



Effect of Exogenously Applied Jasmonic Acid and Kinetin on Drought Tolerance of Wheat Cultivars Based on Morpho-Physiological Evaluation

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Received: 27 July 2020 / Accepted: 22 September 2020 / Published online: 28 September 2020
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Abstract

The population acceleration and better lifestyle submit new challenges for wheat researchers to breed wheat (*Triticum sativum*) cultivars with upgraded yield, quality, and resistance against abiotic stresses such as drought, so exploiting all available natural relatives of cultivated wheat and introducing even sensitive ones may be a useful approach to save time and efforts. Normally, the seedling stage is highly drought vulnerable, but for sensitive cultivars, the situation is more frustrating. We examine the potentiality of two regulating hormones in the upregulation of two wheat cultivars varying in their drought susceptibility at the seedling stage comparatively evaluated by morpho-physiological traits as indicators of drought tolerance. All the studied traits revealed cultivar-dependent variation in response to water deficit where cv. Sids 1 was tolerant and cv. Beni-suef 5 was sensitive. Shoot/root ratio, total water content, total dry weight, chlorophyll stability, total osmotic potential, osmoregulatory components, viz., soluble carbohydrates, soluble proteins and proline, membrane damage trait in terms of LOX, antioxidant defense system enzymatically in terms of APX, CAT, POD, SOD, and total antioxidant as drought tolerance indicators were the troubling shot due to water shortage in both cultivars. The damaging impacts of water deficit on these traits were conceived for sensitive cultivar compared with the tolerant one. Exogenous application of jasmonic acid (JA) or kinetin (K) efficiently conferred drought tolerance to sensitive cultivar to withstand harsh conditions in earlier stages and to perform comparably with tolerant ones. Applied hormones prompted unequivocal inversion from a state of downregulation to upregulation regarding all drought tolerance traits via reallocation of photoassimilates to vegetative sinks, thus promoting growth, increasing the accumulation of some osmoregulation compounds and thus increased tissue vigor and regulated the activity of antioxidant enzymes as well as morphological modulation attained by the restoration of shoot/root ratio. The results would promisingly be supportive of research programs seeking to develop anti-drought stress practices for sensitive wheat cultivars.

Keywords Antioxidant enzymes · Drought tolerance · Jasmonic acid · Kinetin · Osmoregulation · Polyethylene glycol · *Triticum sativum*

1 Introduction

Crop water deficit arises from inadequate rainfall besides the diminution of soil water potential during the growing period,

which acts as a limiting factor to sustain high crop yield (Vadez et al. 2012; Eissa 2014, 2016). Moreover, the predicted climate change and global warming will promote the occurrence of drought and consequently lessen the productivity of agriculture crops (Trenberth et al. 2014; Eissa et al. 2014; El-Mahdy et al. 2018; Eissa and Negim 2018), a matter that makes drought stress an extremely serious issue. The drought influences plant growth and development via hampering crop growth rate, thus adversely impacting biomass acquisition (Damalas 2019); it also impacts plants' water potential and turgor, possibly interfering with normal functions, leading to changes in physiological and morphological traits (Lata et al. 2011; Vurukonda et al. 2016).

Wheat (*Triticum sativum* L.) has a fundamental role in food and feeds in the world (Ahmed et al. 2019) recording the

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largest harvested area of approximately 220.1 million ha and the second bulkiest global production about 749.5 million metric tons (MMT) among food grains (FAOSTAT 2018). Wheat production was estimated as drought stress affected approximately 65 million ha (FAOSTAT 2019). Egypt is a typical example of the drought problem subjected to some arid regions. In these areas, bridging the gap between wheat production and consumption is necessary relatives of cultivated cultivars, even sensitive ones, and increasing their production (Rauf et al. 2007); hence, the establishment of genetic resources provided by naturally resistant varieties and/or breeding the genetically engineered varieties for improving crop adaptation and productivity under water deficit are very costly and time-consuming processes. However, introducing sensitive varieties to dry regions is a risky concern regarding survival and development.

In the case of wheat, the main threatening issue is the deficit of water at the seedling stage, midseason stress, and terminal stress. Drought stress can occur at any growth stage. Therefore, the stage at which genotypes are screened for drought tolerance should be carefully considered. For instance, improving a survival trait under drought conditions at the seedling stage may be appropriate if the drought stress occurs during seedling growth and development (Sallam et al. 2018). In the last 10 years, low rainfall has been noted in Egypt during the planting period, October to March, which coincides with the seedling stage of cultivated wheat (Sallam et al. 2018). Early drought is a major constraint for cultivating the wheat crop in the semi-arid region that results in weak seedlings and poor development and might lead to a complete crop failure with minor ultimate yield under severe drought. Thus, supporting tolerance during such periods of diminishing water resources is a mightily challenging mission.

Plants not only need to grow and develop but also withstand environmental stress to survive and reproduce. Maintaining a balance between plant growth and stress tolerance has many benefits (Zaman-Allah et al. 2011). The very large differences between cultivars in drought tolerance are defined when determining some plant morpho-physiological traits which are very important for selection in a breeding program to improve drought tolerance due to their relation to the adaption for future climate scenarios (Anjum et al. 2017). Drought-tolerant plants try to have a longer root system, higher membrane stability, less water content reduction, and photosynthetic activity. The tolerant group tends to accumulate proline content, soluble sugars, amino acids, chlorophyll content, and non-enzymatic and enzymatic antioxidant activities (Wang et al. 2020).

Several strategies have been handled to refine drought tolerance of crops and mitigate the drought hazards focusing on augmenting sensitive cultivar performances due to the aforementioned reasons (Wang et al. 2020), including the application of plant growth regulators (phytohormones) as foliar

application and seed treatment, which acts as an alternative mechanism for sustainable agriculture under water-deficit conditions (Ilyas et al. 2017). Plant hormones, which are natural and non-toxic compounds, could be applied as safe and environmentally friendly chemical control agents (Wang et al. 2020). Plant growth regulators (PGRs) display a vital role in regulating plant reactions to environmental stresses (Upreti and Sharma 2016). Formerly, it has been proved that exogenously delivered hormones during stress conditions may enhance plant tolerance via increasing the detoxification capacity and physiological attitude of plants (Asgher et al. 2015). The most important phytohormones are methyl jasmonates (MeJAs) and JA (jasmonic acid), a family of important signal transducers that regulate the gene expression involved in defensive responses (Ahmad et al. 2016). Previously, some authors have declared that exogenous application of jasmonates augments plant resistance to water stress conditions (Bandurska et al. 2003) and contributes in the regulation of stomatal closure (Riemann et al. 2015), root development (Fang and Xiong 2015), and elimination of reactive oxygen species (ROS) by raising the antioxidant potential (Nafie et al. 2011). Wang et al. (2020) elucidated that exogenous application of SA and JA may increase the tolerance of drought stress in wheat. Qiu et al. (2014) reported that 3 days of exposure to exogenous JA decreased the membrane damage level in wheat seedlings significantly, improving the tolerance of wheat seedlings to salt stress. Anjum et al. (2016) suggested that MeJA is involved in improving the drought tolerance of wheat by maintaining growth and yield performance of plants at early and late developmental stages.

