International Journal of Horticultural Science and Technology
Vol. 3, No. 2; December 2016, pp 187-197
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2017.213448.147
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Physiological response of Okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress

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(Received: 6 August 2016, Accepted: 17 February 2017)

Abstract

To investigate effects of putrescine (Put) and humic acid (HA) on fruit yield and physiological attributes of okra *(Abelmoschus esculentus* L. 'Kano') under water deficit stress, a field experiment was conducted in split plot based on a randomized complete block design with three replications. Plants were exposed to three different irrigation regimes (33%, 66% and 100% ETc) and were treated with foliar application of Put (0, 0.5, 1 and 1.5 mM) and HA (0, 150 and 300 mg l^{-1}). The results showed that deficit irrigation significantly decreased fruit yield, relative water content (RWC), vitamin C and water use efficiency (WUE), whereas proline content and catalase and peroxidase activities were increased. Foliar application of HA and Put significantly increased fruit yield, RWC, vitamin C and proline contents, catalase and peroxidase activities and WUE. The results suggested that HA at 300 mg l^{-1} and Put at 1.5 mM can improve growth, yield and quality of okra fruits.

Keywords: antioxidant enzyme activity, fruit yield, proline, vitamin C, water use efficiency.

Abbreviations: Put: Putrescine; **HA**: Humic acid; **RWC**: Relative water content; **WUE**: Water use efficiency; **POD**: Peroxidases; **CAT**: Catalase; **ROS:** Reactive oxygen species; **ETc**: Actual evapotranspiration; **I:** irrigation.

Introduction

Okra (*Abelmoschus esculentus* L.) is an annual vegetable crop grown throughout the tropical and subtropical regions of the world. Its cultivation is also extended to dry areas, where drought is limiting the growth and fruit yield (optimum yields of 2-3 t ha^{-1}) of okra (Emuh *et al.,* 2006). Okra is a rich source of carbohydrate, protein, fats, minerals and vitamins that can be largely used in the human diet. World production of okra as a fruity vegetable is estimated to 8.3 million tons per year (FAOSTAT, 2012).

Drought stress is one of the most important environmental stresses that can negatively influence plants photosynthesis, chlorophyll content and as a result growth and total crop yield (Ashraf *et al.*, 2011). Water deficit just prior and during early flowering reduces the number of fruits. This negative effect would be exacerbated when drought stress is concurrent with high temperature and low humidity conditions (Al-Harbi *et al.,* 2008). As an example in

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chickpea, it has been reported that drought stress can negatively influence fruit yield, chlorophyll a, chlorophyll b and total chlorophyll content (Mafakheri *et al.,* 2010).

Leaf water potential and relative water content are two important criterions that generally used to determine plant physiological responses to drought stress (Silva *et al.,* 2010). Both of these parameters are usually decreased in plants under water deficit conditions. Rouphael *et al.* (2008) reported that when water deficit stress applied on grafted watermelon plants, although it caused decreased in yield, biomass and leaf water content, but it led to an increase in water use efficiency.

During exposure to stress conditions, reactive oxygen species (ROS) are one of the major causes of cellular damage in plants. ROSs are generally accumulate in the cells when plants are exposed to environmental stresses (Miller *et al.,* 2010). Plants have evolved a complex array of antioxidant defense systems to prevent ROS-induced oxidative injury, which includes antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) (Wang *et al*., 2009). Compatible solutes such as proline are also produced to protect cells against ROS accumulation under stress conditions (Ashraf and Foolad, 2007).

Accumulation of low molecular weight organic compounds, such as proline is one of the mechanisms that many plant species are used to reduce the negative effects of water stress (Chaves *et al.,* 2003). Tamayo and Bonjoch (2001) suggested that accumulation of proline occurs under drought and other environmental stresses. Accumulation of proline depends on the intensity of stress.

