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Original Research

Enhancement of Physiological and Biochemical Attributes of Okra by Application of Salicylic Acid under Drought Stress

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ABSTRACT

Horticultural crops especially vegetables are most vulnerable to drought due to their higher irrigation needs. Hence, this study was undertaken to evaluate the adverse effects of drought stress and the beneficial effects of salicylic acid (SA) on physiological and biochemical attributes of okra plants under drought stress. For this purpose, a pot experiment was laid out in Complete Randomized Design (CRD) design. Okra seeds were primed with four different SA treatments i.e., 0 (control), 1, 2 and 3 mM and sown in pots (Ø 20 cm). After 14 days of germination, the plants were subjected to two drought levels i.e., 25% and 50% field capacity (FC) and after 20 days of germination regular foliar sprays of SA at 7 days interval were performed with aforementioned SA levels. Physiological parameters like fresh weight, dry weight, and length of plants along with biochemical attributes like chlorophyll ('a', 'b' and total), total carotenoids, total protein and proline contents, and electrolyte leakage were recorded. Results revealed that drought stress (25% FC) significantly reduced all the studied parameters and resulted in the lowest values of fresh weight (5.04 g), dry weight (1.33 g), length of plants (11.68 cm), chlorophyll 'a' content (5.97 mg/g FW), chlorophyll 'b' content (8.86 mg/g FW), total chlorophyll (14.84 mg/g FW), total carotenoids (4.96 mg/g FW) and total protein (1.05 mg/g FW), except proline content (6.81 mg/g FW) and electrolyte leakage (77.31%) which was increased. Application of SA under drought stress reduced the harmful effects of drought and application of 2 mM SA produced the maximum fresh weight (8.60 g), dry weight (2.51 g), length of plants (16.23 cm), chlorophyll 'b' content (14.47 mg/g FW), total protein (3.73 mg/g FW) and proline content (5.11 mg/g FW); whereas application of 3 mM SA showed the highest values of chlorophyll 'a' (8.91 mg/g FW), total chlorophyll content (23.20 mg/g FW) and carotenoids (7.93 mg/g FW), and the lowest value for electrolyte leakage (62.00%).

Keywords: *Abelmoschus esculentus, electrolyte leakage, field capacity, proline content.*

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INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a member of family Malvaceae and an important summer vegetable. It is favoured for its soft and tender green pods, which are commonly consumed as curries and as boiled vegetables (Mounir et al., 2020). Despite of its astonishing beauty of blooms, okra is rich source of nutrients and medicinal properties. The fresh fruit of okra contains carbohydrates (9.6%), protein (2.25%), fibre (1.1%), fat (0.2%) along with many other important vitamins and minerals such as magnesium iron, potassium, calcium, sodium, zinc, nickel, and manganese (Khan and Rab, 2019). Soluble fibre in okra reduces cholesterol and risk of cardiac diseases, whereas insoluble fibre promotes healthy digestive track. Okra helps in slow absorption of sugar, hence can be consumed as anti-diabetic food (Nawaz et al., 2020).

Adverse environmental conditions like drought have many harmful effects on plant growth and yield. Like any other abiotic stress, proline concentration also increases inside the plants under drought stress, which helps them to withstand the stress conditions (Lintunen et al., 2020). Drought decreases rate of leaf growth by making cell walls sclerotic and reduces plant biomass. Plants which are exposed to drought stress also exhibit lower levels of carbohydrates and starch (Qu et al., 2019). Under drought stress, protein degradation starts and reduction in chlorophyll takes place (Dawood et al., 2019). Drought stress mostly causes gathering of ROS (reactive oxygen species) which can lead to oxidative stress in chlorophyll and disrupts normal working of plant cells (Stanley and Yuan, 2019). ROS activities damage lipids, terpenoids, carbohydrates and nucleic acids (Guo et al., 2018). Accumulation of ABA, reduction in leaf area and closing of stomata was also noted in drought affected plants (Meise et al., 2018).

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Salicylic acid (SA) had been categorized as plant hormone due to its important roles in carrying many physiological and biochemical processes. SA is crucial for improving abiotic stress tolerance and significant interests have been developed in scientific community on its use due to its capability to create defense mechanism in plants against abiotic stresses. Many studies support beneficial effects of SA on number of crops which were subjected to abiotic stresses i.e., salinity, drought, and heavy metal toxicity (Klessig et al., 2018).