Kinetin (K) is a synthetically produced cytokinin that shoulders a critical task in stress resistance to a wide array of abiotic stresses and delays the leaf senescence directly by scavenging free radicals (Kaya et al. 2018). Also, it improves both synthesis of photosynthetic proteins and chlorophyll contents in plants, activation of cell division, and modification in apical dominance in plants (Taiz and Zeiger 2006; Bielach et al. 2017).

Polyethylene glycol (PEG) is a group of neutral active osmotic polymers of a distinct molecular weight that is non-ionic, inert, almost impermeable to cell membranes, and can impose representative water stress without inducing physiological damage (Almaghrabi 2012). It has been broadly applied to study crops' response to drought through the germination stage (Basu et al. 2010).

Accordingly, the aims of this research work were to (1) address the sensitivity of the morpho-physiologic parameters of the studied cultivars to drought stress that ultimately determines seedling performance and development, (2) examine the potentiality of JA and/or kinetin (Kin) exogenous application to restore correlated drought tolerance traits particularly in sensitive cultivars at the highly vulnerable seedling stage.

2 Materials and Methods

2.1 Seed Collection and Growth Conditions

Two wheat cultivars (*Triticum sativum* L.) Sids 1 and Beni-suef 5 were handled as seed materials in this investigation. They are all widely cultivated cultivars in Egypt. Seeds were obtained from the Department of Crop Plant, Faculty of Agriculture, Assiut University. Germination experiments were conducted in growth conditions at 10–20 °C temperature under 16 h light and 8 h dark photoperiodic cycle with a light intensity of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and with a relative humidity of 50%. Seeds were surface sterilized with 0.1% sodium hypochlorite for 20 min, extensively washed with sterile distilled H_2O , and then dried.

Seeds of both cultivars were germinated in two folds of filter paper placed in Petri dishes (diameter 12 cm) with 15 ml of the test solutions. Ten seeds were used with four replicates for each treatment. Seeds were germinated under water stress induced by adding inactive osmotica, non-penetrating polymers of PEG 6000 (purchased from Sigma-Aldrich), at the level of -1.03 MPa. This level was chosen based on a previous preliminary experiment consisting of four levels, i.e., -0.15 , -0.49 , -1.03 , and -1.79 (MPa) of PEG 6000. The plants grown in -1.03 MPa recorded a 50% inhibition (EC_{50}).

Jasmonic acid (JA) was obtained from Sigma which was dissolved 0.2 μl in 10 ml 96% ethanol, because JA has low solubility in water, then diluted to 50 ml with distilled H_2O to obtain 0.1 mM (~ 100 μM). On the other hand, 0.5 mM kinetin (Sigma-Aldrich) was prepared by dissolving 0.005 g in 10 ml in 96% ethanol and complete to 50 ml with distilled H_2O .

The 13-day-old seedlings with three leaves were classified into

1. Water (cont).
2. PEG as drought stress at -1.03 MPa.
3. Unstressed seedlings sprayed either with previously mentioned concentrations of jasmonic acid or kinetin.
4. Drought-stressed seedlings sprayed either with jasmonic acid or kinetin.

The green leaves of the treated seedlings (16 days old) were washed with sterile distilled water, harvested, frozen in liquid nitrogen, and stored at -80 °C.

2.2 Growth Parameters

2.2.1 Shoot/Root Ratio and Total Dry Weight

The shoot and root lengths were measured to calculate shoot/root ratios. Subsequently, half of the samples were quickly

dried in an oven at 70 °C to a constant weight and then ground, which was used to determine the total dry weight and further analysis. Another part of the fresh tissue was used immediately for chlorophyll stability and the estimation of the total water content (TWC) of the leaves.

2.3 Physiological and Biochemical Analysis

2.3.1 Determination of Chlorophyll Stability Index (CSI)

Chlorophyll stability index was calculated according to Murphy (1962) as the ratio (expressed in percentage) between the chlorophyll content in the heated sample (56 ± 1 °C for 30 min) and the fresh sample for chlorophyll *a*, which was modified as follows:

$$\text{CSI} = \frac{\text{Chl in heated sample}}{\text{Chl in fresh sample}} \times 100 \text{ Placeholder Text}$$

2.3.2 Determination of Total Osmotic Potential

The leaf sap was obtained by grinding fresh leaves using a Beckman differential thermometer (calibrated to 0.01 °C) then centrifuged at 10,000 $\times g$ for 15 min, and the collected plant extract was used to measure the osmotic potential (Ψ_s) by the cryoscopic method of Ramadan (1998). The osmotic potentials ($-\text{bar}$) were then obtained from tables compiled by Walter (1931).

2.3.3 Determination of Proline and Other Metabolites

Free proline had been determined in dry leaves and determined according to Bates et al. (1973). Leaf samples were homogenized in 6 ml 3% sulfosalicylic acid and the homogenate was centrifuged at 10,000 $\times g$. Two milliliters of supernatant was mixed with 2 ml of ninhydrin and 2 ml of glacial acetic acid. The samples were heated at 100 °C for 1 h and then cooled at room temperature. The reaction mixture was extracted with 4 ml toluene and the free toluene content was quantified at 520 nm and expressed as milligrams per gram of dry weight.

The water-soluble sugars were determined by the method of anthrone–sulfuric acid according to the method of Fales (1951). Anthrone–sulfuric acid reagent: anthrone 0.2 g, 30 ml distilled water, 8 ml absolute ethyl alcohol, and 100 ml concentrated H_2SO_4 were respectively mixed in a conical flask under continuous cooling in an ice bath. Then 30 mg of dry leaves was taken and extracted in 3 ml distilled H_2O which was mixed with 4.5 ml anthrone reagent and boiled in a water bath for 7 min; thereafter, it was directly cooled under tap water. The absorbance of the developed blue-green color was determined at 620 nm against a blank

containing anthrone reagent using a Unico UV-2100 spectrophotometer and expressed as milligrams per gram of dry weight of the seedling leaves. A calibration curve was prepared using pure glucose.

2.3.4 Assay Soluble Protein and Enzyme Activity

Fresh wheat leaves (20 mg) were ground to a fine powder in liquid N₂ and then homogenized with 3 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 g polyvinylpyrrolidone. The homogenate was centrifuged at 18,000 rpm for 10 min. at 4 °C and the supernatants collected and used for the assayed soluble proteins, superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, lipoxygenase, and total antioxidant. All colorimetric measurements (including enzyme activities) were made at 20 °C using a Unico UV-2100 spectrophotometer.

Soluble protein was assayed according to Lowry et al. (1951). Enzymatic extract (0.1 ml) was added to 5 ml of the alkaline reagent solution. Both were mixed thoroughly and allowed to stand at room temperature for 10 min. Thereafter, 0.5 ml of diluted Folin-Ciocalteu's reagent (1: 2 v/v) was added and mixed rapidly. After 30 min, the extinction against appropriate blank was read at 750 nm using a spectrophotometer and expressed as milligrams per gram of fresh weight.