Various strategies have been proposed to help crop production under drought conditions. An alternative and quick strategy to reduce negative effects of drought stress is exogenous application of organic osmolytes or plant growth regulators (Ashraf *et al.,* 2011). Polyamines (e.g. spermine and putrescine) have been identified as essential endogenous plant growth regulators as well as signal molecules in a variety of physiological responses (Davies, 2004; Yang *et al.,* 2007). They are involved in regulation of several physiological processes, including cell division, morphogenesis, senescence, programmed cell death (apoptosis), and secondary metabolism (Yang *et al.,* 2007).

Humic acid (HA), as an organic fertilizers and an important component of humic substances can be used to improve plant growth under water deficit conditions (Senesi *et al.,* 1996). Improvements in growth, yield and quality have been reported following application of humic substances in a number of plant species such as tomato (Yildirim, 2007) and pepper (Karakurt *et al.,* 2009). Under water deficit conditions, foliar fertilization with humic acid can increase leaf water retention, photosynthesis and antioxidant metabolism (Fu Jiu *et al.,* 1995). Farooq *et al.* (2009) showed that exogenous application of polyamines can improve photosynthetic capacity, leaf water content, WUE, and accumulation of free proline in rice plants exposed to water stress conditions.

Although several studies have investigated the effects of drought stress on growth and physiological parameters of okra, no research has been carried out on the relationship between humic acid or putrescine and drought tolerance of okra. Therefore, the objective of this study was to investigate the potential roles of foliar application of humic acid and putrescine on fruit yield, WUE and other physiological parameters of okra under water deficit condition.

Material and Methods

Experimental site

Field experiments were carried out from June to September 2014 at the Research Farm of Agriculture Faculty at the University of Zanjan, Iran. Soil texture was a sandy loam with pH 7.32 (Table 1). Average daily climatic data during the growing season are shown in Table 2.

Table 1. Soil physical and chemical properties on the site of experimental field

Ca (meq l^{-1})	Mg (meq l^{-1})	K (mg/kg)	P (mg/kg)	N (%)	Sand (%)	Silt (%)	Clay (%)	pH (%)	EC (ds m^{-1})
2.3	2.1	281	19.66	0.09	40	27	33	7.32	2

Table 2. Average daily climatic parameters during the growing season (2014) of okra

Climatic parameters	June	July	Aug	Sept
Minimum air temp. (ºC)	7.6	10.7	13.1	6.8
Maximum air temp. (ºC)	35.8	39.5	39.1	35.4
Rainfall (mm)	7.3	17.3	0.1	4.0
Relative humidity (%)	41.5	43.4	37.0	41.4

Plant materials and treatments

Seeds of 'Kano' cultivar were sown on 3-4 cm depth, with 30 cm spacing within row and 60 cm spacing between rows. All necessary management practices such as pests and weeds control were done according to recommended practices during the crop growth. After plant establishment (four leaf stage), irrigation treatments were initiated. At 4th leaf stage, foliar applications of HA and Put were weekly sprayed. For uniformity, deionized water was sprayed on leaves of control plants.

Irrigation treatments were calculated based on actual evapotranspiration (ETc) rates. Three irrigation levels were (1) control or irrigation at 100% crop water requirement (I_{100}), Deficit irrigation at 66% (I_{66}) and at 33% (I_{33}) of control. Foliar treatments include humic acid (HA) at 150 and 300 mg l^{-1} and putrescine (Put) at 0.5, 1 and 1.5 mM were randomly applied on the sub-plots. The experiment design was a split-plot model based on a completely randomized block design (three irrigation levels, six foliar treatments and three replications).

Fruit yield

Number of fruits per plant and fruit weight was measured to determine total yield. The total yield was expressed in kg ha^{-1}.

Vitamin C content

The fruit juices were extracted by fruit pressing. After filteration, the volume was increased to 100 ml using distilled water. Vitamin C content (mg 100 ml^{-1}) was determined by the methods described by Nweze *et al.* (2010).

Proline content

Free proline content was determined colorimetrically in solution of sulfosalicylic acid as described by Bates *et al.* (1973). Mature leaves of plant were sampled 70 days after start of the experiment. Proline was extracted from a sample of 0.5 g fresh leaf samples in 3% (w/v) sulfosalicylic acid solution and was estimated using the ninhydrin reagent. The absorbance of fraction with toluene aspired from liquid phase was read at 520 nm wavelength. Proline concentration was determined using a calibration curve and expressed as μmol g^{-1} fresh weight (FW).

