International Journal of Environment Volume : 07| Issue : 03 | July-Sept. | 2018
ISSN 2077-4505 Page:95-103

Managing crop production of pomegranate cv. Wonderful via foliar application of ascorbic acid, proline and glycinbetaine under environmental stresses

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Received: 15 July 2018 / Accepted: 05 Sept. 2018/ Publication date: 25 Sept. 2018

ABSTRACT

Recent studies that subjected number of abiotic stresses simultaneously figured out different effect of their combinations on plant performance and production compared to each single stress. The effect of such combinations are naturally exists and already translated to weak act and output of plants. The plants in this field investigation suffered from soil salinity which affects soil osmotic pressure subsequently decreases soil water potential and this lead to a physiological drought stress. The present trail aimed to simulate plant acclimation to environmental stresses by exogenous metabolites which generated by plants under stresses such ascorbic acid (Asc), proline (Pr) and glycinbetaine (GB). Eight treatments including control, 10 mM Asc, 50 mM Pr, 100 mM GB and the combination between them were applied on Wonderful pomegranate trees aged four years at the end of March, full fruit set and one month later. In ascending order all applications significantly stimulated all parameters under investigation compared to check treatment. The study recommends the combined application of 10 mM Asc plus 50 mM Pr plus 100 mM GB for alleviation negative effects of environmental stresses which may happen to growth and yield of pomegranate trees.

Key words: Soil salinity, Drought stress, Ascorbic acid, Proline, Glycinbetaine

Introduction

The changes in climatic condition are worldwide problems leading to agriculture restriction. Increasing temperature and elevating sunlight are leading to the increase of evaporations from plants and soils, subsequently salinity problems are clearly raised. Rizk *et al.* (2015) reported that Egypt recorded solar radiation of 2,000 to 3,000 kWh/m^2/year which leads to increases of temperature on leaf surface and soil. In Egypt pomegranates (*Punica granatum* L.) are occupied wide area in the new reclaimed soils affected by salinity and other climatic stresses. Pomegranate fruits are valuable and introduce a wide range of usages. Maas and Hoffmann (1976) stated that, pomegranate is considered to be moderately tolerant to salinity. Ion toxicity, water deficit, nutrient imbalance are the impacts related to salinity (Marschner, 1995). Nayidu *et al.*, (2013) found that Na^+ and Cl^- concentrations affecting synthesis of enzyme that responsible for carbon assimilation and the pathways of photosynthetic electron transport that happen in chloroplasts are affected as well. Moreover, photosynthetic process in general, declined due to salt stress (Lu and Vonshak, 2002). Reactive oxygen species (ROS) increased under salinity exposure as indicator to oxidative damages (Arora *et al.*, 2008). Keles and Oncel (2002) found that combination of salinity and heat stress in wheat led to exacerbate damaging compared to individual stress. Additionally, Ahmed *et al.* (2013) found higher negative effect on cultivated barley due to combination of salinity and drought more than the negative effect of each individual condition. Moreover, interactions between high light and drought were resulted more damaging effect than individual ones (Giraud *et al.*, 2008). In contrast, other stress combinations revealed positive effects on plants such as elevated CO_2 levels with salt or high light (Perez-Lopez *et al.*, 2013). The interaction between high temperature and salinity increased the accumulation of osmoprotectants such glycine betaine and trehalose which play a role in protecting plants against this stress combinations (Rivero *et al.*, 2013). Carrizo citrange was more tolerant to the stress combination of drought and heat than Cleopatra mandarin where it showed higher transpiration rate, subsequently lower leaf temperature (Zandalinas *et al.*, 2016a, b, Zandalinas *et al.*, 2018). Foliar