Okra is one of the most important summer vegetables commonly cultivated in tropical and subtropical plains of Pakistan including Haripur due to its higher nutritional values, ease of cultivation and resistance to harsh environmental conditions. Most of the cultivated land in Haripur region is rainfed and crops depends upon rains (mostly monsoon period) for irrigation and occasionally these areas face moderate to severe droughts during summer when rainfall is low. Despite of huge potential of okra production, above mentioned factors cause a great reduction in yield per unit area and total area under okra cultivation. Hence, the present study was conducted to evaluate the adverse effects of drought and beneficial effects of SA on okra under applied drought.

MATERIALS AND METHODS

Plant material and growth conditions

Current study was conducted at Horticulture Nursery, Department of Horticulture, The University of Haripur during April-June 2020. For this experiment pots of 20 cm diameter and 15 cm depth were used. Each pot was filled with 2 kg potting medium containing sand and clay (1:1). The pots were properly covered by rainout shelters during night-time and during rains to avoid entry of extra moisture to the pot medium. Seeds of okra cultivar Sabz Pari were collected from National Agricultural Research Center (NARC), Islamabad and planted during mid-April 2020. Eight seeds were sown in each pot. Ten days after germination, plants were thinned to 5 plants per pot. The plants were irrigated up to full field capacity (FC) during 14 days after germination to achieve the maximum germination and equilibrium in plant growth. All the cultural practices were kept uniform for all the pots.

Drought application

After 2 weeks of seed germination, the plants were subjected to 2 different levels of drought i.e., 50% (D_1) and 25% FC (D_2), which were maintained on each alternate day. Field capacity was calculated based on saturation percentage as described by Wilcox (1951).

Salicylic acid treatment

Okra seeds were washed and cleaned before SA application to remove any foreign material. Initially the seeds were primed with 0 (control), 1 mM, 2 mM, and 3 mM SA for 24 hours. Later, the seeds were sown in pots and the pots were kept in a lath house. After 20 days of germination, okra plants were sprayed with aforementioned concentrations of SA after every 7 days until the completion of experiment.

Data collection

After 5 weeks of drought application to measure data on physical and biochemical attributes, four plants from each treatment were uprooted and washed properly with tap water to remove any foreign contaminants like mud and dust. These washed plants were placed on tissue paper to absorb extra water and left to dry at room temperature.

Fresh weight per plant (g)

Plant samples were weighed to record their fresh weights by using an electronic weight balance separately for each treatment and then averages were calculated.

Dry weight per plant (g)

After recording fresh weights, the sampled plants were then kept in an oven at 60 ºC for a period of 24 hours. Then these plant samples were taken out and again weighed for their dry weights.

Plant length (cm)

Total length of plants was measure by adding the length of longest root and length of stem (from base to tip) for each treatment and then averages were computed.

Chlorophyll and carotenoids contents (mg/g FW)

Chlorophyll and carotenoids contents were measured by the method explained by Lichtenthaler (1987). Fresh leaves were plucked from each treatment and ground with the help of pestle and mortar. About 0.2 g of ground leaf paste was taken and mixed with 15 mL of acetone (80%) and then filtered by using Whatman No. 42 filter paper. Later, the absorption of filtrate was carried out at 470, 663 and 646 nm with the help of spectrophotometer (Cary-50, Germany). Acetone was used for regulation of device. Chlorophyll and carotenoids concentrations were then measured by employing the following formulas.

Chlorophyll ‘a’ content = 12.25(A 663.2) - 2.79(A646.8)

Chlorophyll ‘b’ content = 21.21(A646.8) - 5.1(A663.2)

Total Chlorophyll content = chl ‘a’ + chl ’b’

$$\text{Carotenoids} = \frac{1000(A470) - 1.82\text{chl 'a'} - 85.02\text{chl 'b'}}{198}$$

Total protein (mg/g FW)

