Journal of Applied Botany and Food Quality 92, 360 - 370 (2019), DOI:10.5073/JABFQ.2019.092.048

[1]Agricultural Botany Department, Faculty of Agriculture, Fayoum University, Egypt
[2]King Khalid University, Faculty of Science, Biology Department, Saudi Arabia
[3]Soil and Water Department, Faculty of Agriculture, Fayoum University, Egypt
[4]Environmental Sustainable Development Department, Environmental Studies and Researches Institute (ESRI), University of Sadat City, Egypt
[5]Assiut University, Faculty of Science, Botany and Microbiology Department, Assiut, Egypt

Salicylic acid and proline enhance water use efficiency, antioxidant defense system and tissues' anatomy of wheat plants under field deficit irrigation stress

Ramadan A. Agami*[1], Saad A.M. Alamri[2], T.A. Abd El-Mageed[3], M.S.M. Abousekken[4], Mohamed Hashem[2,5]

(Submitted: July 29, 2019; Accepted: October 14, 2019)

Summary

Salicylic acid or proline enhances plant performance and encourages resistance to abiotic stress in plants. This investigation tests the influence of soaking kernels in salicylic acid (0.1 mM) or proline (10 mM) on the growth and performance of wheat plants grown in open field under full irrigation (100% of ETc) or deficit irrigation (50% of ETc). The results revealed that plants under field deficit irrigation (FDI) stress showed a decline in growth, kernel yield, relative water content, total content of chlorophylls and carotenoids, as well as negative changes in the anatomy of leaf and stem. Addition of salicylic acid or proline notably increased water use efficiency (WUE) and mitigated the stress created by FDI. Field deficit irrigation stress greatly increased electrolyte leakage, total soluble phenols, proline, and total soluble sugar contents and activities of enzymes SOD, CAT, and POX. Salicylic acid was the more efficient in mitigating FDI stress than proline. The results conclude that salicylic acid, as a growth regulator, could be used to alleviate the negative effect of limited water-availability in soil on wheat as well as improving the growth and yield of the crop.

Keywords: Antioxidants; Anatomy; Field deficit irrigation; Proline; Salicylic acid; *Triticum aestivum* L.

Introduction

Water lowering in Northern Africa and Middle Eastern climate areas will be progressively aggravated by climate change, population increase, and industrial activities. Indeed, water scarcity develops from global climate change is accompanied by more repeated and more severe summer droughts in different regions. Drought is the crucial abiotic stress that reduces growth and yield of crops plant worldwide. Abiotic stress created by water deficiency stress led to a decline in photosynthesis, transpiration and other biochemical processes accompanying with plant growth, development, and outputs (Tiwari et al., 2010). It was stated that plants achieved high activities of antioxidant enzymes have shown a great tolerance to the oxidative damage caused by ROS (Gapinska et al., 2008) because plants have numerous advanced adaptive mechanisms to minimize oxidative injury arising from water deficiency stress, by the biosynthesis of a cascade of antioxidants. One of these mechanisms is the accumulation of specific organic metabolites of low molecular weight in the plants that are known jointly as compatible solutes (Aldesuquy et al., 2011). These osmolytes were found to play an essential role in the cellular osmotic amendment in response to the water lack stress (Misra et al., 2002). Compatible solutes could be classified into three major forms: amino acids (ex. proline, polyol/small sugars such as mannitol and trehalose), and ammonium compounds that are quaternary or tertiary (like as glycine betaine) and dimethylsulfoniopropionate. These solutes are able to work as scavengers for the free-radicals by steady proteins and/or membranes directly (Wang et al., 2003; Agami, 2014).

Proline is an essential amino acid that accumulates in different plant organs, mainly in leaves during the influence of water scarcity stress. The accumulation of proline has a function in the adjustment of osmosis in the plant cell (Meister, 2012). Consequently, proline acts as a signal to water-stress tolerance and its increment in the plant tissues indicates that the plant suffers from water-deficit stress. Exogenous application of proline enhances its endogenous level and therefore, promoting growth and antioxidant defence system of the plant (Shahbaz et al. 2013).

Salicylic acid (SA) is a naturally occurring phenolic compound that plays an essential role in the regulation of plant growth, development, ripening, flowering, and responses to abiotic stresses. The accumulation of SA may have a vital role in the defensive mechanism during water-scarcity stress. Generally, applying of SA at low levels alleviates the sensitivity of plants to abiotic stresses, such as water scarcity stress. (Rivas-San Vicente and Plasencia, 2011).

Wheat crop has importance due to the large use as feed in most regions of the world and it's a moderately drought tolerant crop. Drought is the greatest extensive problem for wheat production. Therefore, enhancing drought tolerance is of specific interest for sustainable wheat production. When wheat grains were soaked in SA, the wheat plants exhibited better resistance to drought stress (Al-Hakimi and Hamada 2001). Under serious water stress, cell elongation may be decelerating due to the breakdown of water gush from the xylem to the adjacent extending cells (Nonami, 1998). Pretreatment wheat plants with 0.5 mM SA considerable alleviated water loss from wheat seedlings, and enhanced their drought tolerance (Kang et al., 2012).

We hypothesized that kernel drenched in salicylic acid or proline as an exogenous application may enhance the performance and yield of wheat plants undergo field deficit irrigation stress. Therefore, this work aimed to evaluate alteration in physio-biochemical attributes and tissues' anatomy under the influence of kernel drenching in salicylic acid (0.1 mM) or proline (10 mM) on wheat plants undergo field deficit irrigation to regulate the relationship between the extent of tolerance and antioxidant system changes. Most of the previous reports focused on exploring the role of proline or salicylic acid individually in improving the stress resistance in different plants. However, in this study we examined the effect of the two substances on the same plant to compare which is the most effective in induction of resisting the field-deficit irrigation stress. The study illustrates the positive effect of soaking wheat grains in proline and salicylic acid on the anatomical structure of wheat plants under conditions of field-deficit irrigation stress in relation to the enhanced resistance to the drought.

* Corresponding author

Materials and methods

Experimental location

Field trials were carried out in the growing season of 2017, at a special Farm situated in Fayoum area, Egypt between latitudes 29´02´´ and 29´35´´ N and longitudes 30´23´´ and 31´05´´ E. As stated by the aridity index, the trial soil is incident under arid conditions (Ponce et al., 2000). The trial soil had the top soil (0-60 cm depth) and classified as a sandy loam in texture (74.8% sand, 11.46 % silt and 14.76% clay), saline (EC = 4.67 dS m^{-1}) and non-calcareous soils ($CaCO_3$% = 6.45), SAR = 12.2, and pH = 7.65 (Tab. 1). The texture was loamy sand with an average bulk density of 1.57 g cm^{-3}. Total available water (%) was averaged 12.98%/40 cm in depth (Tab. 1). The organic matter content in the soil was 0.98% and total N was 0.04%. Physiochemical analysis of the soil was carried out according to Klute (1986) and Page et al. (1982). Meteorological details of El-Fayoum during the time of the trials were shown in Tab. 2.

Irrigation water requirements (IWR)

Wheat plants were irrigated at 15 days intervals by different quantities of irrigation water. The crop water requirements (ETc) were assessed using the crop coefficient as stated by Allen et al. (1998) equation:

$ETc = ETo \times Kc$

Where: ETc = crop water requirements (mm d^{-1}), ETo is the reference evapotranspiration (mm d^{-1}) and Kc = crop coefficient.

ETo was determined according to Allen et al. (1998) as follows:

$ETo = Epan \times Kp$

Where Epan is the evaporation from a class A and Kp is the pan coefficient.

The plots involved irrigation treatments were isolated with 3 m fallow land to avoid the lateral movement of water from irrigation level to another. Subplots within each irrigation treatment were isolated by a distance of 0.5 m fallow land. Seventy-two plots were used, and the area of the experimental plot was 12 m^2 (3 m × 4 m). The amounts of irrigation water applied to each plot during the irrigation regime were determined using the following equation:

$$IWA = \frac{A \times ETc \times Li}{Ea \times 1000}$$

Where IWA is the irrigation water application (m^3), A is the area (m^2), ET_c is crop water requirements (mm d^{-1}), Li is the irrigation intervals (day), and Ea is the application efficiency (%).

