temperature and relative humidity during the growth period of experimentation.

at the 2-4 leaf stage (30 days after sowing) were transplanted to earthen pots of similar size and allowed to establish for 15 days before the initiation of drought stress. Fresh ground water ($EC\ 642\ \mu S\ cm^{-1}$) was used for irrigation. All cultural practices like hoeing, weeding, and fertilizing were evenly applied on all treatments throughout the study. The experiment comprised of four treatments, arranged in completely randomized design (CRD), and repeated four times with one plant per replicate.

Drought stress and SA application. Two soil moisture treatments of 100 % (2,590 ml / pot) and 60 % (1,554 ml / pot) field capacity (FC) were maintained using the gravimetric method (Shehzad *et al.* 2020). After one-week foliar applications of SA ($100\ mg\ L^{-1}$) ($C_7H_6O_3$; Purity $\geq 99\%$; Sigma-Aldrich Ltd. USA), with Tween-20 (0.1 %), were applied to half the plants in each soil moisture treatment (repeated one week later) and the other half (control) were sprayed with distill water + Tween 20.

Biomass. The number of leaves (NOL) of each plant were counted manually and leaf area (LA) was calculated using a portable leaf area meter (Model CI-202, CID Inc. USA). At the end of experiment, the plants were cut at soil level to measure shoot length (SL), shoot fresh and dry weight (SFW & SDW), and root fresh and dry weights (RFW & RDW). The roots were cleaned by careful rinsing in water and root length (RL) measured by measuring scale. Shoots and roots were placed in the oven at $65\ ^\circ C$ for 72 h to determine the dry weight of shoots and roots.

Leaf water status. To measure leaf relative water content (RWC), young fully expanded leaves from each treatment were selected and the fresh weight (FW) immediately recorded. The leaves were then soaked in autoclaved water at $4\ ^\circ C$ for 24 h to determine turgid weight (TW). They were then dried at $65\ ^\circ C$ for 72 h to estimate dry weight (DW). RWC was calculated using the formula 1 (Mayak *et al.* 2004).

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100. \quad (1)$$

For excised leaf water retention (ELWR), young leaves from each treatment were weighed (FW) and incubated at room temperature ($25\ ^\circ C$) for six hours and the leaf wilted weight (LW) recorded following the formula 2 (Lonbani & Arzani (2011)

$$ELWR\ (\%) = \left[1 - \frac{(FW - LW)}{FW} \right] \times 100. \quad (2)$$

Chlorophyll pigments concentration. The stored leaves ($-80\ ^\circ C$) of each treatment (0.5 g) were thawed in ice buckets, then chopped and extracted in 5 mL acetone solution (80 %) at $4\ ^\circ C$ overnight to determine the chlorophyll (Chl *a*, and *b*) and carotenoids (Car) concentrations (Taiz & Zeiger 2002). The extract was centrifuged for 15 min at $4\ ^\circ C$, and absorbance measured at 645, 663 and 680 nm wavelength using a double beam spectrophotometer (Pharmacia, LKB-Novaspec II). The leaf Chl *a*, *b* and Car contents were calculated using the formulas 3, 4 and 5 respectively.

$$Chl\ a\ (mg\ g^{-1}) = 12.7\ (OD_{663}) - 2.69\ (OD_{645}) \times \frac{V}{1000} \times W. \quad (3)$$

Effect of salicylic acid on *Zinnia* under drought stress

$$\text{Chl } b \text{ (mg g}^{-1}\text{)} = 22.9 \text{ (OD645)} - 4.68 \text{ (OD663)} \times \frac{V}{1000} \times W \quad (4)$$

$$\text{Car (}\mu\text{g g}^{-1}\text{)} = \frac{A^{\text{car}}}{E_{\text{max}}^{100}} \quad (5)$$

Antioxidant enzymes. Catalase (CAT) activity was measured following the procedure of Chance & Maehly (1955). The methods of Nakano & Asada (1981) and Elia *et al.* (2003) were used to measure the guaiacol peroxidase (GPX) activity, and SOD activity was measured following the method of Ekler *et al.* (1993).