Method described by Lowry et al. (1951) was implemented to measure the protein content of okra plants. One gram of leaf paste was mixed with 5 mL HCl buffer (0.05 M) and the mixture was centrifuged for 20 min. After centrifugation, the supernatant was separated by process of filtration. The extract was used for evaluating the concentration of protein. For providing biuret agent, 0.1 g coumacyberylliant blue (G 250) was solved in 50 mL 95% ethanol for 1 h. Later, 100 mL of phosphoric acid (85%) was added to the extract and then whole solution was shifted into 1 L distilled water. For standard chart, 1.4 g of cattle protein was

mixed in 1 L distilled water to make 25, 50, 200, 400, 600 mg/L solutions. Absorption of each sample was then carried out by using spectrophotometer at 505 nm wavelength. For specifying protein concentration of unknown samples, standard chart was used. Y = 0.0008X + 0.01 in this relation Y equals read absorption and X equals protein concentration according to milligram on litre.

Proline content (mg/g FW)

Method of Bates et al. (1973) was implemented to evaluate the proline content. Ground leaf sample (5 g) was mixed with 3% sulfosalicylic acid and the mixture was centrifuged (at 10,000 rpm). The obtained supernatant was further utilized for proline calculation. For reaction, supernatant was mixed with acid ninhydrin (2 mL) and glacial acetic acid (2 mL), and the solution was boiled for 1 h at 100 ºC. The reaction was stopped by placing the tubes in ice bath. Then, 4 mL toluene was added to the tube for extraction. The organic phase of the sample containing chromophore was collected and the absorbance at 520 nm was read by using a spectrophotometer (Cary-50, Germany).

Electrolyte leakage (%)

Electrolyte leakage was measured by method of Lutts et al. (1995). Young okra leaves were harvested and washed with double distilled water (DDW) and placed in closed vials filled with 10 mL of DDW. These were incubated for 24 h on a rotatory shaker. After incubation, electrical conductivity (EC1) was measured. Then samples were autoclaved at 120 ºC for 20 min and after cooling, final electrical conductivity (EC2) was measured. The electrolyte leakage was calculated as per the following formula.

$$\text{Electrolyte leakage (\%)} = \frac{EC1}{EC2} \times 100$$

Statistical analysis

The experiment was laid out in a complete block design having four replications. The experimental data were subjected to analysis of variance (ANOVA) using windows software Statistix 8.1. The effects of drought and SA were determined by the least significant difference test (LSD) at $p \leq 0.05$, where the F test was significant (Steel et al., 1997).

RESULTS

Fresh weight per plant

Results regarding fresh weight per plant depicted significant differences ($p<0.01$) between the applied drought and among SA treatments. It was noted that significantly greater fresh weight (9.44 g) was recorded when plants were subjected to 50% FC as compared to those subjected to 25% FC (5.04 g). SA treatments also showed prominent variation with respect to fresh weight of plants and SA (2 mM) produced the highest weight (8.60 g), while the lowest value (5.62 g) was recorded in control (without SA treatment). The combined results of drought and SA on okra plants revealed that okra plants which were subjected to 50% FC and treated with SA (2 mM) produced the maximum fresh weight (11.07 g), while the least fresh weight (4.1 g) were produced by okra plants subjected to 25% FC without SA treatment (Table 1).

Dry weight per plant

Statistical analysis of the data on dry weight per plant also revealed significant differences ($p<0.01$) for the drought and SA treatments. Significantly higher dry weight per plant (2.93 g) was recorded when plants were subjected to 50% FC than to 25% FC (1.33 g). Results regarding SA treatment showed that SA at 2 and 3 mM were not statistically different and had similar value of dry weight (2.51 g). The lowest dry weights of 1.8 and 1.71 g were recorded with SA 1 mM and control (without SA treatment), respectively. The combined effect of drought and SA on dry weight of okra plants demonstrated that okra plants which were subjected to 50% FC treated with 2 mM SA resulted in the maximum dry weight (3.45 g), while least dry weight (0.67 g) were produced by okra plants subjected to 25% FC and under control treatment (Table 1).

Plant length

Length of okra plants significantly differed ($p<0.01$) between drought treatments, among SA levels and their interaction means. The maximum plants length (18.01 cm) was attained at 50% FC, which was significantly greater than that at 25% FC (11.68 cm). Application of SA also showed prominent variation in plant length and 2 mM SA produced the longest plants (16.23 cm), while the shortest length (12.98 cm) was recorded in plants without SA treatment. The interactive results of drought and SA on okra plants exhibited that the plants subjected to 50% FC and treated with SA 2 mM attained greater plant length (20.25 cm), while shortest plant length (9.77 cm) were produced in the plants under 25% FC with no SA treatment (Table 1).