The quantity of irrigation water requirements (IWR) was controlled by a plastic tube of 50 mm in diameter. For each plot, one canal per plot was used to transport water under surface irrigation system. The quantity of water transported through a plastic tube was determined by calculation of Israelsen and Hansen (1962) as:

$Q = CA\sqrt{2gh} \times 10^{-3}$ where,

Q is the discharge of irrigation water (l sec^{-1}), C is the coefficient of discharge, A is traverse section area of irrigation tube (cm^2), g is gravity acceleration (cm sec^{-2}) and h is the average of the effective head of water (cm).

Kernels treatment, trial design, and plant management

Kernels of wheat (*Triticum aestivum* L.) cultivar Sids 12 were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. They were surface-sterilized using 0.1% $HgCl_2$ for 1 min and followed by washing in sterilized deionized water to remove traces of $HgCl_2$. The sterilized kernels were immersed in 0.1 mM salicylic acid (SA) or in 10 mM proline solution for 6 h and dehydrated with forced air under shade. The trials were conducted in a complete randomized block design (Split Plots). Two irrigation treatments were employed (100 and 50% of ETc that were allocated as the main sector) and salicylic acid or proline treatments (i.e., 0.1 and 10 mM, respectively that were assigned to sub-sectors). Thus, six treatments were performed in each experiment as follows:

I_{100}: kernels were soaked in distilled water for 6 h, and the plants were irrigated 100% of Etc.

SA + I_{100}: kernels were soaked in 0.1 mM salicylic acid for 6 h, and the plants were irrigated with 100% of Etc.

Proline + I_{100}: kernels were soaked in 10 mM proline for 6 h, and the plants were irrigated with 100% of Etc.

I_{50}: seeds were soaked in distilled water for 6 h, and the plants were irrigated with 50% of Etc.

SA + I_{50}: kernels were soaked in 0.1 mM salicylic acid for 6 h, and the plants were irrigated with 50% of Etc.

Proline + I_{50}: kernels were soaked in 10 mM proline for 6 h, and the plants were irrigated with 50% of Etc.

Tab. 1: Physical and chemical properties of the studied soils

Layer (cm)	Particle size distribution				Bulk density g cm^{-3}	K_{sat} cm h^{-1}	Soil moisture content at			pH	ECe dS m^{-1}	$CaCO_3$, %	OM, %	N %
	Sand %	Silt %	Clay %	Texture class			F.C %	W.P %	A. w %					
0-40	74.8	11.46	14.76	S.L.	1.57	2.16	24.99	10.01	12.98	7.65	4.67	6.45	0.98	0.04

LS= Sandy loam, F.C=Field capacity, W.P = Wilting point, A.W= Available water and K_{sat} = Hydraulic conductivity and OM= rganic matter.

Tab. 2: Weather data at Fayoum area, Egypt during growing season (2017/2018)

Month	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)	Average relative humidity	Wind speed ms^{-1} (%)	Pan evaporation mmd^{-1}
November	26.2	13.4	19.8	41.8	1.83	2.3
December	21.0	9.5	15.2	43.0	1.65	2.1
January	20.4	8.5	14.5	42.0	2.0	1.6
February	22.4	8.3	15.1	42.0	2.1	2.3
March	29.4	12.7	21.1	37.0	2.3	4.0

Each treatment was reiterated three times and each set contained four replications, making a total of 72 plots for 6 treatments. Kernels of wheat were planted on November 15, 2017, in sandy loam soil. The kernels were sown utilizing an experimental drill in 12 m^2 (3 m × 4 m) sectors comprising of 6 rows with a 20 cm row-space and the seeding rates for trials were about 400 kernels per m^2. The sectors were supplied with 80 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1} at the planting and 80 kg N ha^{-1} at stem elongation. To assure the uniform emergence and growth of wheat, there was no water deficiency throughout the seedling stage until the tillering stage, and then the shortfall treatment was done. Beginning the stage of tillering, the wheat plants were either fully-watered to maintain 100% of ETc or drought stressed by lowering the amount of water supplied to 50% of ETc.

Experimental methods

Analysis of plant growth and water use efficiency (WUE)

From each experimental sector, nine 120-days-old plants from sowing were randomly chosen and assessed for growth attributes. Using a meter scale, length of shoots and spikes (cm) was measured. Numbers of spikelets per spike were counted. Area of flag leaf (cm^2) was measured using a digital leaf meter (LI- 3000 Portable Area meter Produced by LI-COR Lincoln, NE, USA). Fresh weight of shoot per plant was recorded, and then shoots were dried in an oven at 70 °C until constant weight for shoot dry weight measurement. The total content of chlorophylls, carotenoids, proline, soluble protein, soluble sugars, phenols, electrolyte leakage, relative water content and activities of antioxidant enzymes were estimated in the specimen of flag leaves. At the end of the season, plants from each experimental sector were collected to estimate the yield of grain ha^{-1} and 1000-grains weight. WUE values were calculated as kg grain yield m^{-3} of irrigation water used for each irrigation treatment using the formula of JENSEN (1983):

WUE = grain yield (kg ha^{-1}) / water applied (m^3 h^{-1})

An assessment of physio-biochemical attributes

Chlorophyll 'a', chlorophyll 'b' and carotenoids were extracted, and their concentration was determined (in mg g^{-1} FW) following the procedure given by ARNON (1949). Disks (0.2 g) of plant leaves were homogenized in 50 ml 80% (v/v) acetone, and then centrifuged at 10,000 × g for 10 min. Using a UV-160A Visible Recording Spectrometer (Shimadzu, Japan), reads on 663, 645 and 470 nm were recorded to calculate pigment concentrations.

A relative water content (RWC) was estimated as described by MORANT-MANCEAU et al. (2004). The flag leaves excluding midrib were cut, weighed (FM), and floated in distilled water in closed Petri plates and weighed periodically till their full saturation with water (WSM). At the end, they were placed in an oven at 80 °C for 48 h to obtain dry mass (DM), and RWC was calculated as:

RWC [%] = [(FM - DM) / (WSM - DM)] × 100.

The total inorganic ions leaked out from the leaf tissues were determined by a digital conductivity meter (Inolab, Weilheim, Germany) following KORKMAZ et al. (2010). For determination of electrolyte leakage (EL), 20 leaf discs were put in a tube containing 10 cm^3 of deionized water and electrical conductivity was measured (EC_0) after 2 h. The tube was heated to 50 - 60 °C in a water bath and after 25 min EC was measured (EC_1). Then, the content was boiled at 100 °C for 10 min and the EC was again recorded (EC_2). EL was calculated using the formula:

EL [%] = (EC_1 - EC_0) / (EC_2 - EC_0) × 100.

Total soluble sugars were extracted and determined according to IRIGOYEN et al. (1992). A 0.2 g specimen of dry flag leaves was homogenized in 10 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol. The extract was centrifuged at 3,500 × g for 10 min and the supernatant was stored at 4 °C for measurement. Total soluble sugar content was determined by reacting 0.1 ml of the ethanolic extract with 3 ml of freshly prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] and placed in a boiling water bath for 10 min. After cooling, the absorbance of the mixture was recorded at 625 nm using a UV-160A Visible Recording Spectrometer (Shimadzu, Japan).

Flag leaf proline content (in g g^{-1} DW) was determined using the rapid colourimetric method suggested by BATES et al. (1973). Proline was extracted from 0.5 g of each dried flag specimen by grinding in 10 ml 3% (v/v) sulphosalicylic acid and the mixture was then centrifuged at 10,000 × g for 10 min. Two ml of the supernatant was placed in a test-tube, to which 2 ml of a freshly prepared acid ninhydrin solution was added. The tubes were incubated in a water bath at 90 °C for 30 min and the reaction was terminated in an ice bath. Each reaction mixture was extracted with 5 ml toluene and vortex-mixed for 15 s. The tubes were allowed to stand for 20 min in the dark, at room temperature, to allow separation of the toluene and aqueous phases. Each toluene phase was carefully collected into a clean test-tube and its absorbance was read at 520 nm. The free proline concentration in each sample was determined from a standard curve prepared using analytical grade proline and expressed on a DW basis.