Catalase and peroxidase enzyme Activities

Samples were taken from fully expanded leaves in the field and transferred to the laboratory on ice. A leaf sample (0.5 g) was frozen in liquid nitrogen and was grounded using a porcelain mortar and pestle.

Catalase (CAT) activity was determined by following the decomposition of H2O2 at 240 nm using a UV spectrophotometer as described by Havir and McHale (1987). Samples without H2O2 were used as blank. The activity of CAT was calculated by the difference obtained at OD240 values at 30 second interval for 1 min after the initial biochemical reaction.

Peroxidase (POD) activity was measured

with guaiacol at 470 nm using method of Koskeroglu and Tuna (2008) with some modifications. POD activity was expressed as U (unit) mg-1 protein. A change of 0.01 units per minute in absorbance was considered to be equal to one unit POD activity.

Leaf relative water content

Leaf relative water content (RWC) was determined by sampling topmost fully expanded young leaves at noon according to Yamasaki and Dillenburg (1999). Leaves were weighed and immediately immersed into double distilled water for 24 hr to obtain turgid weight. Dry weights of the samples were determined by placing them at 70 °C in an oven. Leaf relative water content was calculated according to Equation (1):

$$RWC\ (\%) = [(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100 \quad (1)$$

Water use efficiency

Water use efficiency (WUE) was calculated for all treatments based on total crop yield and amount of water applied during growth period. WUE was calculated as the ratio of fruit yield (kg ha^{-1}) to the amount of applied irrigation water (m^{-3}) (Stanhill, 1986).

Statistical analysis

A split-plot model with a completely randomized block design was used with three irrigation levels, six foliar treatments, three replications and 10 observations per experimental unit. Data were analyzed using SAS V9 statistical program (SAS Institute Inc., Cary, NC, USA) and means of treatments were compared by Duncan's multiple range tests at 5% probability level. Values were expressed as mean ± SE (standard error).

Results

Yield

Irrigation treatments had significant effects on fruit yield. The highest yield (2433.1 kg ha^{-1}) was obtained in control treatment (100% ETc). Deficit irrigation significantly reduced yield by 45.2% at 66% ETc and by 53.9% at 33% ETc (Table 3). Fruit yield was remarkably influenced by foliar application of HA and Put. The highest yield was obtained in 300 mg l^{-1} HA (2019.8 kg ha^{-1}) and 2 mM Put (1872.8 kg ha^{-1}) respectively (Table 3). Yield was significantly influenced by interactions between irrigation levels and foliar treatments. In 100% ETc irrigation, foliar spray of 300 mg l^{-1} HA resulted in the highest yield (Fig. 1).

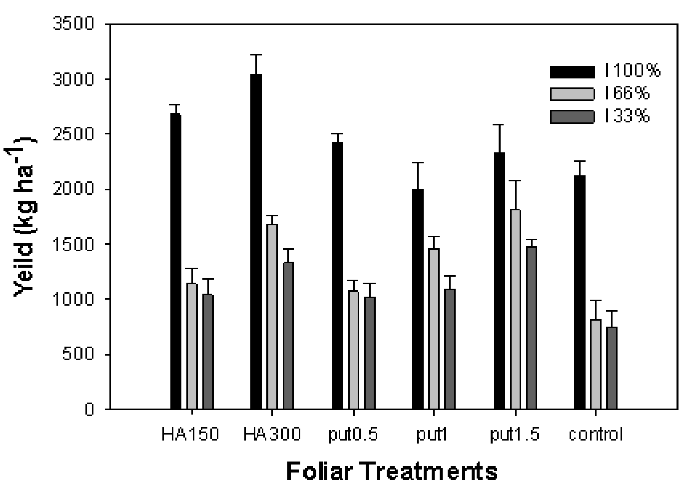

Fig. 1. Effects of foliar application of humic acid (HA) at 150 and 300 mg l^{-1} and putrescin (Put) at 0.5, 1 and 1.5 mM on fruit yield of okra in control (100% ETc) and deficit irrigations (66% and 33% ETc). Values are the means of three replicates and bars represent the standard errors (n = 3).