Chlorophyll 'a' content

Chlorophyll 'a" content of okra leaves was significantly ($p<0.01$) influenced by the applied drought and SA treatments (Table 1).

Table 1: Fresh weight, dry weight and plant height of okra plants as affected by drought stress and salicylic acid treatments.

Treatments	Fresh weight (g)			Dry weight (g)			Plant length (cm)		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
SA_0	7.15 d	4.10 h	5.62 d	2.60 d	0.67 h	1.64 b	16.20 d	9.77 h	12.98 c
SA_1	9.37 c	4.75 g	7.06 c	2.75 c	1.00 g	1.87 b	17.40 c	10.60 g	14.00 b
SA_2	11.07 a	6.12 e	8.60 a	3.45 a	1.57 f	2.51 a	20.25 a	12.22 f	16.23 a
SA_3	10.17 b	5.20 f	7.68 b	2.95 b	2.07 e	2.51 a	18.20 b	14.12 e	16.16 a
Mean	9.44 a	5.04 b	-	2.94 a	1.33 b	-	18.01 a	11.68 b	-

Means sharing similar letter(s) in a group are statistically non-significant at $p \leq 0.05$ (LSD test). D_1 = 50% FC, D_2 = 25% FC, SA_0 = control, SA_1 = 1 mM, SA_2 = 2 mM, SA_3 = 3 mM.

Table 2: Chlorophyll content ('a', 'b' and total) of okra leaves as affected by drought stress and salicylic acid treatments.

Treatments	Chlorophyll 'a' (mg/g FW)			Chlorophyll 'b' (mg/g FW)			Total chlorophyll (mg/g FW)		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
SA_0	9.05 d	5.05 h	7.05 d	16.25 d	7.07 h	11.66 d	25.30 d	12.12 h	18.71 c
SA_1	9.57 c	5.62 g	7.60 c	17.35 c	8.50 g	12.92 c	26.92 c	14.12 g	20.52 b
SA_2	11.12 a	6.20 f	8.66 b	19.20 a	9.75 f	14.47 a	30.32 a	15.95 f	23.13 a
SA_3	10.80 b	7.02 e	8.91 a	18.42 b	10.13 e	14.28 b	29.22 b	17.17 e	23.20 a
Mean	10.13 a	5.97 b	-	17.80 a	8.86 b	-	27.94 a	14.84 b	-

Means sharing similar letter(s) in a group are statistically non-significant at $p \leq 0.05$ (LSD test). D_1 = 50% FC, D_2 = 25% FC, SA_0 = control, SA_1 = 1 mM, SA_2 = 2 mM, SA_3 = 3 mM.

Chlorophyll 'a' content (10.13 mg/g FW) was significantly higher in the leaves of the plants subjected to 50% FC than of those subjected to 25% FC (5.9 mg/g FW). SA treatments also resulted in prominent variation in chlorophyll 'a' content of okra leaves. SA 3 mM resulted in the highest chlorophyll 'a' content (8.91 mg/g FW), while lowest value (7.07 mg/g FW) was recorded in control. The combined results of drought and SA on okra plants revealed that okra plants which were subjected to 50% FC and treated with 2 mM SA had the maximum chlorophyll 'a' content (11.12 mg/g FW), while the least chlorophyll 'a' content (5.05 mg/g FW) was recorded in leaves of the plants subjected to 25% EC and without SA treatment (Table 2).

Chlorophyll 'b' content

Results regarding chlorophyll 'b' content of okra plants depicted that the parameter was significantly ($p<0.01$) affected by the drought and SA treatments. Chlorophyll 'b' content (17.80 mg/g FW) was significantly higher in the leaves of the plants subjected to 50% FC than of those under 25% FC (8.86 mg/g FW). SA treatments also had prominent effect on chlorophyll 'b' content. SA 2 mM treated plants possessed significantly higher chlorophyll 'b' content (14.47 mg/g FW) than untreated control plants (11.66 mg/g FW). The combined results of drought and SA indicated that the plants subjected to 50% FC and treated with SA 2 mM had the maximum (19.20 mg/g FW) while those subjected to 25% FC and without SA treatment had the minimum chlorophyll 'b' (7.07 mg/g FW) in their leaves (Table 2).