Total soluble proteins concentration of the dry flag leaves was determined according to the method described by BRADFORD (1976) with bovine serum albumin as a standard. An amount of 2 g of specimen was ground in a mortar with 5 ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 min. The supernatant of different samples was put in separate tubes. The volume of all samples in tubes was then made equally by adding a phosphate buffer solution and the extraction were stored in the refrigerator at 4 °C for further analysis. After extraction, 30 µl of different samples were taken out in separate tubes and were mixed with 70 µl of distilled water. In all of these separate sample tubes, 2.9 ml of the Coosmassic Brilliant Blue solution was then added and mixed thoroughly. The total volume was 3 ml in each tube. All tubes were incubated for 5 min at room temperature and then, the absorbance was recorded at 600 nm against the Blank. A standard curve of absorbance (600 nm) versus concentration (µg) of total soluble proteins was calculated.

The soluble phenols concentration in wheat flag leaves were extracted as described by HSU et al. (2003). 0.2 g of dry leaves were homogenized in 80 ml methanol and kept overnight. The filtrates were diluted to 100 ml and served as a stock solution. According to SLINKARD and SINGLETON (1997), 200 µl of the stock solution was added to 1.4 ml of distilled water, and 0.1 ml of 50% (1 N) Folin-Ciocalteu phenol reagent. After three min, 0.3 ml of 20% (w/v) sodium carbonate was added. The mixture was allowed to stand for 2 h. After gentle vortex, the absorbance was determined at 765 nm. Total soluble phenols concentration was standardized against tannic acid.

Assays of antioxidant enzymes activities

The method of MUKHERJEE and CHOUDHARI (1983) was used to make extraction from 0.5 g flag leaves specimen. The extracts were frozen in liquid N and then grind in phosphate buffer (100 mM, pH 7.0). Centrifugation of homogenates was done under 15,000 × g at 4 °C for 10 min. Thereafter, the supernatant was stored at 4 °C until being used to estimate peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities. The activity (µmol H_2O_2 min^{-1} g^{-1} protein) of CAT (EC 1.11.1.6) was assayed as described by HAVIR and MCHALE (1987) by observing the reduction in absorbance read at 240 nm because of the breakdown of H_2O_2 ($\varepsilon = 36$ M^{-1} cm^{-1}). The activity (U mg^{-1} protein) of SOD (EC 1.15.1.1) was assayed by measuring its capacity to inhibit nitro blue tetrazolium (NBT) photo-

chemical reduction (BEAUCHAMP and FRIDOVICH, 1971). One unit of SOD activity was allocated as the enzyme amount needed to inhibit half of the NBT photoreduction rate (%). The methods described by MAEHLY and CHANCE (1954) and KLAPHECK et al. (1990) were used to assay the activity of POD (EC1.11.1.7). The guaiacol oxidation rate in H_2O_2 presence read at 470 nm indicates the enzyme activity.

Study of tissues' anatomy

For anatomical observation of stem and leaf, specimens were brought from the fourth internodes and its leaf from the growing point and fixed in FAA solution for 48 h. After that, the specimens were washed in 50% ethanol, dehydrated and cleared in tertiary butanol series, and embedded in paraffin wax. Transverse sections, 25-µm thick, were cut by a rotary microtome (Leitz, Wetzlar, Germany), adhered by a Haupt's adhesive, and then stained with a crystal violet-erythrosin combination (SASS, 1961), cleared in carbol xylene, and mounted in Canada balsam. The sections were examined and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done utilizing a micrometer eye-piece and the average of five readings was calculated.

Statistical analysis of data

Data were analyzed utilizing Genstat 17th edition (VSN INTERNATIONAL, 2015) software package. Where significant differences were detected, means were separated utilizing the least significant difference (LSD) test procedure at the 5% significance level, by the Duncan's protected LSD (DUNCAN, 1955).

Results

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on growth characteristic of wheat plants

Kernels soaking utilizing salicylic acid (SA) or proline were used to determine their ameliorative effects in wheat against field deficit irrigation (FDI) stress. FDI significantly reduced the growth characteristic of wheat plants. The FDI decreased the length of shoots by 12.4 l%, numbers of spike per plant by 36.2%, the area of flag leaf by 44.5%, length of the spike by 16.1% and numbers of spikelets per spike by 6.7% from the unstressed control (Tab. 3). The reduction was significantly decreased because of SA or proline treatment. Treatment of FDI -stressed plants with SA was the most efficient treatment. This treatment alleviated the reduction to 3.2% in length of shoots, 26.1% in numbers of spike per plant, 25.2% in flag leaf area, 3.2% in spike length and 0.0% in numbers of spikelets per spike in relation to the non-stressed control. Compared to the stressed control in absence SA or proline, SA increased the length of shoots, numbers of spike per plant, the area of flag leaf, length of spike and numbers of spikelets per spike by 10.5%, 15.9%, 31.6%, and 15.3%, respectively, under FDI stress. Under unstressed conditions, seed soaking in SA or proline greatly increased these characteristics in most cases compared to those of the control (Tab. 3).

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on yield characteristics and water use efficiency (WUE) of wheat plants

Yield characteristics were considerably decreased, while WUE were significantly increased under stressful conditions (Tab. 4). The reduction was 45.7% in fresh weight of shoot, 72.8% in dry weight of shoot, 28.6% in 1000-kernel weight and 48.1% in kernels yield per hectare in comparison with the non-stressed control. However, kernels pretreatment with SA or proline recovered the stressed yield characteristics and significantly increased them compared to those of the water-stressed control. The SA was the most effective treatment that caused an increase in fresh weight of shoot by 34.1%, dry weight of shoot by 64.8%, the yield of kernels per hectare by 25.1%, and 1000-kernel weight by 31.8%. The increase in growth attributes due to SA or proline under FDI stress reached the growth parameters close to those of the well-watered control, whereas yield characteristics were lower. However, kernels pretreatment with SA or proline significantly increased WUE under FDI compared to the full irrigation conditions (I_{100} treatment). Additionally, the SA + I_{50} or proline + I_{50} treatment showed the highest values of WUE along with an acceptable decrease in kernels yield compared to the non-stressful conditions (SA + I_{100} or proline + I_{100} treatments).

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on leaf photosynthetic pigments of wheat plants

An impairment occurred on the parameters of photosynthetic pigments of flag leaf as affected by FDI treatment (Tab. 5). Under FDI (50% ETc) stress, total chlorophylls, and carotenoids were greatly reduced by 62.9% and 43.7%, respectively compared to the non-stressed control. However, kernels soaking in SA or proline retrieved the stress effects on these photosynthetic attributes and significantly increased them comparing with those of water-stressed control (50% ETc). The SA appeared as the most effective that involved in increasing the total chlorophylls and carotenoids by 36.2% and 16.9%, respectively, to be close to those of the non-stressed control.

Tab. 3: Effect of kernels soaking in 10 mM proline or 0.1 mM salicylic acid (SA) on the growth traits [shoot length (cm), No of spike per plant, flag leaf area (cm^2), spike length (cm) and No. of spiklets per spike] of wheat (*Triticum aestivum* L., cv. Sids -12) plants grown under normal and field deficit irrigation conditions.

Treatments		Parameters				
Water regimes	Kernels soaking	Shoot length	No of spike per plant	Flag leaf area	Spike length	No of spiklets per spike
I_{100}	Control (distilled water)	83.33 ± 1.53^{bc}	6.9 ± 0.15^{c}	30.8 ± 1.5^{c}	10.33 ± 1.15^{bc}	16.7 ± 0.32^{b}
	SA	89.67 ± 6.66^{d}	7.3 ± 0.15^{c}	36.9 ± 1.2^{d}	11.33 ± 0.58^{c}	18.7 ± 0.45^{d}
	proline	87.67 ± 2.08^{cd}	7.0 ± 0.21^{c}	35.1 ± 1.5^{d}	11.00 ± 0.21^{bc}	17.8 ± 0.25^{c}
I_{50}	Control (distilled water)	73.00 ± 1.53^{a}	4.4 ± 0.31^{a}	17.1 ± 0.5^{a}	8.67 ± 0.58^{a}	15.6 ± 0.35^{a}
	SA	80.67 ± 1.53^{b}	5.1 ± 0.20^{b}	22.5 ± 1.0^{b}	10.00 ± 1.00^{abc}	16.7 ± 0.32^{b}
	proline	79.33 ± 1.53^{b}	5.3 ± 0.40^{b}	23.4 ± 1.3^{b}	9.67 ± 0.58^{ab}	17.03 ± 0.21^{b}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; $P \leq 0.05$). Mean pairs followed by different letters are significantly different.