Superoxide dismutase (SOD) and catalase (CAT) activities were mentioned by Fawzy et al. (2017). The activity of SOD (EC 1.15.1.1) was measured in a final volume of 2 ml of a reaction medium containing 50 mM of sodium carbonate buffer (pH 10.2), 0.1 mM EDTA, 100 µl enzymatic extract, and 100 µl of 5.5 mg/ml epinephrine (dissolved in 10 mM HCl, pH 2).

CAT (EC 1.11.1.6) activity was assayed in a reaction medium (4 ml volume) containing 50 mM potassium phosphate buffer (pH 7), 100 µl 10 mM H₂O₂, and 50 µl of enzyme extract. The decrease in absorbance at 240 nm was determined to calculate the specific activity using a spectrophotometer.

Ascorbate peroxidase (APX) and peroxidase (POD) activities were measured spectrophotometrically following the method of Abdel-Wahab et al. (2019).

Assessment of ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined by the rate of hydrogen peroxide-dependent oxidation of ascorbic acid in a reaction mixture that contained 50 mM potassium phosphate buffer (pH 7), 5 mM H₂O₂, 0.1 mM Na₂-EDTA, and 50 µl enzyme extract. The oxidation rate of ascorbic acid was estimated from the decrease in absorbance at 290 nm for 1 min.

POD (EC 1.11.1.7) activity was measured in the reaction mixture (3 ml) that consisted of 30 mM potassium phosphate buffer (pH 7), 6.5 mM H₂O₂, and 1.5 mM guaiacol. The reaction was started by adding 100 µl enzyme extract. The formation of tetraguaiacol was measured at 470 nm.

Lipoxygenase (LOX; EC 1.13.11.12) activity was estimated according to the method of Minguez-Mosquera et al. (1993). For the measurement, the substrate solution was prepared by adding 0.035 ml linoleic acid to 5 ml distilled water containing 0.05 ml Tween-20. The solution was kept at pH 9.0 by adding 0.2 M NaOH until all the linoleic acid was dissolved and the pH remained stable. After pH adjustment to 6.5 by adding 0.2 M HCl, 0.1 M phosphate buffer (pH 6.5) was added to make a total volume of 100 ml. Then 0.05 ml of the enzyme extract was added to 2.95 ml substrate. Solution absorbance was recorded at 234 nm using a spectrophotometer.

2.3.5 Determination of the Total Antioxidant

The total antioxidant contents are described by Prieto et al. (1999). A definite volume of the enzymatic extract was mixed with 3 ml reagent composed of 0.6 M sulfuric acid, 28 mM sodium phosphate Na₂HPO₄, and 4 mM ammonium molybdate and incubated at 90 °C in a water bath for 90 min. The absorbance was measured at 750 nm and expressed in terms of ascorbic acid equivalents (µg g⁻¹ FW) using ascorbic acid (AA) as standard.

2.4 Statistical Analysis

The obtained data were subjected to statistical evaluation using the statistical program package SPSS (version 21). The one-way evaluation of variance followed by a post hoc test (Tukey's multiple range tests) ($p \leq 0.05$) was used for significant differences.

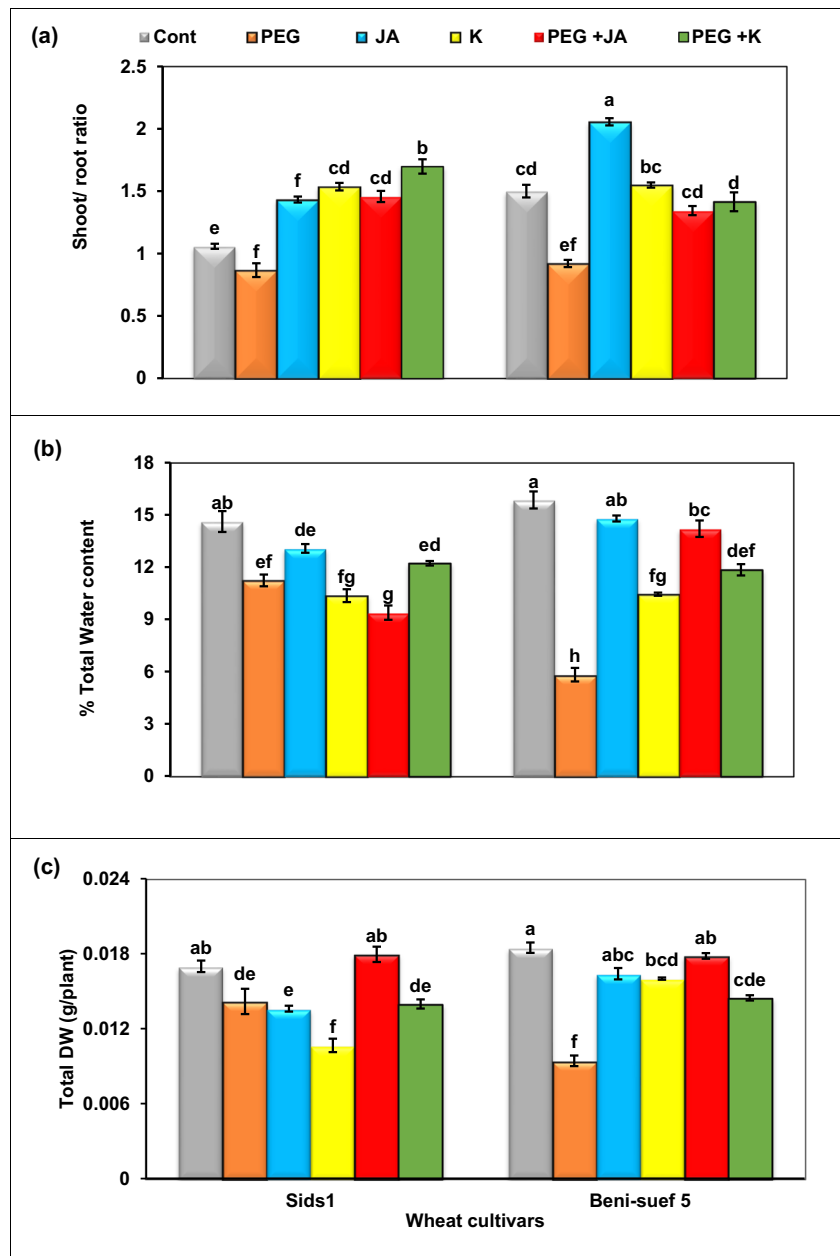
3 Results

3.1 Growth Parameters

3.1.1 Shoot/Root Ratio

Both cultivars exhibited a significant reduction in the shoot/root ratio (Fig. 1a). In cv. Beni-suef 5, an early morphological response to drought was the avoidance mechanism through adjustment of the plant growth rate such as a reduction in the shoot height, which was recorded as 61% when compared with the control and increasing the root growth (low shoot/root ratio) to compensate for the reduction in water uptake. The exogenous application of JA or K significantly restored the shoot/root ratio. This response was more pronounced for investigated seedlings of the sensitive cultivar (cv. Beni-suef 5).