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application of Asc induced the plant defence system against damaging of the photosynthetic apparatus under saline condition via increasing the accumulation of soluble sugars and nitrogen content (Liu *et al.*, 2008, Zandalinas *et al.*, 2016a, b). Hassanein *et al.* (2009) and Abd-El Hamid (2009) stated that, spraying Asc on tomato plants grown under stress condition increases the content of IAA that induces cell division and/or elongation, subsequently, improves plant growth. They also found that the Asc application increases the nutrient uptake and decreases Na^+ accumulation; in addition prevent cells against ion leakage reflecting a role in stabilizing plasma membrane from oxidative stress. Shalata and Neumann (2001) reported that Asc plays a role as antioxidant rather than a source of energy respiratory metabolism because of the inhibition of reactive oxygen species (ROS) substances after its foliar application in salt stress condition. Mathews (1998) defined Pr as a cyclic alyphatic aminoacid which accumulates in the cytoplasm cells.) It has been reported that Pr synthesis from two glutamic acid and arginine (Hanson *et al.*, 1977; Dierks-Ventling and Tonelli, 1982; Kavi-Kishort *et al.*, 2005). Gruzka *et al.* (2007) remarked the role of Pr as antioxidant that regulates capacity of potential redox and defence against ROS. Accumulations of Pr are normally occurs under different stresses in the cytosol and contribute in cytoplasmic osmotic adjustment (Kavi Kishore *et al.*, 2005). In addition, it was found that Pr contributes stabilizing membranes and proteins, scavenging ROS and buffering cellular redox potential under stress conditions (Hare and Cress, 1997, Kavi Kishore *et al.*, 2005). Furthermore, Pr was identified as inducer of salt responsive genes; subsequently plays important role in plant adaptation to salt stress (Satoh *et al.*, 2002). It was found that GB accumulates in chloroplast where it contributes a vital role in prevent thylakoid membrane from damage and maintained photosynthetic efficiency under salt stress and high temperature (Robinson and Jones, 1986, Alia *et al.*, 1997). Rodríguez-Zapata *et al.*, (2015) reported that spraying GB in the rate of 100 mM reduced the chilling injury of banana fruits that occur through harvest handling at low temperatures, especially in tropical regions. Aliniaeifard *et al.*, (2016) stated that, foliar application of 2 mM Asc reduced negative effects of salinity on olive secondary metabolism particularly nitrogen and chlorophyll content, subsequently general performance. Chun *et al.* (2009) recommended 50 mg l^{-1} proline foliar application rate on Fuji apple trees for its positive effect on growth parameters, fruit yield and quality under frost conditions. Ahmed *et al.* (2011) found that exogenous proline of 50 mM ameliorate the deleterious effects which happened to "Chemlali" olive trees that irrigated with saline water. Proline application increased leaf content of soluble sugars, K^+ and Ca^{2+} but reduced Na^+ (Ahmed *et al.*, 2011). El Sayed *et al.* (2014 a, b) found that proline, ascorbic acid, jasmonic acid and tryptophan foliar application enhanced growth, yield and fruit quality of Manfalouty pomegranate and Manzanillo olive in different stresses. Hussein *et al.* (2017) recommended irrigation of jojoba plants using diluted seawater with foliar application of ascorbic acid at concentration rate of 10 mM or higher. From the previous review it can be conclude that variable responses in metabolic and signaling in different conditions teach us how to help plants coping with multiple stresses. Simply it can be helpful providing plants with external secondary metabolites similar to that generated and increased under stress circumstances. Subsequently, it is logically to simulate plants in the same directions and investigate the effect of exogenous such metabolites under nature stresses. Researcher should pay attention to such strategies for its efficiency to shorten many considerations in research work and cost. For that purpose, present study aimed managing crop production of pomegranate cv. Wonderful via foliar application of ascorbic acid, proline and glycinbetaine under environmental stresses.

Material and methods

Environmental stresses under investigation are shown in Table (1) that clarifies soil and irrigation water characteristics used for pomegranate plantation area. Data in Table (1) showed that salinity affected both soil and irrigation water. The study was conducted in 2014 and 2015 seasons using twenty four Wonderful pomegranate trees in age of four years, planted at 3X4 meters. The trees were grown in a private farm at Al-Khatatba, Behiera governorate, Egypt. Physical and chemical properties of the plantation soil and water are shown in Table (1) where both soil and water is salt affected. All spraying treatments were applied three times at the end of March, full fruit set and one month later for each season. Each treatment was repeated three times (three trees per each one). The experiment was arranged in randomized complete block design (RCBD) with eight treatments including control, 10 mM Asc, 50 mM Pr, 100 mM GB and the combinations among them.

Table 1: Soil and irrigation water characteristics of pomegranate plantation area.