I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

Tab. 4: Effect of kernels soaking in 10 mM proline or 0.1mM salicylic acid (SA) on the yield component [shoot fresh weight per plant (g), shoot dry weight per plant (g), kernels yield per hectare (kg), 1000-kernels weight (g) and water use efficiency (WUE) (g L^{-1})] of wheat (*Triticum aestivum* L., cv. Sids -12) plants grown under normal and field deficit irrigation conditions

Treatments		Parameters				
Water regimes	Kernels soaking	Shoot fresh weight per plant	Shoot dry weight per plant	Kernels yield per hectare	1000-kernels weight	WUE
I_{100}	Control (distilled water)	27.6 ± 0.45^{c}	12.5 ± 0.40^{c}	6176 ± 33.81^{d}	42.63 ± 1.14^{c}	1.16 ± 0.01^{a}
	SA	32.3 ± 0.55^{e}	15.8 ± 0.25^{d}	7443 ± 77.67^{f}	46.40 ± 0.75^{e}	1.40 ± 0.02^{d}
	proline	30.0 ± 1.30^{d}	15.4 ± 0.26^{d}	7016 ± 42.85^{e}	44.93 ± 0.64^{d}	1.32 ± 0.01^{c}
I_{50}	Control (distilled water)	15.0 ± 0.26^{a}	3.4 ± 0.38^{a}	3204 ± 10.15^{a}	30.43 ± 0.68^{a}	1.21 ± 0.00^{b}
	SA	18.2 ± 0.75^{b}	4.4 ± 0.10^{b}	4628 ± 48.23^{c}	33.33 ± 0.2^{b}	1.74 ± 0.02^{f}
	proline	17.6 ± 0.44^{b}	4.2 ± 0.10^{b}	4438 ± 20.40^{b}	34.43 ± 0.76^{b}	1.67 ± 0.01^{e}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; $P \leq 0.05$). Mean pairs followed by different letters are significantly different.
I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

Tab. 5: Effect of kernels soaking in 10 mM proline or 0.1 mM salicylic acid (SA) on total chlorophylls (mg g^{-1} FW), total carotenoids (mg g^{-1} FW), proline (mg g^{-1} DW), soluble protein, total soluble phenols (mg g^{-1} DW) and soluble sugars (mg g^{-1} DW) of wheat (*Triticum aestivum* L., cv. Sids -12) plants grown normal and field deficit irrigation conditions

Treatments		Parameters					
Water regimes	Kernels soaking	Total chlorophylls	Total carotenoids	Proline	Soluble protein	Total soluble phenols	Soluble sugars
I_{100}	Control (distilled water)	2.24 ± 0.08^{c}	0.71 ± 0.02^{d}	1.25 ± 0.03^{a}	4.2 ± 0.03^{a}	3.29 ± 0.07^{a}	13.1 ± 0.20^{a}
	SA	2.66 ± 0.17^{d}	0.76 ± 0.02^{e}	1.36 ± 0.02^{b}	4.8 ± 0.10^{b}	3.82 ± 0.11^{cd}	15.1 ± 0.20^{c}
	proline	2.38 ± 0.04^{c}	0.73 ± 0.02^{de}	1.33 ± 0.02^{b}	4.9 ± 0.06^{c}	3.61 ± 0.10^{b}	13.7 ± 0.21^{b}
I_{50}	Control (distilled water)	0.83 ± 0.02^{a}	0.40 ± 0.02^{a}	2.06 ± 0.05^{e}	6.3 ± 0.10^{f}	3.71 ± 0.10^{bc}	20.1 ± 0.25^{d}
	SA	1.43 ± 0.05^{b}	0.59 ± 0.02^{c}	1.78 ± 0.03^{c}	5.4 ± 0.11^{d}	3.90 ± 0.06^{d}	23.1 ± 0.25^{f}
	proline	1.31 ± 0.03^{b}	0.55 ± 0.02^{b}	1.85 ± 0.04^{d}	3.40 ± 0.09^{e}	3.87 ± 0.14^{cd}	21.9 ± 0.20^{e}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; $P \leq 0.05$). Mean pairs followed by different letters are significantly different.
I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on osmoprotectants of wheat plants

It was evident that the FDI treatment had a considerable effect on the total soluble sugars and proline contents of flag leaf (Tab. 5). Under FDI stress, the increase in the content of proline was 64.8% and the total soluble sugars were 53.4% that were higher than the control. However, kernels soaking in SA or proline mitigated the stress effects on these attributes and lowered the content of proline more than non-applied and water-stressed plants (I_{50} treatment). SA was the better treatment that decreased the content of proline by 13.6%. On the other hand, under normal conditions, kernels soaking in SA or proline markedly increased the content of proline by 8.8% and 6.4%, respectively and total soluble sugars by 15.3% and 4.6%, respectively higher than the control (Tab. 5).

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on total soluble phenols and soluble protein of wheat plants

Under FDI stress, the total soluble phenols and soluble protein were notably increased by 12.8 and 50.0%, respectively higher than the non-stressed control (Tab. 5). However, kernels soaking in SA or proline caused an additional increase in total soluble phenols compared to the water-stressed control. SA was the most efficient and caused an increase in the content of soluble phenols by 5.1%. Under non-stressed conditions, kernels soaking in either SA or proline notably increased total soluble phenols by 16.1% and 9.7%, respectively and increased soluble protein by 14.3% and 16.7%, respectively compared with the well-watered control.

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on relative water content (RWC %) and electrolyte leakage (EL %) of wheat plants

All stressful treatments markedly decreased RWC (Tab. 6). In comparison with non-stressed control, RWC in leaf specimen was notably decreased under FDI treatment (I_{50} treatment) by 23.1%. However, EL% increased, RWC% was decreased. The highest RWC was accessed 68.6% at SA + I_{100} treatment. Under FDI (50% ETc) stress, SA or proline pre-treatment lowered the injurious effects of water deficiency stress on the wheat plant and maintained their EL% and RWC% values to close levels of the control.

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on the activity of antioxidant enzymes of wheat plants

In comparison with the full irrigation condition, POD, CAT and SOD activities in stressed plants in absence of SA or proline applied were promoted, and SA or proline application caused additional increase in POD, CAT and SOD activities under the conditions of the FDI stress (Tab. 6). Even in the absence of FDI, kernels soaking utilizing

SA or proline especially SA greatly enhanced the activity of the three enzymes compared to the full irrigation conditions (I_{100}). However, kernels soaking in SA or proline alleviated the stress impacts on these attributes and notably reversed their status, significantly increasing the activities of POD, CAT and SOD higher than the stressed control. The greatest enzymes activities were found because of application of SA under water shortage stress (SOD; 13.3% increase, CAT; 1.2% increase and POD; 13.1% increase).

Effects of kernels soaking in SA or proline and/or field deficit irrigation (FDI) on tissues' anatomy of wheat plants

Under FDI stress, wheat stem diameter was markedly reduced by 23.7% lower than the control (Tab. 7 and Fig. 1). This was primarily caused by the decrease in thickness of sclerenchymatous tissue (12.2%), ground tissue by reducing cell number and diameter, length and width of the vascular bundles (8.9% and 10.9%, respectively), average diameter of metaxylem vessel (19.6%) and average diameter of hollow pith (26.9%) lower than normal control. However, kernels soaking in SA or proline mitigated the stress impacts on these anatomical characteristics and positively altered them compared to those of water-stressed control. Tab. 8 and Fig. 2 show that FDI stress induced a decrease the in blade (by 10.6%), mesophyll (by 15.2%), upper (adaxial) and lower (abaxial) epidermis thickness (by 2.8% and 16.6%, respectively) and length and width of the vascular bundles (by 26.2% and 37.5%, respectively) as well as average diameter of Mx vessels (by 22.2%) lower than the control. However, soaking wheat kernels in either SA or proline caused a considerable amelioration in the mentioned above characteristics and mitigated the adverse impacts of FDI stress as compared with the corresponding FDI treatments. The greatest blade, mesophyll, upper epidermis thickness, and length and width of the vascular bundles, as well as average diameter of Mx vessels, were noticed in SA-treated plants in absence of FDI stress as compared to with other treatments.