Total chlorophyll content

Total chlorophyll content of okra plant exhibited significant differences ($p<0.01$) between drought treatments and among SA levels. Total chlorophyll content (27.94 mg/g FW) recorded was significantly higher in the leaves of the plants under 50% FC than those subjected to 25% FC (14.84 mg/g FW). Among the SA treatments, the plants treated with 3 mM SA had higher chlorophyll content (23.20 mg/g FW) in their leaves followed by 2 mM SA (23.13 mg/g FW), while lower concentration of total chlorophyll content (18.71 mg/g FW) was recorded in SA 0 mM. The combined effect of drought and SA on okra plants indicated that the plants subjected to 50% FC and treated with SA 2 mM had maximum total leaf chlorophyll (30.32 mg/g FW), while the least total chlorophyll content (12.12 mg/g FW) was found in the leaves of okra plants subjected to 25% FC and no SA treatment (Table 2).

Carotenoids content

Data analysis concerning carotenoids of okra leaves depicted significant differences ($p<0.01$) for applied drought and SA treatments. Carotenoids (8.88 mg/g FW) were recorded significantly higher in the plants subjected to 50% FC as compared with those under 25% FC (4.96 mg/g FW). Application of SA @ 3 and 2 mM resulted in significantly higher carotenoids (7.93 and 7.82 mg/g FW, respectively), whereas significantly lower concentration (5.55 mg/g FW) was recorded in untreated (control) plants. The cumulative effect of drought and SA on okra plants revealed that the plants subjected to 50% FC and treated with SA 2 mM contained the maximum carotenoids (10.15 mg/g FW), while the minimum carotenoids (3.40 mg/g FW) were produced by okra plants subjected to 25% FC and no SA treatment (Table 3).

Total protein content

Total protein content of okra leaves significant differed ($p<0.01$) due to applied drought and SA treatments. Significantly higher protein content (4.73 mg/g FW) was estimated in plants under 50% FC than those under 25% FC (1.05 mg/g FW). SA treatments also showed prominent variation and the maximum protein content recorded at 2 mM SA (3.72 mg/g FW), while the minimum (1.77 mg/g FW) when no SA treatment was applied. The combined application of drought and SA revealed that the okra plants subjected to 50% FC and treated with SA 2mM had the maximum protein content (6.30 mg/g FW). On the other hand, the minimum protein content (0.37 mg/g FW) was found

Table 3: Carotenoids, total protein and proline content of okra leaves as affected by drought stress and salicylic acid treatments.

Treatments	Carotenoids (mg/g FW)			Total protein (mg/g FW)			Proline content (µg/g FW)		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
SA_0	7.70 d	3.40 h	5.55 c	3.17 d	0.37 h	1.77 d	1.15h	5.67d	3.41c
SA_1	8.52 c	4.25 g	6.38 b	4.27 c	0.97 g	2.62 c	1.72g	6.25c	3.98b
SA_2	10.15 a	5.50 f	7.82 a	6.30 a	1.17 f	3.73 a	2.10f	8.12a	5.11a
SA_3	9.17 b	6.70 e	7.93 a	5.17 b	1.67 e	3.42 b	2.87e	7.20b	5.03a
Mean	8.88 a	4.96 b	-	4.73 a	1.05 b	-	1.96b	6.81a	

Means sharing similar letter(s) in a group are statistically non-significant at $p \leq 0.05$ (LSD test). D_1 = 50% FC, D_2 = 25% FC, SA_0 = control, SA_1 = 1 mM, SA_2 = 2 mM, SA_3 = 3 mM.

Table 4: Electrolyte leakage (%) from okra leaves as affected by drought stress and salicylic acid treatments.