Statistical analysis. Two-way-factorial (water stress by SA) analysis was employed to statistically analyze the data using STATISTICA Computer Software (Version 8.1) through ANOVA technique. LSD test at 5% probability was employed to compare the treatments' means. A correlation analysis was also done using STATISTICA to find the strong relationship among different variables under drought stress conditions.

Results

Biomass. Drought stress markedly reduced the NOL and LA of *Zinnia* by 12.66 and 31.21 cm², respectively (Table 1). Foliar SA application (100 mg L⁻¹) decreased the negative effects of water stress by increasing the NOL (15.33), LA (46.89 cm²). A significant reduction in SL (21.67 cm) and RL (18.33 cm) was recorded under water stress whereas, SA application (100 mg L⁻¹) improved SL and RL to 25 cm and 26.67 cm respectively. Under water stress conditions, the plants of *Zinnia* supplemented with 100 mg L⁻¹ SA produced maximum SFW (7.60 g) and SDW (0.81 g), whereas minimum SFW (5.60 g) and SDW (0.59 g) in nontreated plants. Likewise, water stress also reduced the RFW, and RDW by 1.22 g and 0.30 g, respectively. In the water-stressed plants SA treatment increased the RFW and RDW by 1.45 g and 0.41 g respectively (Table 1).

Table 1. Effect of foliar applied salicylic acid on number of leaves (NOL), leaf area (LA), shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) in *Zinnia elegans* under drought stress.

Drought stress/Salicylic acid	NOL	LA (cm ²)	SL (cm)	RL (cm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Normal conditions								
N	20.33±0.90 ^a	58.78±1.74 _a	30.33±1.89 ^a	18.67±0.90 ^b	9.81±0.33 ^b	1.27±0.04 ^a	1.72±0.03 ^a	0.47±0.02 ^a
SA	19.67±1.22 ^a	63.24±1.39 _a	34.33±1.22 ^a	19.66±1.22 ^b	11.47±0.47 ^a	1.34±0.03 ^a	1.71±0.02 ^a	0.52±0.01 ^a
Drought stress								
N	12.66±1.23 ^b	31.21±2.27 _c	21.67±1.21 ^b	18.33±1.80 ^b	5.60±0.73 ^b	0.59±0.02 ^c	1.22±0.07 _c	0.30±0.03 ^c
SA	15.33±0.68 ^b	46.89±2.18 _b	25.00±1.56 ^b	26.67±1.24 ^a	7.60±0.43 ^c	0.81±0.04 ^b	1.45±0.03 ^b	0.41±0.02 ^b
<i>P</i> -value								
D	0.0004	< 0.0001	0.0003	0.0509	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SA	0.3528	0.0007	0.0375	0.0124	0.0066	0.0032	0.0069	0.0026
D × SA	0.1388	0.0181	0.8265	0.0356	0.7536	0.0659	0.0050	0.0791
CV ^a	10.33	6.55	9.16	12.08	10.10	5.95	3.41	7.05

Values are mean ± SE and letters represent significant differences at *P* < 0.05 according to LSD test. N, normal water application; SA, salicylic acid under normal water application; D, drought stress without salicylic acid; D+SA, salicylic acid under drought stress. ^aCV, Coefficient of variation.

Leaf water content. SA treated *Zinnia* plants retained maximum 88.46 % and 75.98 % leaf RWC both under normal (100 % FC) and water stress (60 % FC) conditions, respectively (Figure 2A). Whereas, minimum 66.41 % leaf RWC was recorded in water stress *Zinnia* plants. ELWR was also maximum 23.82 % and 31.15 % in SA treated *Zinnia* plants grown under normal (100 % FC) and water stress (60 % FC) conditions respectively. Water stress reduced ELWR of *Zinnia* plants by 22.67 % (Figure 2B).