Discussion

Salicylic acid or proline plays a vital role in the adjustment of plant growth, development and regulation of osmosis in the cell, consequently plant tolerance to environmental stresses. Our findings showed that SA or proline was effective in restricting the impacts of FDI on plant growth and kernels yield. These benefits were more conspicuous in the case of SA pretreatment. Several researchers have stated positive roles of exogenous salicylic acid application (Agami, 2013; Jini and Joseph, 2017) or proline (Huang et al., 2009; Agami, 2014; de Freitas et al., 2018) in mitigate the unfavorable influence of drought on the growth and yield of numerous plant species.

Deficit irrigations (50% ETc) stress lead to a great reduction of wheat

Tab. 6: Effect of kernels soaking in 10 mM proline or 0.1 mM salicylic acid (SA) on relative water content (RWC %), electrolyte leakage (EL%), and the activities of peroxidase (μg g^{-1} FW min^{-1}) superoxide dismutase (U mg^{-1} protein) and catalase (μmol H_2O_2 min^{-1} g^{-1} protein) of wheat (*Triticum aestivum* L., cv. Sids -12) plants grown under normal and field deficit irrigation conditions

Treatments		Parameters				
Water regimes	Kernels soaking	Relative water content	electrolyte leakage	POD activity	SOD activity	CAT activity
I_{100}	Control (distilled water)	65.4±0.30^{d}	7.20±0.10^{b}	77.56±0.31^{a}	49.4±0.44^{a}	186.4±0.26^{a}
	SA	68.6±0.31^{f}	6.80±0.10^{a}	79.63±0.25^{c}	52.1±0.55^{b}	188.7±0.45^{b}
	proline	67.1±0.25^{e}	6.93±0.15^{a}	78.20±0.10^{b}	51.7±0.32^{b}	188.3±0.21^{b}
I_{50}	Control (distilled water)	50.3±0.26^{a}	11.10±0.20^{e}	93.66±0.25^{d}	68.5±0.25^{c}	193.8±0.25^{c}
	SA	53.6±0.38^{c}	9.10±0.20^{c}	105.86±0.21^{f}	77.6±0.35^{e}	196.1±0.25^{e}
	proline	52.2±0.45^{b}	9.80±0.10^{d}	103.56±0.32^{e}	75.2±0.10^{d}	194.6±0.35^{d}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; P ≤0.05). Mean pairs followed by different letters are significantly different.
I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

Tab. 7: Effect of kernels soaking in 10 mM proline or 0.1 mM salicylic acid (SA) on stem anatomical structure of wheat plants (*Triticum aestivum* L., cv. Sids -12) grown under normal and field deficit irrigation conditions

Treatments		Parameters								
Water regimes	Kernels soaking	Stem diameter (μm)	Sclerenchyma thickness (μm)	Ground tissue			Vascular bundles		Av. diameter of mx vessels (μm)	Av. diameter of hollow pith (μm)
				Thickness (μm)	No. of cell layers	Av. diameter of cells (μm)	Length (μm)	Width (μm)		
I_{100}	Control (distilled water)	2117±4.36^{d}	93.87±1.70^{d}	315.7±3.00^{b}	8.0±0.00^{bc}	39.47±0.32^{ab}	165.1±1.81^{b}	147.8±1.62^{c}	41.17±0.71^{d}	1254±4.04^{e}
	SA	2390±2.30^{f}	108.50±0.60^{e}	392.5±2.26^{e}	8.4±0.17^{d}	46.73±0.74^{d}	172.4±1.31^{c}	160.6±1.21^{d}	44.10±0.66^{e}	1378±3.00^{f}
	proline	2192±4.66^{e}	89.97±1.66^{c}	356.3±2.10^{d}	8.2±0.17^{cd}	43.43±0.68^{c}	172.1±1.70^{c}	134.4±1.06^{b}	36.63±0.67^{b}	1228±2.5^{d}
I_{50}	Control (distilled water)	1615±3.15^{a}	82.40±1.37^{a}	294.7±4.08^{a}	7.4±0.17^{a}	39.83±1.10^{b}	150.3±1.40^{a}	131.7±1.10^{a}	33.10±0.75^{a}	917±4.34^{b}
	SA	1766±3.80^{c}	95.57±0.87^{d}	345.2±3.39^{c}	8.2±0.17^{cd}	42.13±0.65^{c}	171.9±1.66^{c}	161.2±1.20^{d}	36.73±0.57^{bc}	878±3.00^{a}
	proline	1689±1.21^{b}	85.80±1.57^{b}	295.5±1.48^{a}	7.8±0.21^{b}	38.07±1.11^{a}	172.3±1.20^{c}	134.5±0.91^{b}	37.83±0.31^{c}	940±2.62^{c}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; P ≤0.05). Mean pairs followed by different letters are significantly different.
I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