Fig. 1 Effects of polyethylene glycol (PEG), exogenous application of jasmonic acid (JA) or kinetin (K), and exogenous application of JA or K to stressed seedlings (PEG + JA and PEG + K, respectively) on shoot/root ratio (a), percentage of total water content (b), and total dry weight (c) of two wheat cultivars (Sids 1 and Beni-suef 5). The data are means of four replicates ± SD. Means followed by the same letters are not significantly different according to Tukey’s test ($p \leq 0.05$)



3.1.2 Total Water Content

Under drought conditions, the percentage of total water content in seedlings of cv. Beni-suef 5 recorded (76.8%) was decreased more than cv. Sids 1 (36.6%) when compared with the control (Fig. 1b). Under unstressed conditions, the exogenous application of JA or K caused a significant increment in the total water content in the seedling of cv. Sids 1 reached 89% and 70%, respectively, while under the previously mentioned condition, the application of K had a significant decrease in the total water content in seedlings of cv. Beni-suef 5 that amounted to 65.8% when compared with control. Under stress conditions, the exogenous application of JA caused a

significant inhibition of the total water content in the case of cv. Sids 1; in contrast, either the application of JA or K showed highly significant stimulation in the total water content of cv. Beni-suef 5 when compared with drought-stressed seedlings.

3.1.3 The Total Dry Weight of Seedling

In this investigation, drought caused a significant inhibition in the total dry weight of both wheat cultivars, but seedlings of cv. Sids 1 was less affected than cv. Beni-suef 5 as evidenced by 83.4% and 51%, respectively, when compared with the control (Fig. 1c). Likewise, under unstressed conditions, the

exogenous application of JA or K decreased the total dry weight of cv. Sids 1 compared with the control, recorded as 80% and 62.7%, respectively, while under previously mentioned conditions, seedlings of cv. Beni-suef 5 exhibited a significant stimulation of 88.7% and 86.4%, respectively, as compared with the control. Only treated stressed seedlings of cv. Beni-suef 5 with exogenous application of JA was a significant enhancement of the total dry weight. Furthermore, treated drought-stressed seedlings of both wheat cultivars with K induced a significant stimulation of the total dry weight when compared with the stressed seedling.

3.1.4 Chlorophyll Stability Index (CSI)

The CSI indicates how well chlorophyll (Chl.a SI) performs under stress conditions (Fig. 2a). The drought-tolerant cultivar (Sids 1) had higher CSI values than the drought-susceptible one (Beni-suef 5) under control and drought stress conditions as well. Under drought-stressed condition, seedlings of Sids 1 and Beni-suef 5 cultivars exhibited a significant reduction (50% and 19.8%, respectively) when compared with the control. Exogenous application of both hormones was a sufficient tool to improve this trait in the case of drought-susceptible cultivar to be comparable with the control, and application of JA without drought recorded the highest value of Chl.a SI in leaves of cv. Beni-suef 5 (202.5%) when compared with the control. On the contrary, the exogenous application of

hormones negatively affected Chl.a SI under either stressed or unstressed seedlings of the drought-tolerant cultivar (Sids 1).

3.2 Osmotic Potential and Osmo-Metabolic Compounds

3.2.1 Total Osmotic Potential

The present results of the total osmotic potential represented in Fig. 2b revealed that significant adaptation of both cultivars to drought stress was pronounced through the increasing of total osmotic potential to be maximally recorded for cv. Sids 1 rather than cv. Beni-suef 5, which recorded 107% and 90%, respectively, when compared with the control. When both cultivars were exposed to exogenous application of JA or K, it significantly decreased the total osmotic potential under stressed and unstressed conditions especially cv. Sids 1, which is more responsive to the exogenous application of JA or K.

3.2.2 Proline

Drought stress induced a significant inhibition of the proline content in leaves of cv. Sids 1 which was recorded as 7%, while this condition significantly increments proline content in leaves of cv. Beni-suef 5 which was recorded as 644%, when compared with the control (Fig. 3a). The exogenous application of hormones under unstressed condition altered

Fig. 2 Effects of polyethylene glycol (PEG), exogenous application of jasmonic acid (JA), or kinetin (K) and exogenous of JA or K to stressed seedlings (PEG + JA and PEG + K, respectively) on percentage chlorophyll stability index of Chl.a SI (a) and total osmotic potential (b) in leaves of two wheat cultivars (Sids 1 and Beni-suef 5). The data are means of four replicates \pm SD. Means followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$)

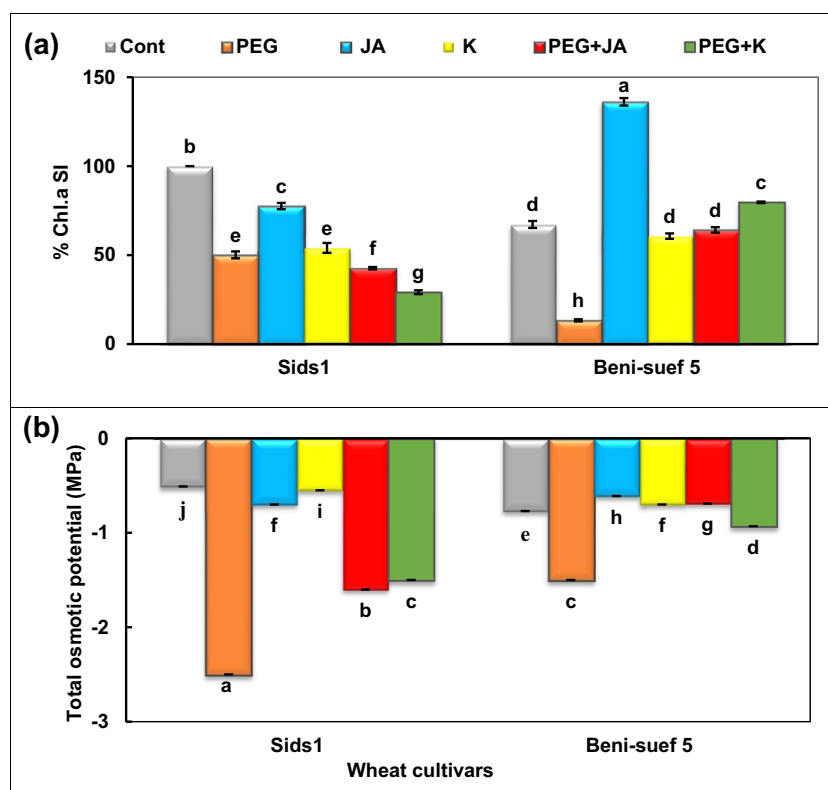
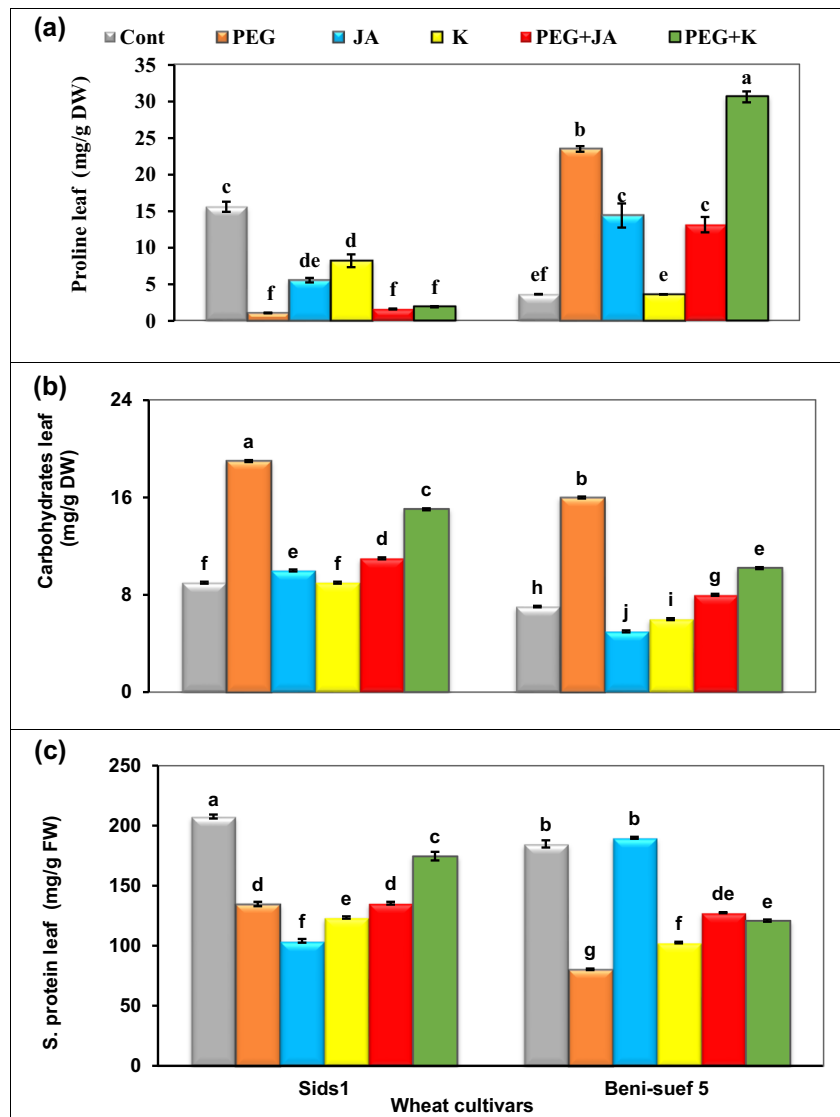