Vitamin C

Deficit irrigation significantly reduced vitamin C content (Table 3). The highest vitamin C content (12.21 mg 100 ml^{-1}) was recorded in 100% ETc treatment (Table 3). As expected, the vitamin C content was significantly increased by all foliar applications of HA and Put on the non-stressed control plants. The highest increase (5.56%) was recorded in plants treated with 300 mg l^{-1} HA. Interaction between irrigation levels × foliar treatments had no significant effect on vitamin C content.

Proline

The level of proline was significantly increased in all plants sprayed with HA and Put under water deficit stress. Proline accumulation was positively dependent on the levels of water deficit stress (Table 3). There was a significant difference in proline content among foliar treatments and the highest proline level was observed in 300 mg l^{-1} HA (3.1 μmol g^{-1} FW) and 2 mM Put (3.06 μmol g^{-1} FW) (Table 3). The highest proline content was detected for I_{33} × 2 mM Put followed by I_{33} × 300 mg l^{-1} HA treatments, which were not significantly different at $P<0.05$ (Fig. 2).

Catalase and Peroxidase activity

Water deficit had significant effect on catalase (CAT) activity (Table 3). Its activity was increased by 17.1% in response to 33% ETc irrigation. Significant differences were observed among foliar treatments as well. Foliar application of 1.5 mM Put resulted in 10.63% increase in CAT activity followed by 9.9% increase by foliar application of 300 mg l^{-1} HA as compared to control plants (Table 3). CAT activity increased under deficit irrigation in all foliar treatments compared to control plants. Interactions between irrigation levels and foliar treatments had no significant effect on CAT activity. Similar to CAT, peroxidase (POD) activity was increased in response to an increase in water deficit stress (Table 1). The highest POD activity (0.54 unit g^{-1} FW min^{-1}) was found in plants sprayed with 300 mg l^{-1} HA under 33% ETc irrigation (Fig. 3). However, results indicated that POD activity was significantly reduced by foliar application of Put (Table 1, Fig. 3).

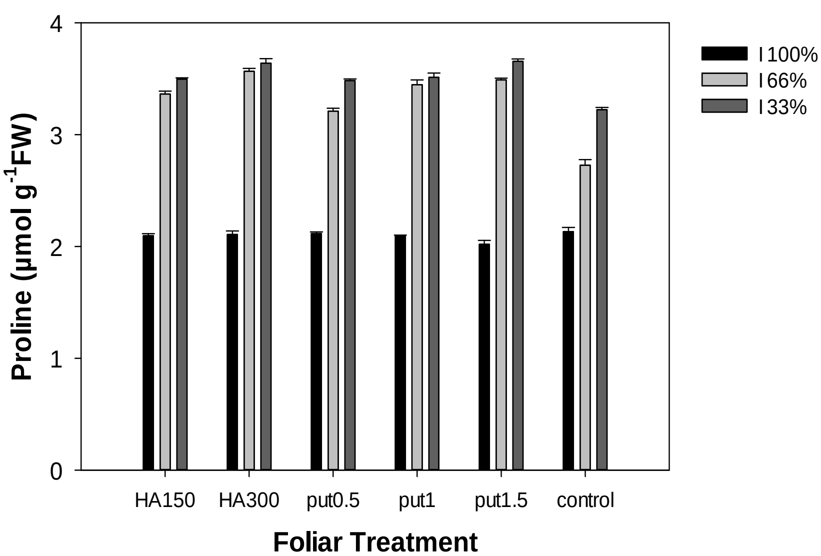

Fig. 2. Proline content of okra fruit in response to foliar spray of humic acid (HA) at 150 and 300 mg l^{-1} and putrescin (Put) at 0.5, 1 and 1.5 mM in control (100% Etc) and deficit irrigations (66% and 33% ETc). Values are the means of three replicates and bars represent the standard errors (n = 3).