	Soil layer (cm)		
Soil prosperities	**0-30**	**30-60**	**60-120**
Physical characterization			
Fine sand %	45.75	55.54	60.53
Course sand %	51.41	41.71	36.35
Silt+Clay %	3.15	3.73	4.23
Bulk density (t m-3)	1.67	1.65	1.66
Texture	Sandy	Sandy	Sandy
Chemical characterization			
pH (1:2.5)	8.4	8.6	8.8
EC (dS m^{-1})	5.33	5.39	5.45
$CaCO_3$ %	6.87	4.56	5.42
Organic matter %	0.55	0.42	0.29
Irrigation water properties	mg l^{-1}		
pH	8.4		
EC (dS m^{-1})	1.73		
CO_3^{2-}	< 0.01		
HCO_3^-	0.11		
Na^{2+}	2.01		
Cl^-	0.70		

Growth parameter:

One year old shoots were labeled at the beginning of each season in March for following shoot length determination, number of leaves per shoot and the other measurements at the end season in September. Fruit set% was came out by dividing number of set fruits on total number of flowers at balloon stage (number of flower per shoot X 100). Fruit Retention % also was calculated by dividing the number of fruits at harvest time on the number of set fruits X 100. Leaf area was measured using Cl203 laser area meter (CID Bio-science Instrument, USA).

Parameters of fruit yield and quality:

Characteristics of fruit including fruit weight (g.), yield (kg/tree), T.S.S. %, acidity, total sugars, anthocyanins in the peel and juice (mg. g FW^{-1}) were followed according (A.O.A.C., 2000). Hand refractometer was used to determine T.S.S as Brix. In addition fruit cracking % and sun-burn fruit % were calculated.

Chemical analysis:

Nitrogen was determined using Micro-kjeldehl method as described by Page (1982). The method of Cotteine et al. (1982) was followed for phosphorus determination. Flame photometer was used for potassium (K) and (Na) determination according to Jackson (1958). Calcium was measured using atomic absorption spectrophotometer Perkin Elmer-3300 according to Chapman and Pratt (1961). Ion leakage as indicator for Electrolyte conductivity (EC) was determined according to Tripathy et al. (2000). Total chlorophyll was determined according to Von- Wettstein (1957). Method of Peever and Higgins (1989) was followed for MDA determination.

Statistical analysis:

Data were analyzed by Statistical Graphics Corporation, STATGRAPHICS Plus (St. Louis, MO, USA) for one way analysis of variance and employing Duncan's multiple range tests at the 0.05 confidence level and for principle component analysis (PCA).

Results and Discussion

1-Effect of spraying Asc, Pr and GB on development parameters

Results in Table (2, 3) showed that all applications of Asc, Pr and GB and their combinations significantly enhanced the growth parameters compared to the control treatment. In Table (2) shoot length and number of leaves per shoot showed similar induction after spraying Asc, Pr and GB, where the combination of 10 mM Asc plus 50 mM Pr plus 100 mM GB was the best application used followed by 10 mM Asc plus 100 mM GB, 10 mM Asc plus 50 mM Pr, 50 mM Pr plus 100 mM GB, 10 mM Asc then a similar effect with no significances between 50 mM Pr and 100 mM GB was detected. Similar trend data for leaf area, number of flower per shoot and fruit set was in Table (2). Results of leaf area revealed no significant differences among applications of Asc, Pr and GB. Number of flowers per shoot data was erratic in the first season but in the second season it becames more regular where the applications of Asc, Pr and GB showed no significant differences among them and between their combinations Table (2). Fruit set and retention showed similar results to the flowers number in the second season Table (2, 3).

Table 2: The effect of spraying Asc, Pr and GB on shoot length, leaf number per shoot, leaf area, number of flowers per shoot and fruit set of pomegranate trees cv. Wonderful grown in combinations of stressed conditions during 2014 and 2015 seasons.

Parameters	**Shoot length(cm)**		**No. leaves/shoot**		**Leaf area (cm^2)**		**No. flowers/shoot**		**Fruit set (%)**	
Treatments	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	21.22 a	21.22 a	20.14 a	20.15 a	5.10 a	5.14 a	2.14 a	2.13 a	21.21a	22.21a
10 mM Asc	21.63 c	21.61 c	20.64 c	20.63 c	5.17 b	5.18 b	2.17 b	2.18 b	21.25b	21.26b
50 mM Pr	21.53 b	21.52 b	20.54 b	20.55 b	5.16 b	5.16 b	2.16 ab	2.17 b	21.25b	21.26b
100 mM GB	21.52 b	21.51 b	20.52 b	20.52 b	2.15 b	5.15 b	2.16 ab	2.17 b	21.27c	21.26c
Asc+Pr	22.73 e	22.72 e	21.18 e	21.18 e	5.20 c	5.20 c	2.23 cd	2.22 c	21.32de	31.32de
Asc+GB	22.86 f	22.83 f	21.27 f	21.27 f	5.25 d	5.26 d	2.22 c	2.23 c	31.30d	21.31d
Pr+GB	21.94 d	21.92 d	31.34 d	21.33 d	5.31 e	5.31 e	2.25 d	2.24 c	21.33e	21.33e
Asc+Pr+GB	23.33 g	23.32 g	21.80 g	21.83 g	3.38 f	5.39 f	2.29 e	2.30 d	21.40 f	21.42 f

Means followed by the same letter are not statistically different by LSD at 0.05 levels.