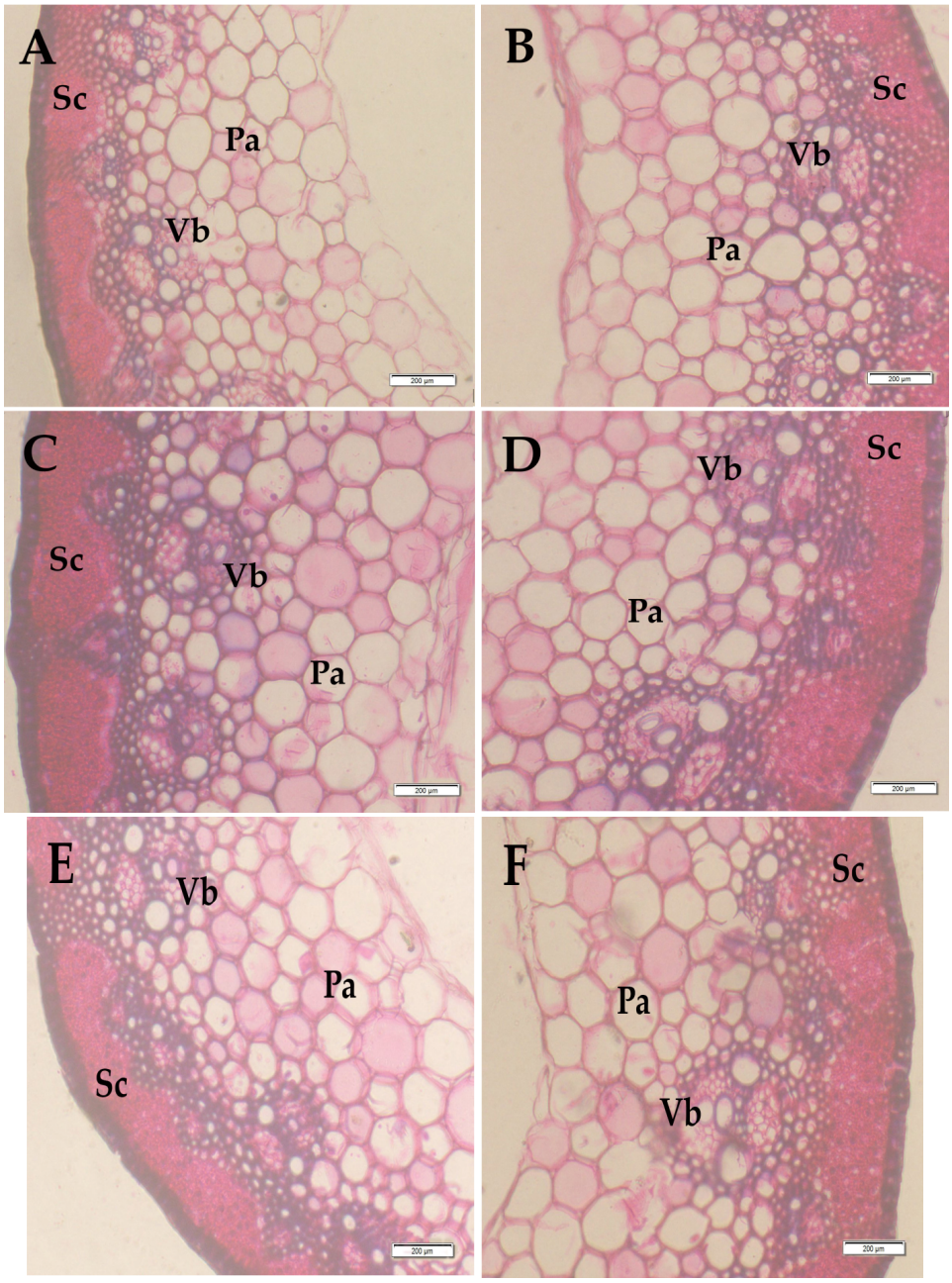

Fig. 1: Photographs of wheat stem section as affected by salicylic acid or proline application grown under normal and field deficit irrigation conditions. A) I_{60}; B) I_{100}; C) SA + I_{60}; D) SA + I_{100}; E) proline + I_{60}; F) proline + I_{100} pa, parenchyma cells; sc, sclerenchyma cells; and vb, vascular bundle, scale bars = 200 µm.

growth. The lowering in the growth of the plant and its productivity is created by the metabolic process disturbances and raised respiration rate as a result of increased energy requirements, resulting in reductions of the meristematic activity and cell enlargement (AGAMI et al., 2018). Under deficiency irrigations stress, proline or SA greatly enhanced the growth and yield approximately up to the growth and yield of the non-stressed controls. This effect may result because proline was effective in lessening the oxidative damage by modulating the antioxidative systems including enzymatic and non-enzymatic antioxidants. The improvement of growth characteristics and yield of the drought-stressed wheat plants could be because proline acts to modify organic solutes' content and photosynthetic parameters (Tab. 3, 5). The positive influences of SA on wheat performance and yield was greater than that of proline. This because SA increased the synthesis of proline in addition to increment assimilation a number of useful nutrients such as nitrogen and sulphur, which are essential for enhancing plant growth and development. Also, SA leads to the cumulating of proline which in turn protects the photosynthetic machinery from water shortage stress by stabilizing the structure of Rubisco (NAZAR et al., 2015). In our study, it was noticed that wheat performance and yield notably correlated with photosynthetic parameters under FDI stress suggesting that exogenously application of SA or proline keeps photosynthesis through weakened the unfavorable impacts of water-scarcity stress on photosynthetic machinery. The increased yield of drought-stressed wheat plant may have been connected with increased metabolic products when grains were developed. High chlorophyll content coupled with high compatible solutes content of SA treated-plants may be responsible for the im-

Tab. 8: Effect of kernels soaking in 10 mM proline or 0.1 mM salicylic acid (SA) on leaf anatomical structure of wheat plants (*Triticum aestivum* L., cv. Sids -12) grown under normal and field deficit irrigation conditions

Treatments Parameters								
Water regimes	Kernels soaking	Lamina thickness (µm)	Mysophyll thickness (µm)	Upper epidermis thickness (µm)	Lower epidermis thickness (µm)	Vascular bundles		Av. diameter of mx vessels (µm)
						Length (µm)	Width (µm)	
I_{100}	Control (distilled water)	429.5 ± 1.90^{d}	324.0 ± 3.00^{d}	68.10 ± 1.49^{a}	51.07 ± 1.31^{b}	132.0 ± 1.27^{d}	120.6 ± 2.29	42.27 ± 0.50^{c}
	SA	558.1 ± 2.40^{f}	387.9 ± 2.30^{f}	84.30 ± 1.22^{e}	50.10 ± 1.61^{b}	166.5 ± 1.76^{f}	144.2 ± 1.92	48.27±0.80^{e}
	Proline	447.6 ± 2.82^{e}	344.3 ± 0.70^{e}	78.87 ± 1.53^{d}	42.97 ± 0.97^{a}	148.1 ± 1.21^{e}	124.1 ± 1.83	43.07±0.91^{cd}
I_{50}	Control (distilled water)	383.9 ± 1.95^{a}	274.8 ± 2.00^{a}	66.20 ± 1.42^{a}	42.57 ± 0.8 ^{a}	97.4 ± 1.08^{a}	87.7 ± 0.68	32.87 ± 0.21^{a}
	SA	420.7 ± 1.76^{c}	294.6 ± 2.31^{b}	73.33 ± 1.17^{b}	56.40 ± 1.54^{c}	113.1 ± 1.87^{b}	96.6 ± 1.37	44.57±0.95^{d}
	Proline	407.8 ± 1.73^{b}	317.5 ± 1.97^{c}	76.10 ± 1.85^{c}	49.53 ± 1.30^{b}	115.7 ± 1.16^{c}	99.9 ± 1.26	39.57±1.58^{b}

Values are means ± SD (n = 9) and differences between means were compared by the Duncan's multiple range test (LSD; P ≤0.05). Mean pairs followed by different letters are significantly different.
I_{100} = irrigation with 100% of ETc and I_{50} = irrigation with 50% of ETc.

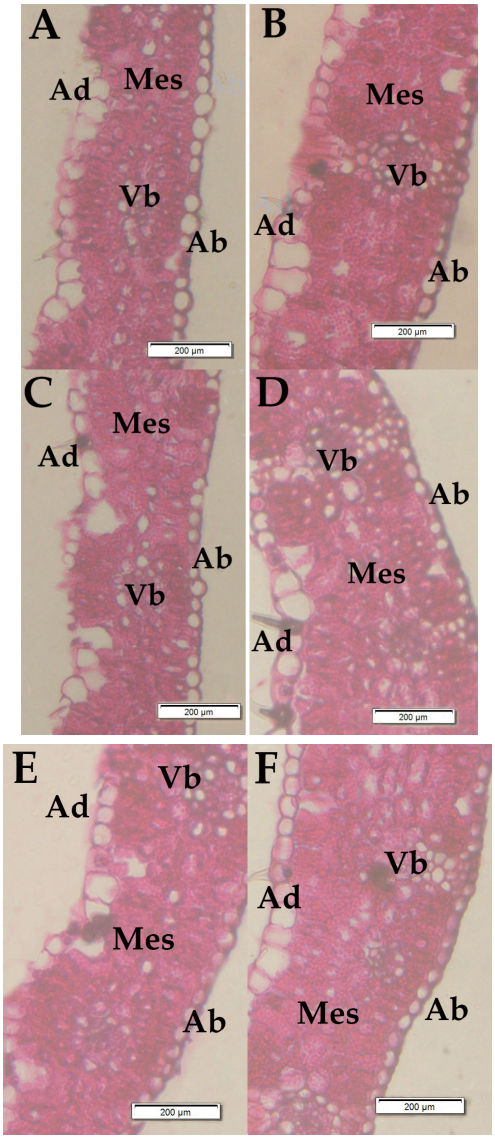

Fig. 2: Photographs of wheat leaf section as affected by salicylic acid or proline application grown under normal and field deficit irrigation conditions. A) I_{60}; B) I_{100}; C) SA + I_{60}; D) SA + I_{100}; E) proline + I_{60}; F) proline + I_{100} Ab, abaxial surface; Ad, adaxial surface; Mes, mesophyll cells and vb, vascular bundle, scale bars=200 µm.

proved dry matter accumulation in wheat plants under drought stress. KHAN et al. (2012) mentioned that soaking kernels of wheat in SA could be utilized as a potential promoter of water shortage tolerance in wheat. We confirmed that SA or proline markedly enhanced WUE under FDI stress conditions that are essentially due to improved photosynthesis by SA or proline (Tab. 4, 5). RWC is a common method to evaluate water balance in plant leaves during periods in which water is insufficient. Keeping RWC in cells and tissues at a healthy status induces the metabolic activity to be continuous by osmotic adjustments and other adaptations like salinity and/or water deficiency (SLABBERT and KRÜGER, 2014). Our results documented that FDI stress induced the decrease in RWC, but SA or proline applications could alleviate water deficiency stress by a higher value of WUE. Applications of SA or proline on plants grown under field deficit irrigation stress lead to a reduction in EL in the plant tissues. This is a signal about the stability of membranes in the drought-stressed wheat plants by SA or proline.