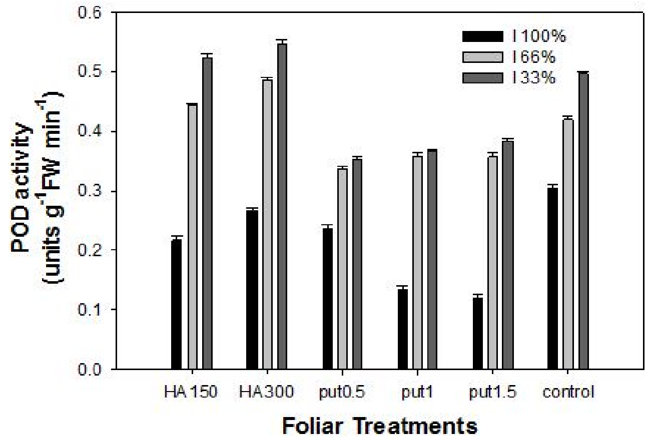

Fig. 3. Peroxidase (POD) activity of okra in response to foliar application of humic acid (HA) at 150 and 300 mg l^{-1} and putrescin (Put) at 0.5, 1 and 1.5 mM in control (100% ETc) and deficit irrigations (66% and 33% ETc).Values are the means of three replicates and bars represent the standard errors (n=3).

Relative Water Content (RWC)

RWC was significantly affected by water deficit conditions. Compared with the control treatment (I_{100}), RWC of leaves in I_{66} and I_{33} was decreased by 8.7% and 11.2%, respectively (Table 3). For all foliar treatments, significant increases in RWC were observed in comparison with control plants. The highest increase in RWC was observed in plants sprayed with 300 mg l^{-1} humic acid. In addition, data of the interaction between water deficit stress and foliar treatments indicated that there was no significant difference in RWC.

Water Use Efficiency (WUE)

WUE was significantly decreased by increasing water deficit. The highest WUE (0.51 kg m^{-3}) was calculated in irrigation level of 100% ETc. Foliar treatments had a significant effect on WUE. Maximum WUE of 0.53 and 0.51 kg m^{-3} were obtained in 300 mg l^{-1} HA and 2 mM Put, respectively. Interactions between irrigation levels and foliar treatments were statistically significant. Plants sprayed with 300 300 mg l^{-1} humic acid exhibited higher WUE under 100% ETc irrigation (Fig. 4).

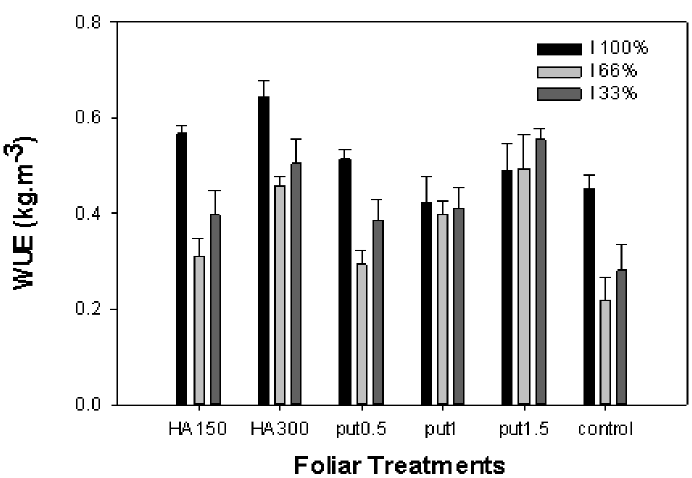

Fig. 4. Water use efficiency (WUE) of okra in response to foliar application of humic acid (HA) at 150 and 300 mg l^{-1} and putrescin (Put) at 0.5, 1 and 1.5 mM in control (100% Etc) and deficit irrigations (66% and 33% ETc). Values are the means of three replicates and bars represent the standard errors (n=3).

Table 3. Main effects of deficit irrigations based on evapotranspiration (ETc) rates on proline content, relative water content (RWC), vitamin C content, catalase (CAT) and peroxidase (POX) enzymes activities, yield, and water use efficiency (WUE) in okra.