Treatments	D_1	D_2	Mean
SA_0	65.75 e	84.00 a	74.87 a
SA_1	65.75 e	78.50 b	72.12 b
SA_2	59.25 f	75.50 c	67.37 c
SA_3	52.75 g	71.25 d	62.00 d
Mean	60.87 b	77.31 a	-

Means sharing similar letter(s) in a group are statistically non-significant at $p \leq 0.05$ (LSD test). D_1 = 50% FC, D_2 = 25% FC, SA_0 = control, SA_1 = 1 mM, SA_2 = 2 mM, SA_3 = 3 mM.

in leaves of the okra plants subjected to 25% FC and without SA treatment (Table 3).

Proline content

Results regarding proline content of okra leaves exhibited significant variation ($p<0.01$) for applied drought and SA treatments. Proline content (6.81 µg/g FW) recorded was significantly greater in the leaves of the plants subjected to 25% FC than of those under 50% FC (1.96 µg/g FW). Pertaining to SA treatments, the maximum proline content was recorded in the leaves of the plants treated with 2 mM and 3 mM SA (5.11 and 5.03 µg/g FW, respectively), while the minimum value (3.41 µg/g FW) was observed in leaves of untreated (control) plants. The interactive effect of drought and SA treatments indicated that the okra plants subjected to 25% FC and treated with SA 2 mM accumulated greater proline content (8.12 µg/g FW), while lesser proline content (1.15 µg/g FW) was noted when okra plants were subjected to 50% FC and no SA application (Table 3).

Electrolyte leakage

Statistical analysis of the data on electrolyte leakage from okra leaves illustrated significant difference ($p<0.01$) between drought and among SA treatments. Electrolyte leakage was significantly greater (77.31%) from the leaves of the plants subjected to 25% FC than in those of subjected to 50% FC (60.87%). SA also showed prominent variation regarding Electrolyte leakage of plants and SA0 produced highest Electrolyte leakage was the maximum in the leaves of the control (untreated with SA) plants (74.87%) and the minimum (62.00%) in leaves of 2 mM SA treated plants. The interactive effect of drought and SA treatments revealed that 25% FC and no SA treatment resulted in greater electrolyte leakage (84.00%), while 50% FC and 2 mM SA resulted in the least electrolyte leakage (52.75%) from the leaves of okra plants (Table 4).

DISCUSSION

Reduction in morphological growth parameters is a clear indication of drought stress in plants and indicates their sensitivity towards waters deficiency. Our study also confirmed that at higher water stress, a decrease in plant growth parameters i.e., fresh, and dry weights per plant and plant length was observed. Wilting, closing of stomata to prevent transpiration and reduction in cell growth are some key responses of plants to drought stress which are produced due to lesser water content, reduced turgor pressure and lower water potential which causes reduced fresh and dry weights and plant height (Guo et al., 2018). Water deficiency also reduces cell division, cell differentiation and cell growth which also cause reduction in fresh and dry weight and height of okra plants. The dying of plant leaves under water deficiency reduces rate of photosynthesis, which makes plants unable to acquire desirable biomass and height, as suggested by Tanveer et al. (2019). Okra plants treated with SA showed more morphological growth as compared to untreated plants. SA accumulation in plants escalates their adaptation towards abiotic stresses (El-Shafey, 2017). As SA increases cell division and number of cells, which cause increase in leaf area and hence produces more plant biomass and height (Elhakem, 2019). SA also reduces anti-oxidant characteristics and therefore reduces oxygen radical activities (Li et al., 2019). Parveen et al. (2020) also suggested that application of SA on plants might increase the amounts of auxins and ABA and prevented the reduction of cytokinin under drought stress conditions, which in return increased the morphological attributes.

Drought also affected the chlorophyll content of okra. Chlorophyll (a, b and total) pigments are responsible for collection and conversion of sunlight into food and energy. Structural and functional integrity of chlorophyll is directly related to water availability, and at higher drought a decreased pigment contents were observed as reported earlier (Peiró et al., 2020; Hussain et al., 2019). Decrease in photosynthetic pigments can be attributing to lesser relative water content of leaves and lower water potential (Trueba et al., 2019). Similarly, stomatal impairment in water deficit plants is responsible for reduced chlorophyll pigment content (Dąbrowski et al., 2019). Drought stress also destabilizes the integrity of protein complexes and increases activity of chlorophyllase, a chlorophyll degrading enzyme. This eventually leads towards reduced chlorophyll concentration. Our results indicate that SA application increased the chlorophyll (a, b, total) pigments under drought stress; this increase in chlorophyll could be attributed to stimulatory effects of SA on Rubisco activity and photosynthesis (Farhangi-Abriz and Ghassemi-Golezani, 2018). Salicylic acid also enhances the synthesis of protein kinases, which play an important role in regulating cell division, differentiation and morphogenesis and hence cause increase chlorophyll concentrations (Faried et al., 2017).