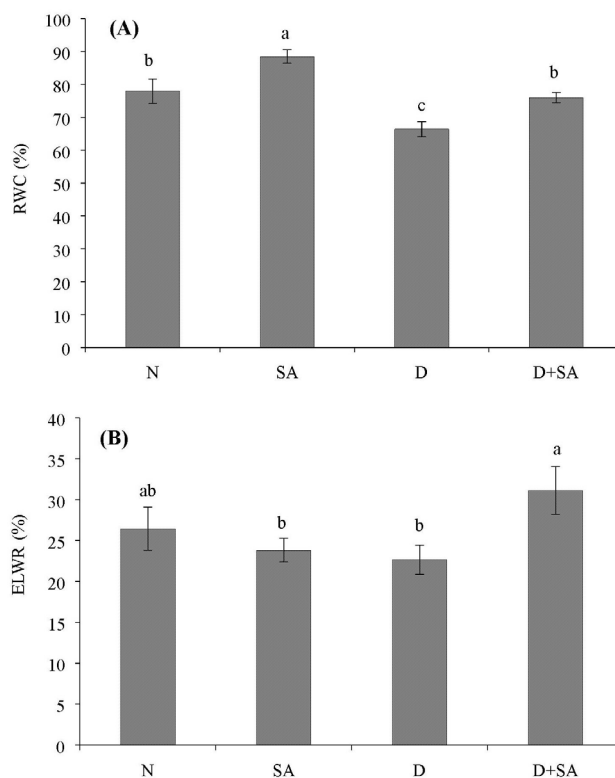


Figure 2. Effect of foliar applied salicylic acid on leaf relative water contents (RWC) (A) and excised leaf water retention (ELWR) (B) in *Zinnia elegans* under drought stress. Values are mean \pm SE and letters above the bars represent significant differences at $P < 0.05$ according to LSD test. N, normal water application; SA, salicylic acid under normal water application; D, drought stress without salicylic acid; D+SA, salicylic acid under drought stress.

Pigments content. Water stress (60 % FC) reduced Chl_a , Chl_b and Car concentrations by 0.52, 0.25 and 0.96 mg g^{-1} respectively (Figure 3A-B-C). SA treatment of the water stressed treatments resulted in higher leaf Chl_a (0.68 mg g^{-1}), Chl_b (0.38 mg g^{-1}) and Car (1.10 mg g^{-1}) concentrations. Well water (100 % FC) *Zinnia* plants treated with SA improved Chl_a , Chl_b and Car to 0.83, 0.50 and 1.27 mg g^{-1} respectively.

Antioxidant activity. Water stress (60 % FC) significantly ($P < 0.05$) increased the activities of the antioxidant-related enzymes of CAT, GPX and SOD by 91.33 mg g^{-1} , 46.33 $\text{U min}^{-1} \text{mg}^{-1}$ protein and 74.33 $\text{U min}^{-1} \text{mg}^{-1}$ protein respectively (Figure 4A-C). Foliar applied SA (100 mg L^{-1}) of the water stressed treatments (60 % FC) resulted in considerably improved activity of 116.67 mg g^{-1} , 72 $\text{U min}^{-1} \text{mg}^{-1}$ protein and 93 $\text{U min}^{-1} \text{mg}^{-1}$ protein for CAT, GPX and SOD respectively. Foliar SA application also produced maximum CAT (78.07 mg g^{-1}), GPX (35.33 $\text{U min}^{-1} \text{mg}^{-1}$ protein) and SOD (58.33 $\text{U min}^{-1} \text{mg}^{-1}$ protein) in well water (100 % FC) *Zinnia* plants.

The leaf RWC and ELWR was positively correlated with CAT and GPX activities in SA treated plants, but there was no significant correlation with SOD activity under water stress conditions (Table 2). A strong and positive cor-

relation of Chl_a and Chl_b concentrations with LA and SDW was noted in the water stress treatments, but not with Car. Similarly, SA treatment of the water stressed plants showed strong positive correlations with CAT activity, and GPX with LA and SDW while non-significant for SOD.

Discussion

Drought induced growth inhibition of annual flowering (Kaur *et al.* 2015), however, there is little information on the effects of stress ameliorants on physiological processes of summer annuals plants. The present study identified an effective approach to improve plant growth of *Zinnia* under water stress.