Treatments	Proline (µmol g^{-1} FW)	RWC (%)	Vitamin C (mg 100 ml^{-1})	CAT (µmol H_2O_2 g^{-1} FW min^{-1})	POX (units g^{-1} FW min^{-1})	WUE (kg m^{-3})	Yield (kg ha^{-1})
Irrigation rate							
100% ETc	2.09±0.01c	89.04±0.34 a	12.21±0.066a	2.8±0.03c	0.21±0.016c	0.51±0.022a	2433.1±104.9a
66% ETc	3.3±0.06 b	80.25±0.74 b	11.81±0.105b	3.28±0.047b	0.39±0.013b	0.36±0.027c	1331.9±100.5b
33% ETc	3.5±0.03a	77.82±1.16 c	11.06±0.094c	3.37±0.043a	0.44±0.019a	0.42±0.026b	1120.4±70.1c
Foliar treatments							
Hum 150 mgl^{-1}	2.98±0.22cd	82.71±1.76a	11.8±0.19ab	3.05±0.08bc	0.39±0.046c	0.42±0.042b	1620.6±272.3b
Hum 300 mgl^{-1}	3.1±0.25a	84.37±1.76a	11.95±0.18a	3.3±0.12a	0.43±0.042a	0.53±0.033a	2019.8±270.2a
Put 0.5 mM	2.93±0.2 d	81.99±1.66a	11.56±0.21bc	3.06±0.083bc	0.3±0.018d	0.39±0.035b	1506.7±235.1b
Put 1 mM	3.01±0.23bc	83.04±1.73a	11.67±0.22ab	3.17±0.104b	0.28±0.038e	0.41±0.022b	1519±156.1b
Put 1.5 mM	3.05±0.26ab	84.29±1.42a	11.85±0.14ab	3.31±0.11 a	0.28±0.041e	0.51±0.028a	1872.8±163.9a
Control	2.69±0.15e	77.82±2.88b	11.33±0.22c	3.01±0.08c	0.4±0.028b	0.31±0.041c	1232±236.02c

Means in columns followed by the same letter are not significantly different at P<0.05 according to the Duncan's multiple range tests.

Discussion

In the present study, growth and the physiological response of okra plants were investigated after exposure to different levels of water deficit. Furthermore, evaluation of foliar applications of humic acid and putrescin on yield, proline, water use efficiency and antioxidant activity under different levels of water deficit conditions were done in the current study.

Water deficit stress caused significant reductions in yield. Control treatment (without water deficit) was resulted in the maximum fruit yield (Table 3). These results are in agreement with those reported by Yang *et al.* (2007) who observed that rice yield was significantly reduced when plants were subjected to long-term water stress. Sarker *et al.* (2005) suggested that yield is affected by water stress and is significantly decreased by intensified water stress conditions. Drought stress can progressively decrease CO_2 assimilation rates due to closing of stomata and reduced leaf area, consequently decreases photosynthetic pigment content and activity. Drought stress also induces reduction in the content and activity of photosynthetic carbon reduction cycle enzymes, including its key enzyme ribulose-1, 5-bisphosphate carboxylase/ oxygenase (Kumudini, 2010).

Foliar application of HA and Put during drought stress period increased plant adaptation to stress conditions. In agreement, It has been reported that endogenous application of polyamines (putrescine) significantly improved grain yield of rice under water stress condition (Yang *et al.,* 2007). Polyamines play an important role in the regulation of a variety of physiological processes, including cell division, morphogenesis, senescence, programmed cell death (apoptosis), and secondary metabolism (Yang *et al.,* 2007). Brunetti *et al.* (2007) found a positive correlation between wheat grain yield and the components of HA. HA substances affect the solubility of many nutrient elements by building complex forms or chelating agents of humic matter with metallic cations (Lobartini *et al.,* 1997).