Drought stress causes a series of physical and bio-chemical changes in plant organs and tissues. Carotenoids reduction is one of them which depend upon severity of drought, duration of exposure, phase of plant growth and genetic resistance of plants (Plazas et al., 2019). Khan et al. (2019) proposed that chloroplast degradation, photo-oxidation of chloroplasts, chlorophyll synthesis inhibition and increased chlorophyllase activity are major reasons of reduced carotenoids. Ahmad et al. (2019) suggested that activation of LOX (Lipoxygenase) and degradation of β-carotene caused the degradation of carotenoids under drought conditions. In this study it was noted that okra plants exposed to drought have lower carotenoids but application of SA increased carotenoids. SA may momentarily reduce the intensity of oxidative stress in stressed plants, which act as a hardening process, by enhancing the anti-oxidative capacity of the plant cells and tissues and help them to stimulate

the production of protective compounds such as carotenoids (Ahmadi et al., 2020).

Water deficiency causes decrease in protein content of plants because water shortage seriously affects nitrogen metabolism inside plants, this can be due to reduction in polysomal complexes in plant tissues because of lower tissue water content (Khan and Rab, 2019). Production of ROS (Reactive oxygen species) also causes collapses of protein structure, hence causing an oxidative stress which might be responsible for reduced protein content in drought affected okra plants. However, application of SA increased protein content under drought conditions. Faraz et al. (2020) suggested that SA application increases the activity and concentration of nitrate reductase and nitrate content which in turn enhances plant N levels and helps in production of more proteins. Similarly, SA also affects the hormonal system of plants which balances the protective reactions of plants and accelerates the repairing process of biochemical molecules and hence increases the protein content (Ahmadi et al., 2020).

During this study, an increase in proline content was recorded in plants subjected to drought. Proline synthesis is greatly associated with the plant's response towards stress. Production of osmolytes by plants is a common mechanism adopted to lower the stress. Proline is one of these osmolytes which in case of drought stress act as organic reservoir (Zhang et al., 2014). It is reported that increase in proline can protect turgor pressure and prevents membrane damage on plants. So, proline accumulation is an adaptation of plants which amplify the tolerance toward drought stress (Singh et al., 2019). SA treatment to drought stressed okra plants exhibited an increase in proline concentration. The protective action of SA during water deficit was demonstrated by the enhanced proline production in stressed plants. Many studies have reported that treatment of SA during stress condition increases accumulation of proline content which produces resistance against losing leaf water and increases plant growth under stress conditions (Ignatenko et al., 2019).

Electrolyte leakage helps to assess the injury occurred to cell membranes due to applied stress. The integrity of cell membranes allows plant to survive during continuous or random water deficiencies (Oraee and Tehranifar, 2020). Decrease of electrolyte leakage in plants indicates the tolerance of plants towards drought stress (Jafarnia et al., 2018). Our results indicate that increase in drought stress increased electrolyte leakage from okra leaves which could be due to production of ROS, and oxidation of cell membranes which caused damage to membrane stability and integrity (Meena et al., 2017). On other hand, okra plants treated with SA showed lower electrolyte leakage from leaves under water deficit conditions. This response of SA can be attributed to the fact that it acts as facilitator for maintenance of membrane integrity. This facilitation helps in induction of antioxidant response and elevation of Ca^{2+} uptake by the plants that might protect them from oxidative damage by ROS (Elhakem, 2019). SA also helps in osmotic adjustment and maintaining of cell turgor, while ROS scavengers are involved in decreasing damage to cell membranes (Pourghayoumi et al., 2017).

CONCLUSION

Drought stress significantly reduced growth and biochemical attributes of okra cultivar Sabz Pari and induced prominent reductions in fresh and dry weights, plant length, chlorophyll , carotenoid and protein contents and increased proline content and electrolyte leakage. The application of salicylic acid at a level of 2 and 3 mM successfully reduced the harmful effects of drought.

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