One of the important mechanisms used in the plant to fight the harmful effects of water deficiency and to create compatible solutes (RAMANJULU and BARTELS, 2002). Accumulation of compatible solutes such as sugars and proline in the cells of wheat leaves facilitates water uptake and thus permits the maintenance of turgor pressure under FDI stress conditions as well as scavenge ROS. The pretreatment of kernels with SA or proline caused a high increase in sugars in wheat leaves; however, the increase was clearer in the case of SA than that in the non-stressed control. The increases in compatible solute contents following SA application facilitated water uptake by osmoregulation, therefore, stress generated by FDI was alleviated. Similar results were obtained by CHANDRA et al. (2007) who showed that addition of SA to cowpea plants markedly increased total soluble sugar. Water shortage enhances the accumulation of proline in several plant species, where proline is probably the most widely-distributed osmolyte (REDDY et al., 2004). Proline accumulation could be a protecting action, due to the protecting role of proline in the inhibited stress-caused water shortage. (KUZNETSOV and SHEVYAKOVA, 1997). Field deficit irrigation stress induced a significant increase in the content of total soluble phenols of wheat leaves. Our results are in accordance with former studies on wheat (KELES and ONCEL, 2004) and potato (DANESHMAND et al., 2010). Phenolic compounds can do as an antioxidant to scavenge ROS in plants under stressful condition (SOLECKA, 1997). The results revealed an improve antioxidant status indicating forming of antioxidants in all treatments.

In our investigation, wheat plants showed high activity of SOD, CAT and POD enzymes under FDI stress. This activity was higher with SA or proline applications in comparison to the control plants. This evidence improved redox defense status to scavenge ROS damage. Treatment with SA plays a role in the induction of antioxidant defense responses by promoting several antioxidant enzymes, which are necessary for plant protection against water and salt stresses as well as other stress (NOREEN et al., 2009). The results obviously proved that SA lowered the stress status inside the wheat plant by regulating the physiological mechanism. Thus, the increased level of antioxidant enzymes by FDI stress was increased by SA application.

Considerable negative changes were noticed in the anatomy of stem and leaf of the wheat plant due to exposure to FDI stress. However, FDI stress caused a considerable reduction in the thickness of the stem diameter. This was principally due to the lowering in thickness of sclerenchymatous tissue, ground tissue thickness by lowering both cell number and diameter, the average diameter of metaxylem vessels and dimension of vascular bundles in compare to plants undergo to FDI alone (I_{60} treatment). This reduction in the preceding mentioned characters probably due to the suppression of the procambial activity of vascular tissues as well as a decrease in the cell number and size of stem tissues. The decrease in cell number and size of wheat stem tissues caused by FDI stress may be attributed to decrease in phytohormones level such as indole acetic acid (IAA) and cytokinins, and consequently decrease in cells division and expansion. SA or proline caused a positive change in the proceeding mentioned features and resisted the adverse effect of FDI stress compared with the corresponding FDI level, because salicylic acid increased the content of IAA, which causes cells division and expansion. All enhanced stem anatomical parameters due to SA or proline applications reflected a high translocation of the absorbed water and nutrients into cells to be utilized in various metabolic processes, which positively affected the performance and yield of the wheat plant. FDI stress induced a great decrease in midvein, blade, mesophyll, upper and lower epidermis thickness as well as the average diameter of vessels as compared to the control. However, SA or proline induced a marked improvement in the proceeding mentioned attributes and confrontation the undesirable impacts of FDI stress. The lowering in mesophyll and epidermal cells thickness of the FDI stressed leaves may result from the lessening in volume of mesophyll and epidermal cells as a result of decrease in endogenous of IAA content. The decline in blade thickness may also be related to a little volume of mesophyll cells under FDI stressed leaves. Similarly, TODD et al. (1974) showed that leaf thickness was reduced by water shortage stress. MERKULOV et al. (1997) noticed that lamina of sugar beet genotypes undergoes to water deficiency stress exhibited smaller epidermal cells, thicker cuticle and a higher number of layers of smaller mesophyll cells. Our result demonstrated a close correlation between amelioration in the anatomy of leaf and stem and physio-biochemical traits supporting the superior performance of wheat plant under FDI stress. To the best of the authors' knowledge, this study is the first attempt to illustrate the positive effect of soaking wheat kernels in proline and salicylic acid on the anatomical structure of wheat plants under conditions of field deficit-irrigation stress, enabling wheat plants to overcome the negative impact of the deficit-irrigation stress.

Conclusion

The results conclude that pretreatment of wheat kernels with SA or proline could effectively increase performance, kernel yield, and water use efficiency of wheat plants under field deficit irrigation stress. The enhancement of different parameters like total chlorophyll and carotenoid contents, proline accumulation, soluble phenols, soluble protein, and sugars contents, as well positive alternations in the anatomy of leaf and stem additionally reducing oxidative stress by ROS, are among the important manifestations of SA or proline pretreatment. Soaking kernels in SA or proline implicated in suppression of oxidative damage in wheat cells through enhancement of enzymatic antioxidants responsible for modulating of ROS during field deficit irrigation stress. Promotion of the antioxidative defense systems, at least in part, protected the photosynthetic machinery and enhanced the tolerance of the wheat plants to the field deficit irrigation stress. The results suggest using SA may serve as an efficient pretreatment for the amelioration of physiological and metabolic processes that may improve tolerance to water deficiency stress in wheat plants.

Acknowledgments

We acknowledge the support from the Deanship of Scientific Research at King Khalid University and the Agricultural Botany Department, Faculty of Agriculture, Fayoum University for the sponsorship, and encouragement.

Funding

This work was supported by the Deanship of Scientific Research at King Khalid University for funding this study through the research groups program under grant number R.G.P. 1/43/40.

References

Agami, R.A., 2013: Alleviating the adverse effects of NaCl stress in maize seedlings by pretreating seeds with salicylic acid and 24-epibrassinolide. S. Afr. J. Bot. 88, 171-177. DOI: 10.1016/j.sajb.2013.07.019

Agami, R.A., 2014: Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. Biol. Plant. 58, 341-347. DOI: 10.1007/s10535-014-0392-y

Agami, R.A., Alamri, S.A.M., Abd El-Mageed, T.A., Abousekken, M.S.M. Hashem, M., 2018: Role of exogenous nitrogen supply in alleviating the deficit irrigation stress in wheat plants. Agr. Water Mange. 210, 261-270. DOI: 10.1016/j.agwat.2018.08.034

Aldesuquy, H.S., Haroun, S.A., Abo-Hamed, S.A., Al-Saied, A.A., 2011: Physiological studies of some polyamines on wheat plants irrigated with waste water. I. Osmolytes in relation to osmotic adjustment and grain yield. Phyton 50, 263-268.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998: Crop Evapotranspiration guidelines for computing crop water requirements. Irrigation and Drainage Paper 56, FAO, Rome.

Al-Hakimi, A.M.A., Hamada, A.M., 2001. Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. Biol. Plant. 44, 253-261.

Arnon, D.I., 1949: Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris* L. Plant Physiology 24, 1-5.

Bates, L.S., Waldeen, R.P., Teare, I.D., 1973: Rapid determination of free proline for water stress studies. Plant and Soil 39, 205-207.

Bradford, M.M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Beauchamp, C., Fridovich, I., 1971: Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44 (1), 276-287.

Chandra, A., Anand, A., Dubey, A., 2007: Effect of salicylic acid on morphological and biochemical attributes in cowpea. J. Environ. Biol. 28, 193-196.

Daneshm, F., Arvin, M.J., Kalantari, K.M., 2010: Physiological responses to NaCl stress in three wild species of potato in vitro. Acta Physiol. Plant. 32, 91-101. DOI: 10.1007/s11738-009-0384-2

de Freitas, P.A.F., de Souza Miranda, R., Marques, E.C., Prisco, J.T., Gomes- Filho, E., 2018: Salt tolerance induced by exogenous proline in maize is related to low oxidative damage and favorable ionic homeostasis. J. Plant Growth Regul. 37, 911-924. DOI: 10.1007/s00344-018-9787-x

Duncan, D.B., 1955: Multiple range and multiple F-test. Biometrics 11, 1-42.