Fig. 3 Effects of polyethylene glycol (PEG), exogenous application of jasmonic acid (JA) or kinetin (K), and exogenous application of JA or K to stressed seedlings (PEG + JA and PEG + K, respectively) on proline content (a), soluble carbohydrate content (b), and soluble protein content (c) in leaves of two wheat cultivars (Sids 1 and Beni-suef 5). The data are means of four replicates ± SD. Means followed by the same letters are not significantly different according to Tukey’s test ($p \leq 0.05$)



proline content depending on the cultivar type. Both hormones efficiently reduced proline content in the drought-tolerant cultivar. Whereas the exogenous application of JA triggered proline accumulation in sensitive cultivar (Beni-suef 5), under the previously mentioned condition, on the other hand, application with K recorded a non-obvious change in proline content when compared with control seedlings. The exogenous application with either JA or K to drought-stressed seedlings of Sids 1 exhibited non-significant effects on proline content. However, drought-stressed (Beni-suef 5) seedlings triggered proline content by K which shoulders the regulation of proline metabolism in sensitive cultivar under drought condition rather than JA which exerted the opposite effect.

3.2.3 Soluble Carbohydrates

Drought stress significantly increased the soluble carbohydrate content in the leaves of Sids 1 and Beni-suef 5 cultivars

to 211% and 227%, respectively, when compared with the control (Fig. 3b). Under unstressed conditions, the exogenous application of JA or K has no obvious effects on the soluble carbohydrate content of both cultivars. In contrast, exogenous applications of JA or K to drought-stressed seedlings significantly reduced soluble carbohydrate content in the leaves of both cultivars when compared with drought-stressed seedlings.

3.2.4 Soluble Proteins

Treatment of both wheat cultivars with PEG caused significant decreases in leaf content of soluble proteins (Fig. 3c). It is worth mentioning that cv. Sids 1 was affected by drought stress less than cv. Beni-suef 5 that amounted to 64% and 43%, respectively, when compared with the control. Under the unstressed condition, exogenous application of JA showed different responses with two wheat cultivars; this treatment

caused a significant inhibition in the soluble protein content in leaves of cv. Sids 1 that reached 50%, whereas the previous condition stimulated soluble protein content in leaves of cv. Beni-suef 5 which was recorded as 102.7% when compared with the control. The exogenous application of K significantly suppressed the soluble protein content in the leaves of both cultivars when compared with the control, while in drought-stressed seedlings of cv. Sids 1, K application showed better responses than the JA application, which stimulated the soluble protein content more than stressed seedlings. Either exogenous application with JA or K significantly increased the soluble protein content of stressed seedlings (Beni-suef 5) when compared with stressed ones.

3.3 The Specific Activity of Enzymes

Data in Table 1 describe specific activities of APX, CAT, POD, and SOD enzymes of two wheat cultivars, which had different responses under drought stress imposed by PEG at –1.03 MPa. The specific activities of APX and CAT in the tolerant cv. Sids 1 was significantly inhibited by drought stress and amounted to 49% and 50.8%, respectively, while the sensitive one (Beni-suef 5) exhibited a significant increase in the specific activities of these enzymes recorded as 153% and 1087%, respectively, when compared with the control seedlings. On the other hand, drought stress significantly stimulated POD and SOD specific activity in cv. Sids 1, which was recorded as 144% and 126%, respectively, and also cv. Beni-suef 5 exhibited the same attitude and recorded the highest activity of these enzymes at 288.31% and 360%, respectively, when compared with the control.

The exogenous applications of JA or K under unstressed conditions had a different behavior on the specific activities of these enzymes in two wheat cultivars. Under previously mentioned conditions, JA application to leaves of cv. Beni-suef 5 exhibited high and significant stimulation in the activity of CAT, POD, and SOD enzymes to 333%, 128.9%, and 148%, respectively. Also, the exogenous application of K to unstressed seedlings caused a significant increase in the specific activity of APX, POD, and SOD enzymes in leaves of Sids 1 cv. to 121%, 128.9%, and 122.2%, respectively, when compared with the control. On the other hand, applications of JA or K under unstressed conditions significantly inhibited the activity of APX in cv. Beni-suef 5 which was recorded as 25.6% and 32.4%, respectively, and under previous conditions, a significant inhibition was observed in the CAT activity in leaves of cv. Sids 1 (39.4% and 78.3%, respectively) when compared with the control.

The exogenous applications of JA or K to stressed seedlings were effective upon cv. Beni-suef 5 more than those of cv. Sids 1. It was observed that treated Sids 1 seedlings with JA application manifested significant suppression in the activities of CAT and SOD enzymes. The application of K had the same attitude on the activity of APX, CAT, POD, and SOD. On the other hand, the treated stressed seedling of Beni-suef 5 cv. with JA exhibited a significant inhibition in the activities of CAT, POD, and SOD enzymes. Also, the exogenous application of K to this cultivar had the same effects on the activity of APX, CAT, POD, and SOD when compared with stressed seedlings.