Table 2. Correlation (r) among different *Zinnia elegans* traits following salicylic acid (SA) under drought stress conditions.

X-variable	Y-variable	Drought stress
Water relations		
ELWR	CAT	0.81*
	GPX	0.80*
	SOD	0.67 ^{ns}
RWC	CAT	0.88*
	GPX	0.95**
	SOD	0.77 ^{ns}
Pigments		
Chl <i>a</i>	LA	0.90*
	SDW	0.82*
Chl <i>b</i>	LA	0.92**
	SDW	0.78 ^{ns}
Car	LA	0.78 ^{ns}
	SDW	0.65 ^{ns}
Antioxidants		
CAT	LA	0.89*
	SDW	0.85*
GPX	LA	0.98***
	SDW	0.96**
SOD	LA	0.90*
	SDW	0.76 ^{ns}

LA, leaf area; SDW, shoot dry weight; CAT, catalase; GPX, guaiacol peroxidase; SOD, superoxide dismutase; ELWR, excised leaf water retention; RWC, relative water contents; chl, chlorophyll *a*, *b*; Car, carotenoids. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, non-significant

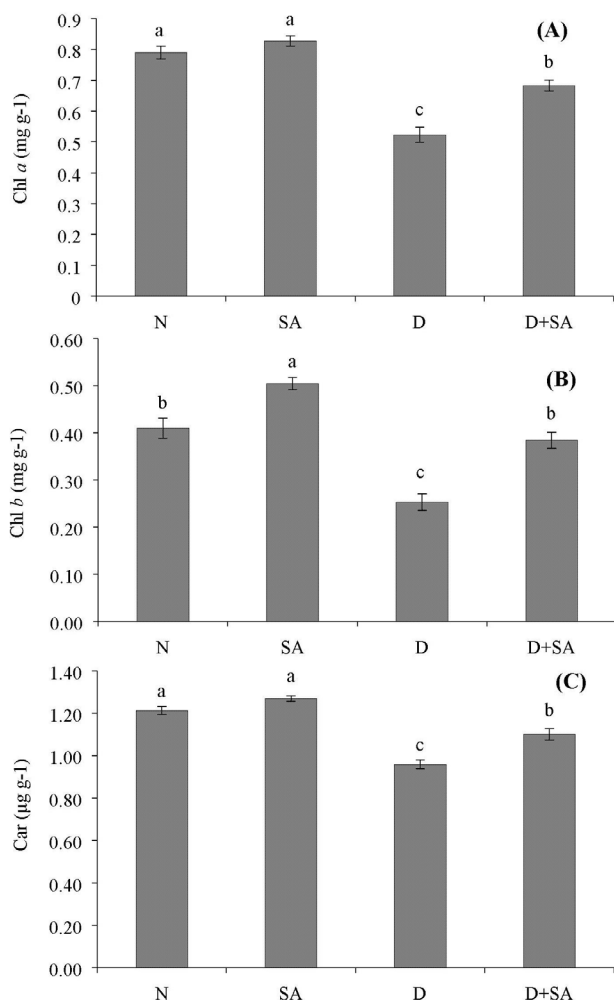


Figure 3. Effect of foliar applied salicylic acid on chlorophyll (*a*, *b*, A,B), and carotenoids (Car, C) contents in *Zinnia elegans* under drought stress. Values are mean \pm SE and letters above the bars represent significant differences at $P < 0.05$ according to LSD test. N, normal water application; SA, salicylic acid under normal water application; D, drought stress without salicylic acid; D+SA, salicylic acid under drought stress.