Gapinska, M., Sklodowska, M., Gabara, B., 2008: Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots. Acta Physiol. Plant. 30, 11-18. DOI: 10.1007/s11738-007-0072-z

Havir, E.A., McHale, N.A., 1987: Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84, 450-455.

Huang, Y., Bie, Z., Liu, Z., Zhen, A., Wang, W., 2009: Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. Soil Sci. Plant Nutr. 55, 698-704. DOI: 10.1111/j.1747-0765.2009.00412.x

Hsu, C.I., Chen, W., Weng, Y.M., Weng, C., Weng, Y., 2003: Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 83, 85-92.

Irigoyen, J.J., Emerich, D.W., Sanchez-Diaz, M., 1992: Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plant. 8, 455-460. DOI: 10.1111/j.1399-3054.1992.tb08764.x

Israelsen, O.W., Hansen, V.C., 1962: Irrigation principles and practices. John Wiley & Sons Inco., New York, U.S.A.

Jensen, M.E., 1983: Design and operation of farm irrigation systems. ASAE, Michigan, USA.

Jini, D., Joseph, B., 2017: Physiological mechanism of salicylic acid for alleviation of salt stress in rice. Rice Science. 24, 97-108. DOI: 10.1016/j.rsci.2016.07.007

Kang, G.Z., Li, G.Z., Liu, G.Q., Xu, W., Peng, X.Q., Wang, C.Y., Zhu, Y.J., Hara, M., Furukawa, J., Sato, A., Mizoguchi, T., Miura, K., 2012: Abiotic stress and role of salicylic acid in plants. In: Parvaiza, A., Prasad, M.N.V. (eds.), Abiotic stress responses in plants, 235-251. Springer, New York.

Keles, Y., Oncel, I., 2004: Growth and solute composition in two, wheat species experiencing combined influence of stress conditions. Russ. J. Plant Physiol. 51, 203-208.

Khan, S.U., Bano, A., Ud-Din, J.U., Gurmani, A.R., 2012: Abscisic acid and salicylic acid seed treatment as potent inducer of drought tolerance in wheat (*Triticum aestivum* L.). Pak. J. Bot. 44, (Special Issue), 43-49.

Klapheck, S., Zimmer, I., Cosse, H., 1990: Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol. 31, 1005-1013. DOI: 10.1093/oxfordjournals.pcp.a077996

Klute, A., 1986: Methods of soil analysis. Part-I: Physical and mineralogical methods, 2nd ed. American Society of Agronomy Madison, Wisconsin, USA.

Korkmaz, A., Korkmaz, Y., Demirkiran, A.R., 2010: Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. Environ. Exp. Bot. 67, 495-501. DOI: 10.1016/j.envexpbot.2009.07.009

Kuznetsov, V.V., Shevyakova, N.I., 1997: Stress responses of tobacco cells to high temperature and salinity: Proline accumulation and phosphorylation of polypeptides. Physio. Plant. 100, 320-326. DOI: 10.1111/j.1399-3054.1997.tb04789.x

Maehly, A.C., Chance, B., 1954: The assay of catalase and peroxidase. In: Glick, D. (ed.), Methods of biochemical analysis, 357-425. John Wiley & Sons, Inc, Hoboken, NJ.

Merkulov, L. j, Ivezi, C.J., Krsti, C.B., Kovacev, L., Pajevi, C.S., 1997: Structural characteristics of leaf blade of differentially drought-tolerant sugar beet genotypes. Proceedings: Drought and Plant Production, Agricultural Research Institute "Serbia", 487-492.

Meister, A., 2012: Biochemistry of the amino acids, 2nd Edit., Elsevier.

Misra, A.N., Biswal, A.K., Misra, M., 2002: Physiological, biochemical and molecular aspects of water stress in plants, and their biotechnological applications. Proc. Natl. Acad. Sci. 72, 115-134.

Morant-Manceau, A., Pradier, E., Tremblin, G., 2004: Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. J. Plant Physiol. 161, 25-33. DOI: 10.1078/0176-1617-00963

Mukherjee, S.P., Choudhari, M.A., 1983: Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant 58, 116-170. DOI: 10.1111/j.1399-3054.1983.tb04162.x

Nazar, R., Umar, S., Khan, N.A., Sareer, O., 2015: Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. S. Afr. J. Bot. 98, 84-94. DOI: 10.1016/j.sajb.2015.02.005

Nonami, H., 1998: Plant water relations and control of cell elongation at low water potentials, J. Plant Res. 111, 373-382. DOI: 10.1007/BF02507801

Noreen, S., Ashraf, M., Hussain, M., Jamil, A., 2009: Exogenous application of salicylic acid enhances antioxidative capacity in salt stressed sunflower (*Helianthus annuus* L.) plants. Pak J. Bot. 41, 473-479.

Page, A.I., Miller, R.H., Keeny, D.R., 1982: Methods of soil analysis part II. Chemical and Microbiological Methods, 2nd ed. American Society of Agronomy, Madison, WI, USA, 225-246.

Ponce, V.M., Pandey, R.P., Ercan, S., 2000: Characterization of drought across the climate spectrum. J. Hydrol. Eng. ASCE 5 (2), 222-224. DOI: 10.1061/ (ASCE) 1084-0699(2000) 5:2(222)

Ramanjulu, S., Bartels, D., 2002: Drought- and desiccation-induced modulation of gene expression in plants. Plant Cell Environ. 25, 141-151.

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004: Drought-

induced responses of photosynthesis and antioxidant metabolism in higher plants: Review. Plant Physiol. 161, 1189-1202.

Rivas-SanVicente, M., Plasencia, J., 2011: Salicylic acid beyond defence: its role in plant growth and development. J. Exp. Bot. 62, 3321-3338. DOI: 10.1093/jxb/err031

Sass, J.A., 1961: Botanical Microtechnique, 3rd ed. The Iowa State University Press, Ames.

Shahbaz, M., Mushtaq, Z., Andaz, F., Masood, A., 2013: Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L.)? Sci. Hortic. 164, 507-511.

Slabbert, M.M., Krüger, G.H.J., 2014: Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed *Amaranthus* leaves. S. Afr. J. Bot. 95, 123-128. DOI: 10.1016/j.sajb.2014.08.008

Slinkard, K., Singleton, V.L., 1997: Total phenol analysis: automation and comparison with manual methods. Amer. J. Enol. Viticult. 28, 49-55.

Solecka, D., 1997: Role of phenyl propanoid compounds in plant responses to different stress factor. Acta Physiol. Plant. 19, 257-268. DOI: 10.1007/s11738-997-0001-1

Tiwari, J.K., Munshi, A.D., Kumar, R., Pandey, R.N., Arora, A., Bhat, J.S., Sureja, A.K., 2010: Effect of salt stress on cucumber: Na^+/K^+ ratio, osmolyte concentration, phenols and chlorophyll content. Acta Physiol. Plant. 32, 103-114. DOI: 10.1007/s11738-009-0385-1

Todd, G.W., Richardson, P.E., Sensgupta, S.P., 1974: Leaf and stem anomalies in a drought susceptible species *Impatiens balsamina* under conditions of drought stress. Bot. Gaz. 135, 121-126.

VSN International, 2015: GENSTAT for Windows 17th Edition. (VSN International: Hemel Hempstead, UK), Available at www.GENSTAT.co.uk. Retrieved from www.GENSTAT.co.uk

Wang, W., Vinocur, B., Altman, A., 2003: Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 218, 1-14. DOI: 10.1007/s00425-003-1105-5

ORCID:
Ramadan A. Agami https://orcid.org/0000-0002-2646-2276
Saad A.M. Alamri https://orcid.org/0000-0001-9228-188X
T.A. Abd El-Mageed http://orcid.org/0000-0002-8691-748X
M.S.M. Abousekken http://orcid.org/0000-0002-2544-616 X
Mohamed Hashem https://orcid.org/0000-0003-2593-3387

Address of the corresponding author:
Ramadan A. Agami, Agricultural Botany Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt
E-mail: rag01@fayoum.edu.eg