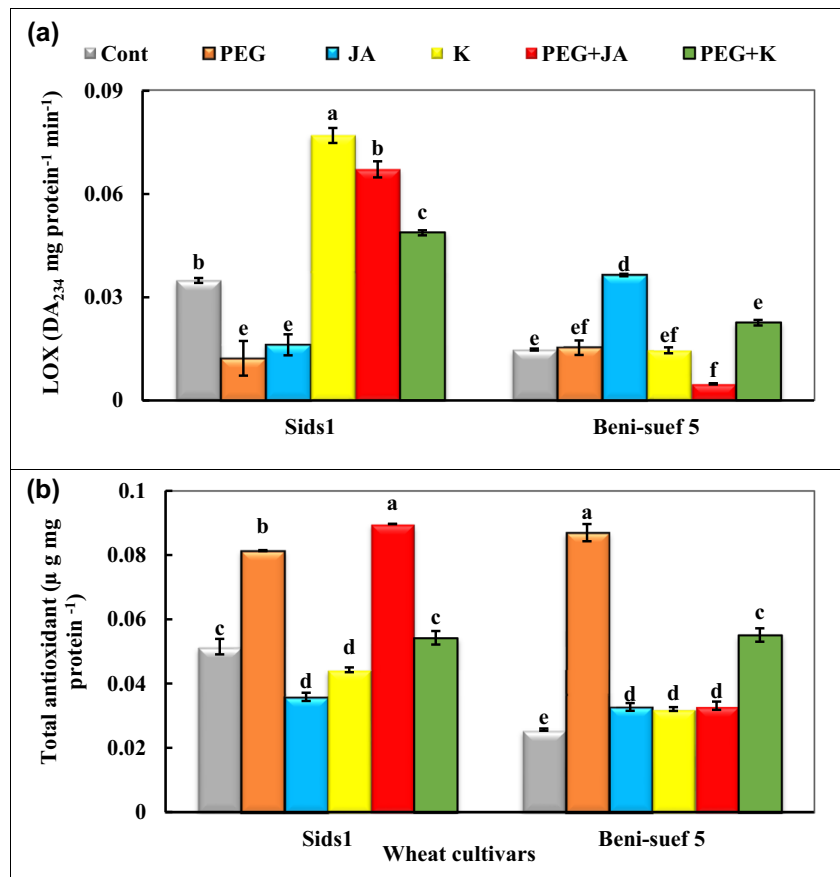
Table 1 Effects of polyethylene glycol (PEG), exogenous application of jasmonic acid (JA) or kinetin (K), and exogenous application of JA or K to stressed seedlings (PEG + JA and PEG + K, respectively) on

ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities in leaves of two wheat cultivars (Sids 1 and Beni-suef 5)

Wheat cultivars	Treatments	APX (DA ₂₉₀ mg protein ⁻¹ min ⁻¹)	CAT (DA ₂₄₀ mg protein ⁻¹ min ⁻¹)	POD (DA ₄₇₀ mg protein ⁻¹ min ⁻¹)	SOD (DA ₄₈₀ mg protein ⁻¹ min ⁻¹)
Sids 1	Control	0.09 ± 0.002 ^b	0.072 ± .009 ^c	0.043 ± 0.001 ^f	0.034 ± 0.0007 ^{cd}
	PEG	0.044 ± 0.005 ^d	0.037 ± .0001 ^{def}	0.062 ± 0.002 ^d	0.042 ± 0.002 ^{de}
	JA	0.082 ± 0.0002 ^b	0.028 ± .003 ^{efg}	0.111 ± 0.0001 ^a	0.053 ± 0.003 ^a
	K	0.11 ± 0.005 ^a	0.057 ± 0.01 ^{cd}	0.066 ± 0.003 ^{cd}	0.041 ± 0.002 ^b
	PEG + JA	0.058 ± 0.003 ^c	0.027 ± 0.002 ^{efg}	0.068 ± 0.0001 ^c	0.037 ± 0.002 ^{bc}
	PEG + K	0.027 ± 0.004 ^e	0.016 ± 0.0001 ^{fg}	0.054 ± 0.0006 ^{cd}	0.025 ± 0.0007 ^e
Beni-suef 5	Control	0.062 ± 0.004 ^c	0.013 ± 0.001 ^g	0.03 ± 0.0003 ^h	0.007 ± 0.0003 ^g
	PEG	0.095 ± 0.002 ^b	0.16 ± 0.005 ^a	0.086 ± 0.0008 ^b	0.027 ± 0.0007 ^e
	JA	0.016 ± 0.0007 ^e	0.045 ± 0.004 ^{de}	0.038 ± 0.0005 ^g	0.011 ± 0.0004 ^{fg}
	K	0.02 ± 0.0001 ^e	0.077 ± 0.008 ^c	0.05 ± 0.0005 ^e	0.012 ± 0.0008 ^{fg}
	PEG + JA	0.088 ± 0.004 ^b	0.018 ± 0.0009 ^{fg}	0.052 ± 0.0004 ^e	0.015 ± 0.0002 ^f
	PEG + K	0.025 ± 0.0003 ^e	0.111 ± 0.004 ^b	0.038 ± 0.00004 ^g	0.013 ± 0.0008 ^{fg}

The data are means of four replicates ± SD. Means followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$)

Fig. 4 Effects of polyethylene glycol (PEG), exogenous application of jasmonic acid (JA) or kinetin (K), and exogenous application of JA or K to stressed seedlings (PEG + JA and PEG + K, respectively) on lipoxygenase enzyme (LOX) activity (a) and total antioxidant (b) in leaves of two wheat cultivars (Sids 1 and Beni-suef 5). The data are means of four replicates \pm SD. Means followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$)



The data of LOX activity in leaves of both wheat cultivars are represented in Fig. 4a. The drought-stressed seedlings of Sids 1 cv. exhibited a significant inhibition in the specific activity of LOX, which recorded 35%, while the same condition did not cause a noticeable change in the activity of this enzyme in leaves of cv. Beni-suef 5 when compared with control seedlings. Exogenous application of JA to unstressed seedlings of cv. Sids 1 caused a significant inhibition in the activity of this enzyme to 46.5%. In contrast, under the previous condition, the application of K to this cultivar increased the activity of this enzyme significantly to 220.8% when compared with the control. On the other hand, application of JA to unstressed seedlings of cv. Beni-suef 5 recorded the significant value of LOX activity in leaves to 247%, while exogenous application of K caused no obvious change in the activity of this enzyme when compared with the control.

Treated drought-stressed seedlings of cv. Sids 1 with exogenous application of JA or K caused a significant increase in the activity of LOX when compared with stressed seedlings. Under the previously mentioned condition, application of JA to cv. Beni-suef 5 exhibited a significant inhibition. In contrast, K caused a non-significant inducement in LOX activity when compared with stressed seedlings.

3.4 The Total Antioxidants

Drought stress caused a significant increase in the accumulation of total antioxidants in leaves of Sids 1 and Beni-suef 5 cultivars by 158% and 339%, respectively, when compared with control seedlings (Fig. 4b). Also, exogenous applications of JA or K to unstressed drought seedlings of (Sids 1) decreased the total antioxidants of leaves by 69.6% and 86%, respectively, whereas these treatments exhibited significant increases in the content of total antioxidants in leaves of cv. Beni-suef 5 (127.5% and 125%, respectively) when compared with the control. When drought-stressed seedlings of both cultivars were treated with JA or K, it significantly decreased the total antioxidant content in leaves when compared with drought-stressed seedlings.