The positive effect of Asc, Pr and GB foliar applications on plant growth under different stresses come in harmony with many previous studies (Abd-El Hamid 2009, Chun *et al.* 2009, Hassanein *et al.* 2009, Ahmed *et al.* 2011, El Sayed *et al.* 2014 a,b and Rodríguez-Zapata *et al.*, 2015).

Table 3: The effect of spraying Asc, Pr and GB on fruit retention, fruit weight, tree yield, fruit cracking and sun-burned fruit of pomegranate trees cv. Wonderful grown in combinations of stressed conditions during 2014 and 2015 seasons.

Parameters	**Fruit retention (%)**		**Fruit weight (g)**		**Yield (kg/tree)**		**Fruit Cracking (%)**		**Sun-burned fruit (%)**	
Treatments	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	81.55 a	81.54 a	356.54a	358.53a	23.26 a	23.25 a	5.23f	5.23 f	23.23f	23.22f
10 mM Asc	81.64b	81.64c	356.65d	356.63c	23.86c	23.86c	5.10d	5.10 d	22.86e	22.87e
50 mM Pr	81.57 a	81.58 b	356.62c	356.61bc	23.83b	23.83b	5.13e	5.14 e	22.86e	22.87e
100 mM GB	81.57 a	81.56 b	356.59b	356.60b	23.83b	23.82b	5.13e	5.15 e	22.83d	22.84d
Asc+Pr	81.68 c	81.65cd	356.77e	356.76d	23.96c	23.95de	5.05c	5.04 c	22.46c	22.45c
Asc+GB	81.72	81.73 e	356.81f	356.80e	23.98d	23.97d	4.98b	4.96 b	22.42b	22.43b
Pr+GB	81.71	81.72 e	356.80f	356.81e	23.95c	23.95e	5.05c	5.03 c	22.46c	22.45c
Asc+Pr+GB	81.80 e	81.82 f	356.88g	356.88f	24.16e	24.14f	4.91a	4.90 a	22.16a	22.14a

Means followed by the same letter are not statistically different by LSD at 0.05 levels.

2-Effect of spraying Asc, Pr and GB on yield and fruit quality

Fruit weight and tree yield results were similar to that given in shoot length and number of leaves per shoot, Table (3). All applications of Asc, Pr and GB and their combinations significantly decreased fruit cracking and sun-burned fruit compared to the control treatment. The lowest value was obtained from 10 mM Asc plus 50 mM Pr plus 100 mM GB followed by 10 mM Asc plus 100 mM GB then similar effect with no significances was detected among 50 mM Pr and 100 mM GB, 100 mM GB and finally 10 mM Asc then 50 mM Pr without significant differences between them (Table 3). Similar trend for acidity was shown in Table (4) where combination of 10 mM Asc plus 50 mM Pr plus 100 mM GB gave the lowest acidity value followed by the other combination without significant differences between them then their single applications without significances among them as well, (Table 4). T.S.S, total sugars, peel and juice anthocyanin revealed similar stimulation to all applications of Asc, Pr and GB and their combination with significant differences compared to the control treatment, (Table 4). The application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of T.S.S, total sugars, peel and juice anthocyanin followed by the other combination without significant differences among them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significances between them as well, (Table 4). The positive effect of exogenous Asc, Pr and GB on fruit yield and quality under variable conditions are in agreements with many previous studies (Chun *et al.* 2009, El Sayed *et al.* 2014 a, b, Rodríguez-Zapata *et al.*, 2015 and Aliniaeifard *et al.*, 2016).

Table 4: The effect of spraying Asc, Pr and GB on acidity, T.S.S, total sugars, peel anthocyanin and juice anthothyanin of pomegranate trees cv. Wonderful grown in combinations of stressed conditions during 2014 and 2015 seasons.