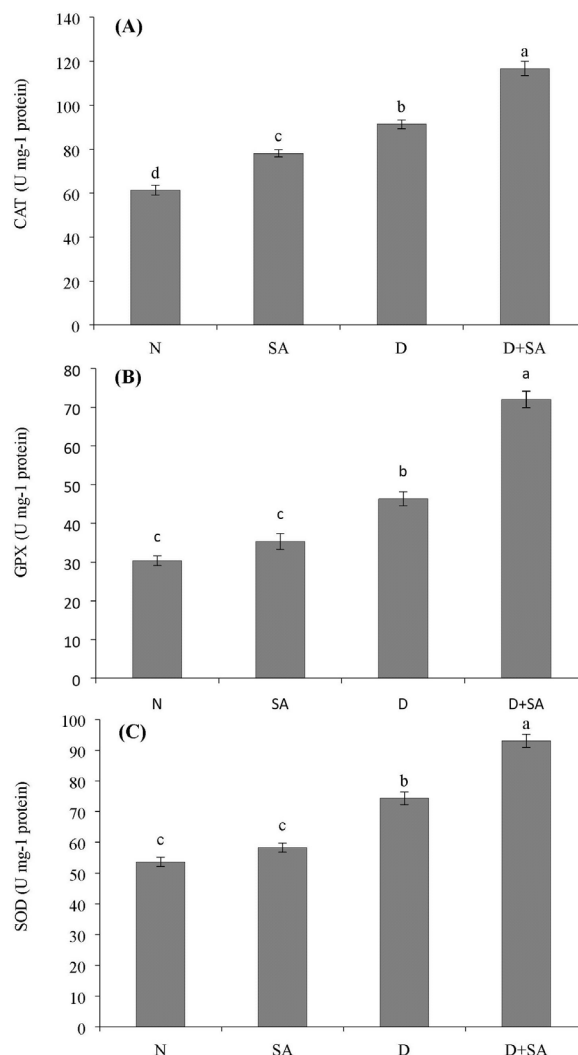


Figure 4. Effect of foliar applied salicylic acid on catalase (CAT, A), guaiacol peroxidase (GPX, B), and superoxide dismutase (SOD, C) in *Zinnia elegans* under drought stress. Values are mean \pm SE and letters above the bars represent significant differences at $P < 0.05$ according to LSD test. N, normal water application; SA, salicylic acid under normal water application; D, drought stress without salicylic acid; D+SA, salicylic acid under drought stress.

The present study showed that SA-mediated alterations in physiological processes markedly increased the growth attributes of *Zinnia* under water stress conditions. A considerable decline in growth of *Zinnia* under drought stress might be due to a decrease in water relations resulting in significant loss of turgor, thereby preventing the transport of nutrients and photosynthates to and from the leaves (Shehzad *et al.* 2020). The leaves are one of the primary plant organs that are most vulnerable to severe effects of drought stress. The immediate plant responses to drought stress involve physiological and metabolic changes in leaves (Conti *et al.* 2019). It was also evident by the reduction in NOL and LA in current study that drought stress significantly reduced photosynthetic capacity causing less CO₂ fixation and biomass production. Similar effects have been reported for calendula (*Calendula officinalis*), brazilian cherry (*Eugenia uniflora*), purple passionflower (*Passiflora incarnate*), and red tip photinia (*Photinia \times fraseri*) (Toscano *et al.* 2016, Garcia-Castro *et al.* 2017, Akhtar *et al.* 2019).

Exogenous SA application induces diverse physiological adaptations in plants in relation to water stress (Zarghami *et al.* 2014). These are associated with biochemical changes responsible for growth inhibition under water stress (Li *et al.* 2014). In current study, foliar SA application mitigated the effects of water stress by enhancing LA, SFW and SDW. This was likely via increasing photosynthetic capacity since SA plays a critical role in maintaining stomatal regulation and biosynthesis of photo-assimilates (Fakheri *et al.* 2019).

It was found that the positive effects of SA were also evident on root biomass and root length, possibly through a reduction in reactive oxygen species (ROS) production in roots (Tamas *et al.* 2015) and increased synthesis of osmolytes such as proline and free fatty acids that promote root growth (Azooz & Youssef 2010, Chen *et al.* 2014). Our findings are similar to those of Hosseini *et al.* (2015), which showed SA reduces electrolyte leakage and increases biomass, pigments and antioxidative enzymes activity in lolium (*Lolium perenne*) under water stress conditions.