4 Discussion

Morpho-physiological traits are very important indicators to enhance drought tolerance due to their relation to the adaption for future climate scenarios. Drought upsets crop productivity by restricting different physiological and morphological features of the plant (Abd-Elmabod et al. 2020). In the present experiment, hormone application was effective in enhancing

shoot/root ratio, total water content, total dry weight, chlorophyll stability, total osmotic potential, osmoregulatory components, soluble carbohydrates, soluble protein, and proline accumulation of wheat cultivars, which was more efficient under deficit irrigation and for the tolerant cultivar Sids 1 compared with cv. Beni-suef 5. Such cultivar-dependent effect in response to drought stress was similar to the finding of Abeed and Dawood (2020).

One of the renovating mechanisms of hormone application under drought stress was the restoration of shoot/root ratio. The exogenous application of either JA or K efficiently restored the shoot/root ratio in both cultivars. The previous study of Wang et al. (2020) manifested that the increased applied concentration of JA led to a dramatic alteration in gene expression involving root growth, flower development, and formation of secondary metabolites. Also, the findings of Niakan and Ahmadi (2014) showed that kinetin application increased shoot and root length in tomato plants under moderate and strong drought stress.

Water status as an indicator of drought tolerance under water deficit was significantly reduced in terms of total water content for sensitive cultivars more than tolerant ones. This agreed with the results of Slama et al. (2007) who assumed that the inhibitory effects of PEG on whole-plant development and water relationships were predominantly related to limitation of the capacity of roots to supply water to the leaves, while the exogenous application of JA or K had more positive effects on this trait. Mahmood et al. (2012) manifested that JA plays a crucial role in restoring plant water content and growth under water-deficit conditions. Elsewhere, Pazurkiewicz-Kocot et al. (2011) concluded that there was a positive correlation between the application of kinetin and biomass of fresh, dry weight and water content in *Zea mays* seedlings. Reducing leaf water loss in response to JA under drought could be due to the possible regulatory effect of JA on stomatal closure, thus enhancing tissue ability for water maintenance (Mahmood et al. 2012).

It was clear from the data that drought stress had negative effects on the total dry weight of both cultivars, especially sensitive ones. This agreed with the results of Ullah et al. (2020) who evidenced that all growth parameters such as plant height, leaf area, dry and fresh weight of root, and shoot fresh weight were significantly reduced under various deficit irrigations, whereas exogenous applications of both hormones under unstressed and stressed conditions improved these traits of this cultivar. Wang et al. (2020) suggested that the application of JA to some plants might control the rate of photosynthesis by regulating the opening and closing of stomata. Also, Hussein et al. (2015) registered that the foliar application of salicylic acid (SA) or kinetin (Kin) increased the dry mass of drought-stressed sesame plants.

Maintaining chlorophyll concentration is one of the physiological measurements of plant tolerance to water-deficit

stress, as the chlorophyll content and chlorophyll stability index in wheat plants decrease under drought stress conditions (Qaseem et al. 2019). This investigation showed that under drought-stressed condition, seedlings of the sensitive wheat cultivar (Beni-suef 5) exhibited significant reductions when compared with the tolerant one. In contrast, the exogenous application of both hormones improved this trait in the case of drought-susceptible cultivars more than tolerant ones. It agrees with the results of Krishna Surendar et al. (2013) who observed that both tolerant and moderately tolerant banana cultivars and hybrids appeared to be less affected than those of control in chlorophyll stability index response to irrigation at 50% soil water content, while susceptible cultivars and hybrids of banana showed lowest CSI of up to 19% due to water deficit than control. The hormone application restrained the value of CSI to be comparable with control indicating the sensitivity of CSI as drought tolerance trait, hence improving this trait reflected on drought tolerance degree.

This could be due to enhancement of Chl synthesis, inhibition of its degradation through the inhibition of chlorophyllase activity, and influence on the synthesis of protochlorophyllide and formation of 5-aminolevulinic acid that is mediated by K (Sayed 1999). The diminution in osmotic potential is reflected as a potential cellular mechanism of drought resistance as it assists turgor maintenance and growth permanence (Bajji et al. 2000).

Differential adaptation of both cultivars to water-deficit stress appeared through increasing of total osmotic potential for the tolerant cultivar (Sids 1) rather than of cv. Beni-suef 5 which is logically explained by higher osmolytes produced for the former under drought stress compared with the latter cultivar; however, when both cultivars were exposed to exogenous application of hormones, they suffered less from water stress by decreasing total osmotic potential. This can be explained as the whole-cell energy sinks to plant production or these biomolecules may be exploited as radical scavenging and/or osmoprotectant. In this respect, the MeJA-induced tolerance against drought stress might also be attributed to minimizing the negative effects of osmotic potential due to the accumulation of some solutes as a response to drought, which might help to protect the metabolic processes, and contribute to plant growth through conserving cell turgor (Wang et al. 2020). Our results are in agreement with the results of Gadallah and Sayed (2001) who found that kinetin-sprayed *Sorghum bicolor* plants had higher relative water content than control plants.

Some researchers advocate that, under conditions of water-deficit stress, plant accumulated proline to protect itself from its negative effects or to help preserve osmotic potential, eliminating free ROS radicals, protecting macromolecules from denaturation, and regulating cellular pH (Shirmohammadi et al. 2020). In the present study, under drought stress conditions, proline content in leaves of sensitive cultivars

accumulated more than tolerant ones, and exogenous application of both hormones supplied proline content in leaves of the sensitive cultivar. Cia et al. (2012) stated that under drought stress, there is a wide controversy on the protective properties of proline, and after 10 days of water stress, the tolerant sugarcane varieties exhibited less proline accumulation compared with sensitive plants.

Results go parallel with those of Bidabadi et al. (2013) who noticed that the proline content of four Iranian grapevine cultivars under varying levels of MeJA (0.5 and 1.0 g L⁻¹) showed a significant decrease compared with control plants. Khan et al. (2015) found that glycine-betaine and kinetin foliar spraying enhanced the content of proline in the leaves of *Oryza sativa* L. under water stress and helped to overcome the physiological limitation by proline accumulation. It is probably due to the involvement of kinetin in the osmotic adjustment under water stress (Khan et al. 2016).

Plants respond to drought stress by the accumulation of osmoregulation substances in plant cells, such as soluble sugars, that can control osmotic potential, maintain cell turgor pressure, scavenge radicals, serve as a pool for energy and carbon as well as confer protection to the stability of membrane structures and metabolism-related enzymes (Sun et al. 2016). In this experiment, drought stress induced the accumulation of soluble carbohydrates in leaves of both cultivars. The results of this investigation are in harmony with the study of Talaat (2020) on maize plants exposed to drought stress that resulted in an increase in total soluble sugar concentration, hence soluble sugar can be used as a trait for screening the betterment of drought tolerance in durum wheat (Al Hakimi et al. 1995), while the exogenous application of both hormones significantly decreased this trait. This situation may indicate that cultivars foliar-applied with hormones suffered less from water stress to direct whole-cell energy toward plant production or these metabolites may be directed to be osmoprotectant and/or radical scavenging (Dawood and Abeer 2020). These agreed with the results reported by Niakan and Ahmadi (2014) who found that exogenous kinetin application reduced soluble sugars in tomato leaves. This might be due to the involvement of kinetin for enhancing levels of invertase which has a role in the movement of sugars into the developing reproductive organs of the plant (Khan et al. 2015).