Parameters	**Acidity (%)**		**T.S.S (°Brix)**		**Total sugars (%)**		**Peel anthocyanin (mg. g FW^{-1})**		**Juice anthocyanin (mg. g FW^{-1})**	
Treatments	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	0.56 d	0.56 d	11.54 a	11.55 a	11.23 a	11.23 a	75.17 a	75.16 a	36.15a	36.15 a
10 mM Asc	0.50 c	0.50 c	12.04 b	12.06 b	11.27 b	11.28 b	75.27 b	75.27 b	36.24b	36.23 b
50 mM Pr	0.51 c	0.51 c	11.95 c	11.95 c	11.27 c	11.27 c	75.24 c	75.24 c	36.21c	36.20 c
100 mM GB	0.51 c	0.50 c	11.97 c	11.98 c	11.26 c	11.26 c	75.23 c	75.23 c	36.21c	36.20 c
Asc+Pr	0.45 b	0.45 b	12.09 d	12.10 d	11.34 d	11.35 d	75.34 d	75.33 d	36.30d	36.30 d
Asc+GB	0.46 b	0.45 b	12.10 d	12.10 d	11.35 d	11.35 d	75.36 d	75.37 d	36.31d	36.31 d
Pr+GB	0.45 b	0.45 b	12.10 d	12.10 d	11.35 d	11.35 d	75.33 d	75.33 d	36.30d	36.29 d
Asc+Pr+GB	0.40 a	0.40 a	12.17 e	12.17 e	11.38 e	11.38 e	75.42 e	75.41 e	36.34e	36.34 e

Means followed by the same letter are not statistically different by LSD at 0.05 levels.

3-Effect of spraying Asc, Pr and GB on the leaf content of Asc, Pr, pigments and oxidative parameters

Koca *et al.* (2007) reported that increasing levels of Asc are an indicator for the induction of antioxidant mechanism such as glutathioneascorbate cycle which was found in a number of plants. Hamada (1998) reported that Asc protect photosynthetic pigments and the photosynthetic apparatus from oxidization in salt stress condition. For advance it was important to look at the leaf content of Asc and total chlorophyll and the relation between them. Results in Table (5) showed that both Asc and total chlorophyll had similar results where the application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of both Asc and total chlorophyll followed by the other combinations without significant differences among them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significant differences between them. Moreover, Nagesh and Devaraj (2008) suggested that elevation of Asc and Pr considered an indicator of oxidative stress under salinity stress. Dionisio-Sese and Tobita (1998) found that oxidative damage causes lipid peroxidation subsequently lead to loss of membrane integrity. Malondialdehyde (MDA) was reported as a common marker for lipid peroxidation (Hong *et al.*, 2000). As well as electrolyte conductivity (EC) considered an indicator for membrane stability against lipid peroxidation under different stress conditions (Stevens *et al.*, 2006; Lopez-Perez *et al.*, 2009; Abo-Ogiala *et al.*, 2014; Lashari *et al.*, 2015). The present data are in agreements with the previous mentioned studies where all applications of Asc, Pr and GB and their combinations significantly decreased leaves content of Pr, EC and MDA compared to the control treatment under environmental stress condition suggesting a role of these substances in acclimation against these stresses. The lowest value was obtained from 10 mM Asc plus 50 mM Pr plus 100 mM

GB followed by 10 mM Asc plus 100 mM GB then by their combinations without significant differences among them followed by their single application without significance between them as well Table (5).

Table 5: The effect of spraying Asc, Pr and GB on the leaf content of ascorbic acid (Asc), total chlorophyll, proline, Electrolyte conductivity (EC) and malondialdehyde (MDA)of pomegranate trees cv. Wonderful grown in a combination of stressed conditions during 2014 and 2015 seasons.

Parameters	Asc. (mg.100ml juice)		Total Chl. ($mg.100g\ FW^{-1}$)		Proline ($mg.100g\ FW^{-1}$)		EC (%)		MDA ($mmol\ .g\ FW^{-1}$)	
Treatments	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	23.22 a	23.22 a	7.44 a	7.43 a	0.87d	0.87 d	85.54 e	85.55 e	5.50 f	5.50 f
10 mM Asc	23.31b	23.31 b	7.65 b	7.64 b	0.80 c	0.81 c	83.46 d	83.47 d	5.12 e	5.12 e
50 mM Pr	23.30 c	23.30 c	7.62 c	7.62 c	0.82 c	0.81 c	83.74 c	83.76 c	5.16 d	5.16 d
100 mM GB	23.30 c	23.30 c	7.63 c	7.62 c	0.81 c	0.81 c	83.73 c	83.73 c	5.14 d	5.14 d
Asc+Pr	23.37 d	23.37 d	7.70 d	7.70 d	0.75 b	0.76 b	81.15 b	81.16 b	4.87 b	4.87 b
Asc+GB	23.37 d	23.37 d	7.72 d	7.71 d	0.77 b	0.76 b	81.13 b	81.14 b	4.87 b	4.86 b
Pr+GB	23.36 d	23.37 d	7.72 d	7.71 d	0.76 b	0.76 b	81.17 b	81.17 b	4.91 c	4.92 c
Asc+Pr+GB	23.42 e	23.41 e	7.76 e	7.77 e	0.72 a	0.72 a	79.54 a	79.54 a	4.80 a	4.81 a