Drought typically decreases stomatal conductance and as a result it decreases net photosynthesis. The decrease in net photosynthesis may result in a reduced transport of primary metabolites as the major source of precursors for biosynthesis of phenolic compounds, carotenoids, and ascorbate, towards the fruits (Fanciullino *et al.,* 2014). In contrast with our results, Wahb-Allah (2014) proposed that vitamin C of tomato fruits is positively affected by water deficit conditions. In our study, exogenous application of HA and Put also resulted in increased vitamin C content. In agreement with our results increased vitamin C content through HA application has been also reported in hot pepper fruits (Aminifard *et al.,* 2012),

Applications of HA and Put were positively affected foliar level of proline in okra plant under water deficit conditions. Our results showed that the highest level of foliar proline concentration was recorded in plants sprayed with 300 mg l^{-1} HA. Proline accumulation is one of the plants responses to drought stress conditions. Proline is an amino acid that accumulates in the cytoplasm of many plant species in response to environmental stress, and plays a significant role in alleviating the adverse effects of stress in plants and protects cell membranes from oxidative stresses by enhancing activities of various antioxidants (Ashraf and Foolad, 2007). Sarker *et al.* (2005) reported that proline accumulates by plant exposure to stress condition and returns back to initial levels after stress recovery. It seems to act as a survival mechanism. Mafakheri *et al.* (2010) showed that proline accumulates by increasing drought stress in chickpea. In our study, accumulation of proline was occurred in response to foliar treatments. This improved tolerance to drought stress and as a result lead to improved fruit yield, while plants that accumulate lower or negligible amount of such solutes were more sensitive to water deficit conditions. Reddy *et al.* (2004) indicated that proline acts as a free radical scavenger, reduces damage to thylakoid membranes and acts as a simple osmolyte which help plants to cope with stress conditions.

Our results showed that the activity of CAT and POD enzymes increased under water stress conditions. These results are in agreement with Murshed *et al.* (2013), who reported that CAT activity in tomato fruits increased by water stress treatments. Decreased activities of POD and CAT in leaves were generally observed for untreated plants. Scandalios (2005) found that similar to other environmental stress conditions, ROS is involved in the damaging effects of water stress on plants. Enzymatic antioxidants including CAT, POD, and superoxide dismutase (SOD) are activated in response to stress conditions to minimize the damaging effects of ROS.

HA and Put applications led to increase in CAT and POD activities, which is consistent with previous studies that reported induction of antioxidant activity by HA (Aminifard *et al.,* 2012). Mechanisms of ROS detoxification in plants can be categorized as enzymatic (CAT and POD) and non-enzymatic (Reddy *et al.,* 2004) detoxifications. It has been reported that polyamines (PAs) can act as free radical scavengers. Their activities are in correlation with the activities of antioxidant enzymes such as SOD, CAT, and POD (Liu *et al.,* 2004). It has been well documented that polyamines play important roles in plants defense against diverse environmental stresses (Kasukabe *et al.,* 2004; Groppa and Benavides, 2008). The reduced levels of polyamines, especially Put and Spermidine, were related to higher stress injury and decreased water content in soybean (Nayyar *et al.,* 2005). High levels of PAs could confer plant tolerance to abiotic stresses by acting as direct ROS scavengers or by binding to antioxidant enzyme molecules to indirectly scavenge free radicals (Duan *et al.,* 2008; Groppa and Benavides, 2008).

WUE is another important plant adaptation under water stress, which also has been proposed as an effective selection criterion to identify and/or develop drought tolerant plants (Ray *et al.,* 2004; Cao *et al.,* 2007). Our results indicated that deficit irrigation caused a reduction in WUE. Lowest WUE was obtained at I_{66} (Table 3). Lower WUE under I_{66} is due to reduction in RWC. Decrease in RWC and leaf water potential can negatively affect leaf photosynthetic rate (Lawlor and Cornic, 2002). However, stomatal limitations have been accepted as the main cause of photosynthetic reduction under drought stress condition (Lawson *et al.,* 2003).

Conclusions

The results obtained in this study suggest that Okra requires considerable amount of water for its growth and development. Therefore, it is sensitive to water deficit stress during its entire growing period. Under water deficit, decrease in the leaf relative water content is related to decrease in the fruit yield and WUE. Beneficial effects of HA and Put on fruit yield of okra plants were related to improvements in physiological parameters. Foliar treatments of HA and Put through increasing RWC, proline accumulation and antioxidant enzyme activities improved fruit yield of okra under different level of water deficit conditions.

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