The regulatory metabolic role of hormone application on the studied metabolites was clearly explained by soluble protein content. Drought stress negatively affects the soluble protein content of both cultivars. Faraji and Sepehri (2020) found wheat leaf soluble protein content significantly declined under the drought-stressed condition, while both hormones caused a significant increment in the leaf soluble protein contents that were more sounded for cv. Beni-suef 5. Piotrowska et al. (2010) treated *Wolffia arrhiza* L. with 0.01–1 μM JA and enhanced production of high levels of protein. Similarly, the

previous study of Wasternack (2007) reported that jasmonates have a notable effect on the induction synthesis of jasmonate-induced proteins (JIPs). Likewise, Hussein et al. (2015) found that the addition of kinetin caused a little increase in the content of proteins in drought-stressed sesame plants. Besides, Kudryakova et al. (2013) suggested kinetin activation by the accumulation of many proteins and involved in the metabolism of vascular plants.

Abiotic stress-induced ROS accumulation in the cells can explore oxidative damage to the plants. Plants have a sophisticated innate mechanism for controlling excessive ROS production by controlling the activities of the antioxidant enzymes (Farid et al. 2020). It has been reported that various stresses enhanced activities of many antioxidant enzymes in plants to cope with oxidative stress, such as SOD, CAT, APX, and POD (Rezayian et al. 2018). In this experiment, it was observed that the activities of CAT, APX, SOD, and POD exhibited similar patterns of change under stressed conditions which significantly enhanced the activities of these enzymes, whereas exogenous application of JA or K to unstressed and stressed seedlings suppressed these traits when compared with stressed ones in leaves of the sensitive cultivar. Contrary to the observation of the present study, Piotrowska et al. (2010) pronounced that treating *W. arrhiza* with 0.1 μM MeJA alone caused a greater peroxidase activity. Also, Yasser et al. (2016) found that kinetin-treated sesame plants under drought stress showed a slight increment in the activities of peroxidase when compared with the control plant.

It is apparent that hormone application of wheat cultivars prevented oxidative stress, thus sufficed with antioxidant enzyme activity lower than produced under stress, so hormone application could act as ROS scavenger rather than mitigated the ROS production via exacerbation of antioxidant enzyme activity, so hormones application did not act as an elicitor of the antioxidant production. Guschina et al. (2002) revealed that, under stress conditions, phytohormones interfere with membrane phospholipids to stabilize the membranes, thus increasing plant tolerance against oxidative stress. MeJA impacts the activity and/or pools of stress enzymes and results in the mitigation of oxidative stress (Jung 2004). Likewise, kinetin functions as a direct free radical scavenger or acts as an antioxidative mechanism associated with the preservation of purine breakdown, and contribution of genes in stress responses is often indicated from modification in the transcript multitude in response to a given stress trigger (Abd El-Samad 2016).

In this investigation, the drought-stressed seedlings of cv. Sids 1 exhibited a significant inhibition in the activity of LOX, while the same condition did not cause a noticeable change in the activity of this enzyme in leaves of cv. Beni-suef 5 when compared with control seedlings. On the other hand, treatment with exogenous application of JA and K under the unstressed and stressed conditions to sensitive cultivar had negative and

negligible effects on the activity of this enzyme whereas treating seedlings with jasmonic only had a positive effect. It was manifested that singlet oxygen and superoxide anions that can be formed through LOX catalyzed the oxidation of fatty acids (Lynch and Thompson 1984). A similar reduction was found by Egert and Tevini (2002) who found that the specific LOX activity was significantly inhibited by 60% in leaves of drought-stressed *Allium schoenoprasum* plants. The reduction in LOX activity under stressed conditions resulting from a simple inhibition or a downregulation can be counted as beneficial for the plant.

A recent study of Fugate et al. (2018) on sugarbeet observed that MeJA lowered leaf lipid peroxidation levels in both watered and drought-stressed plants. Furthermore, Eser and Aydemir (2016) observed that the treatment of plants with kinetin under stress triggers the activities of antioxidant enzymes. Most plants that encountered drought stress can elicit some or more of the physiological alternations such as increasing the level of non-enzymatic antioxidants and boosting the activity of the antioxidant enzymes (Osman 2015). Based on our results, drought-stressed seedlings of both cultivars exhibited significant stimulation LOX activity, while exogenous application of JA or K had highly stronger suppression effects on this enzyme of the sensitive cultivar than on the tolerant one. The results of this investigation are in agreement with the study of Anjum et al. (2017) who demonstrated that drought stress enhanced LOX activity in three maize hybrids because accumulation of ROS and membrane damage seemed to be higher under drought stress. Also, Gholamreza et al. (2019) found that the foliar application of MeJA on water-stressed peppermint improved the content antioxidative capacity. Nafie et al. (2011) mentioned JA has a protective effect in plants by prompting antioxidant enzyme activity in drought-induced oxidative damage. The exogenously applied plant growth regulators, particularly kinetin, improve plant drought tolerance by raising various antioxidant enzyme production (Ogweno et al. 2010; Yasser et al. 2016).

5 Conclusion

Shoot/root ratio, total water content, total dry weight, chlorophyll stability, total osmotic potential, osmoregulatory components, soluble carbohydrates, soluble protein, proline, membrane damage trait in terms of LOX, enzymatic antioxidant defense system in terms of APX, CAT, POD, and SOD, and total antioxidant were manifested as sensitive morpho-physiologic parameters judging drought tolerance and could be used as selection criteria in wheat for drought tolerance at the seedling stage. Beni-suef 5 was found to be drought susceptible due to low performance whereas Sids 1 which possessed a high percentage of total water content and chlorophyll stability index, and high capacity of antioxidant enzyme

activity resist more against drought. Although exogenously applied hormones did not have an apparent stimulatory role on well-watered plants, unequivocal inversion from a state of downregulation to upregulation was distinct under water stress and highly sounded for a sensitive cultivar. Morpho-physiological adaptation of wheat cultivars having great importance in their growth, survival, and yield under drought stress was effectively prompted by hormone application in sensitive wheat cultivar as well as tolerant ones. Hopefully, these results would be supportive of research programs seeking to develop anti-drought stress practices for sensitive wheat cultivars for maximum exploitation of all-natural plant resources that could be realized via introducing the sensitive cultivars along with hormone application in earlier stages to drought exposure and follow-up to increase the productivity of these cultivars.

Acknowledgments The authors are very grateful for Prof Afaf Hamada Abdel Rahman—professor of plant physiology, Botany and Microbiology Dept., Faculty of Science, Assiut University (afafhamada@yahoo.com), for her critical reading and revision of this research article. Also, the authors are very grateful for the insightful and helpful comments, constructive suggestions, and careful corrections made by the editor-in-chief and the anonymous referees for further improvements of this manuscript.

Funding This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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