Leaf RWC and ELWR can decrease significantly in various chive, turfgrass, and shrubs species subjected to water stress (Toscano *et al.* 2016). The decreased leaf RWC and ELWR in drought stressed *Zinnia* might be associated to protoplasm dehydration (Shabbir *et al.* 2016), damages to cell wall through lipid peroxidation (Cechin *et al.* 2015), reduction in photosynthetic capacity (Hussain *et al.* 2016), or increased water retention and enhanced stomatal closure (Miura & Tada 2014). Our study showed a marked effect of SA in maintenance of leaf RWC. Previous studies showed that SA positively interacts with abscisic acid (ABA) signaling pathways to induce stomatal closure, thereby improving drought tolerance in plants. In addition, SA application promotes root growth (also observed in the present study) which will improve water absorption and availability to the shoots

Estimating photosynthetic pigment concentrations including leaf Chl_a, Chl_b and Car provides a good indication of drought-induced damage to photosynthetic activity (Pellegrini *et al.* 2011, Toscano *et al.* 2016). Drought reduced photosynthetic pigments in callistemon (*Callistemon citrinus*) (Álvarez *et al.* 2011), marigold (*Tagetes erecta*) (Riaz *et al.* 2013) phoebe (*Phoebe bournei*) (Ge *et al.* 2014), and sunflower (*Helianthus annuus*) (Shehzad *et al.* 2020). Similarly, Aldesuquy *et al.* (2012) suggested that enhanced drought tolerance in plants treated with SA was correlated with improved photosynthetic activity. Similar results were observed in the current study for Chl_a, Chl_b and Car. Foliar SA application substantially improved the pigments in *Amaranthus tricolor* and *Borago officinalis*, and *Hordeum vulgare* (Habibi 2012, Khandaker *et al.* 2011, Shemi *et al.* 2021). The protective role of SA during water stress involves regulation of metabolic reactions responsible for maintenance of membrane integrity and photosynthetic pigments (El-Tayeb 2005). Moreover, SA directly influences the expansion of grana, coagulation of stroma and increases the volume of the chloroplast to reduce chlorophyll degradation and fragmentation under water stress (Uzunova & Popova 2000, Cheng *et al.* 2016), and stimulates the up-regulation of stress-responsive genes involved in signal transduction, photosynthesis and protein metabolism under water stress (Kang *et al.* 2012).

Exposure to water stress promotes intracellular ROS accumulation that induces oxidative stress and significantly damages membrane integrity (Miura & Tada 2014, Nawaz *et al.* 2015). Excessive ROS also triggers ABA production that influences K⁺ transport in guard cells to cause stomatal closure (Kwak *et al.* 2003), thereby reducing CO₂ assimilation and decreasing biomass production in *Zinnia* (Ge *et al.* 2014). However, excessive SA application may also promote ROS production by decreasing the ability of antioxidative enzymes to overcome ROS (Miura & Ohta 2010). Hence, it is crucial to apply the appropriate SA dose to improve stress tolerance in plants. In the present study, foliar SA (100 mg L⁻¹) was found effective to reduce the damaging effects of water stress in *Zinnia*, as evidenced by the increased activities of CAT, GPX and SOD. The accumulation of CAT, SOD, GPX as well as heat shock proteins (HSP) provide an ecological adaptation against ROS-induced oxidative stress (Ramel *et al.* 2012, Nawaz *et al.* 2015). Increased antioxidant-related enzymes activity would likely have contributed to lower ROS accumulation and the increased biomass production observed in this study. Similar responses have been observed on tomato (Hayat *et al.* 2008), sugar beet (Salami & Saadat 2013), zoysia grass Chen *et al.* (2014), and jasmine (Shahmoradi & Naderi 2018).

Taken together, the current study is the first report on SA mediated physiological and biochemical processes to improve drought tolerance in *Zinnia*. A marked reduction in growth was observed in *Zinnia* upon exposure to water stress; however, foliar SA applications significantly mitigated the adversities of water stress through improved leaf

water status, photosynthetic pigments, and antioxidant-related enzymes. Our results provide evidence that SA application is an effective, efficient, and feasible approach to reduce water stress in annual flowers like *Zinnia*. The results would be of relevance to the floriculture industry as well as researchers and breeders interested in developing drought tolerant annual flowers.

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