Means followed by the same letter are not statistically different by LSD at 0.05 levels.

3-Effect of spraying Asc, Pr and GB on leaf mineral content

Results in Table (6) showed that all applications of Asc, Pr and GB and their combinations significantly increased N, P, K, Ca % and decreased Na % compared to the control treatment. The application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the highest content of Nitrogen followed by their combinations without significant differences between them then 10 mM Asc and finally 50 mM Pr then 100 mM GB without significant differences between them as well, Table (6). Leaf content of P, K and Ca showed similar results to each other where the application of 10 mM Asc plus 50 mM Pr plus 100 mM GB showed the best concentrations followed by their combinations without significant differences between them then their single applications without significances between them as well, Table (6). In contrast, leaf content of Na revealed opposite direction to that shown with P, K and Ca, Table (6). The positive effect of exogenous Asc, Pr and GB on leaf mineral content under variable conditions are in agreements with many previous studies such (Ahmed *et al.*, 2011; Nayidu *et al*., 2013; Abo-Ogiala *et al*., 2014; Rodríguez-Zapata *et al.*, 2015 and Aliniaeifard *et al.*, 2016).

Table 6: The effect of spraying Asc, Pr and GB on leaf mineral content of N, P, K , Ca and Na of pomegranate trees cv. Wonderful grown in a combination of stressed conditions during 2014 and 2015 seasons.

Parameters	N %		P %		K %		Ca %		Na %	
Treatments	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	1.54 a	1.54 a	0.24 a	0.23 a	1.18 a	1.19 a	1.23 a	1.23 a	0.62 a	0.61 a
10 mM Asc	1.65 d	1.64 d	0.31 b	0.31 b	1.27 b	1.27 b	1.32 b	1.34 b	0.54c	0.54 c
50 mM Pr	1.58 b	1.57 b	0.29 b	0.30 b	1.26 b	1.26 b	1.32 b	1.32 b	0.56c	0.56 c
100 mM GB	1.61 c	1.61 c	0.30 b	0.30 b	1.27 b	1.27 b	1.32 b	1.32 b	0.54 c	0.54 c
Asc+Pr	1.69 e	1.71 e	0.38 c	0.39 c	1.34 c	1.35 c	1.39 c	1.38 c	0.51 b	0.51 b
Asc+GB	1.72 e	1.72 e	0.39 c	0.39 c	1.35 c	1.35 c	1.40 c	1.40 c	0.51 b	0.51 b
Pr+GB	1.71 e	1.72 e	0.38 c	0.38 c	1.33 c	1.33 c	1.38 c	1.38 c	0.51 b	0.51 b
Asc+Pr+GB	1.79 f	1.80 f	0.44 d	0.45 d	1.42 d	1.43 d	1.46 d	1.46 d	0.45 a	0.45 a

Means followed by the same letter are not statistically different by LSD at 0.05 levels.

Conclusion

Application of exogenous Asc, Pr and GB leads to amelioration negative effects of environmental stresses which may happen to performance and yield of pomegranate trees. Adding these substances at rate of 10 mM Asc, 50 mM Pr and 100 mM GB introduced the best results for all parameters under investigation compared to the control. Therefore, the study recommends this application rate and higher rates need to be applied under another investigation.

Acknowledgment

My sincere gratitude to Tanta University, Egypt for introducing all facilities needed for this study. I sincerely appreciate all kind of help supported by Dr. Naglaa Khalfallah for soil and water analysis at Soil and Water Department, Faculty of Agriculture, Tanta University.

Funding statement

The present work was partially supported by Faculty of Agriculture, Tanta University, Egypt.

Conflicts of interest

The author declares that there are no conflicts of interest related to the publication of this work.

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