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RESEARCH ARTICLE

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Foliage applications of jasmonic acid modulate the antioxidant defense under water deficit growth in sugar beet

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Abstract

Aims of study: Water deficit (WD) is becoming an alarming problem in many regions of the world. Jasmonic acid (JA) is considered an important intracellular plant growth regulator. The aim of the current research was to investigate the important role of JA in mitigating the negative effects of WD on plant growth.

Area of study: Sugar beet production systems of two locations in Chaharmahal-Bakhtiari province, Iran.

Material and methods: A field trial was conducted to assess the foliar applications of JA (0, 5 µM and 10 µM) and WD (50%, 75%, 100% plant water requirements) effects on physiological yield components of sugar beet (*Beta vulgaris* L.) plants.

Main results: WD significantly ($p<0.05$) increased catalase, ascorbate and peroxidase activities, and malondialdehyde, hydrogen peroxide and white sugar content (WSC); however, it caused a reduction in white sugar yield and root yield (RY). JA foliage applications further enhanced the enzymes activity in WD treated plants resulting in higher WSC, potassium concentrations, white sugar and final RY. Interestingly the effects of JA applications were more pronounced under severe WD (50%) compared to mild (75%) or well-watered plants (100%). JA (10 µM) foliage applications increased the RY and white sugar production by 21% and 24% under severe WD.

Research highlights: JA can ameliorate the adverse effects of WD and increase the WD tolerance of sugar beet crop by upregulating the antioxidant enzyme activities to withstand adverse environmental conditions.

Additional keywords: *Beta vulgaris*; antioxidant enzymes; irrigation requirement; lipid peroxidation; root yield; white sugar yield.

Abbreviations used: ABA (abscisic acid); APX (ascorbate peroxidase); CAT (catalase); FW (fresh weight); GSH (glutathione); JA (jasmonic acid); MDA (malondialdehyde); MeJA (methyl jasmonate); MS (molasses contents); POX (peroxidase); ROS (reactive oxygen species); RY (root yield); SC (sugar content); SOD (superoxide dismutase); TBA (thiobarbituric acid); TCA (trichloroacetic acid); WD (water deficit); WSC (white sugar content); WSY (white sugar yield).

Authors' contributions: HG, JR and MRT conceptualized the research. HG conducted the fieldwork, data collection and laboratory analyses. HG, MN and MC prepared the first draft, did statistical analyses, improved the discussion and finalized the current manuscript. All authors read and approved the final manuscript.

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Introduction

Crop plants face different abiotic stresses during their growth cycle (Leufen *et al.*, 2016); however, water deficit (WD) is considered the most deleterious among all abiotic stresses, that limits the plant growth and final productivity in agricultural crop systems worldwide (Leufen *et al.*, 2016; Liu *et al.*, 2018; Ghaffari *et al.*, 2019). The current situation is becoming more alarming due to climate change where many parts of the world are facing WD challenges to sustain crop production to meet the growing needs of the food and feed (Mancosu *et al.*, 2015). At present, the decline in surface and ground water resources is further aggravating WD scenario and is a major threat to crop production and food security across the globe (Mishra & Singh, 2010). Decline in water resources could negatively affect the agricultural productivity through various mechanisms; for instance, reduction in turgor and water contents in the growing plant, as a result of imbalanced osmotic pressure and cell homeostasis (Din *et al.*, 2011; Leufen *et al.*, 2016), production of reactive oxygen species (ROS) along with enhanced levels of malondialdehyde (MDA) in plant cells (Nahar *et al.*, 2016). Such adverse changes cause cell membrane damages in plants (Moussa & Abdel-Aziz, 2008; Cunhua *et al.*, 2010), resulting in lower final productivity in agricultural systems. However, inbuilt defense systems have been reported in growing plants to combat the negative effects of WD including oxidative stresses (Ghaffari *et al.*, 2019). Oxidative defense systems include enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), superoxidase dismutase (SOD) and peroxidase (POX), whereas non-enzymatic antioxidants include lower molecular weight compounds, such as vitamins (vitamins C and E), β-carotene, uric acid, and glutathione (GSH), a tripeptide (l-γ-glutamyl-l-cysteinyl-l-glycine) that comprises a thiol (sulfhydryl) group (Birben *et al.*, 2012; Ghaffari *et al.*, 2019) that allows the scavenging of superoxide radicals and H_2O_2.

Sugar beet (*Beta vulgaris* L.) is the second most important sugar crop after sugarcane (Iqbal & Saleem, 2015). Sugar beet production mainly depends on the precipitation and irrigation in many areas of world (Fotouhi *et al.*, 2017; Ghaffari *et al.*, 2019). Although the sugar beet is relatively tolerant to adverse environmental conditions (Fotouhi *et al.*, 2017); however, severe WD could result in major losses in sugar beet yield (Chołuj *et al.*, 2014; Moosavi *et al.*, 2017; Ghaffari *et al.*, 2019). For instance, up to 30% loss has been reported in arid, and semi-arid regions (Ober, 2001; Mansuri *et al.*, 2018). In a previous study (Ghaffari *et al.*, 2019), we observed 35% yield reduction under severe WD when sugar beet was grown under field conditions. Fotouhi *et al.* (2017) reported an increase in sugar percentage under WD compared to well water supplies; however, WD caused significant yield losses, which might be due to the reduction in root yield (RY) (Mahmoud *et al.*, 2018).

Different agronomic and molecular approaches are being applied to mitigate the detrimental effects of WD on growing plants (Hasanuzzaman & Fujita, 2011; Alam *et al.*, 2014; Ghaffari *et al.*, 2019). Foliage applications of different osmoprotectants are being considered as potential strategies to mitigate WD effects under various climatic conditions (Gholami Zali & Ehsanzadeh, 2018; Ghaffari *et al.*, 2019). Jasmonic acid (JA) and methyl jasmonate (MeJA), commonly referred as jasmonates, are considered as osmoregulators and play crucial role in regulating environmental stresses (Muñoz-Espinoza *et al.*, 2015; Pazoki, 2015). Alam *et al.* (2014) concluded that JA is an important signaling molecule and shows plant responses responsible in a wide range of morphological, physiological, and biochemical mechanisms. Keeping in view the importance of sugar beet and increasing events of drought stress, the current study was planned to evaluate the effects of foliar application of JA on different growth stages (sugar beet growth stage BGS 16-32, BGS 19-34 and BGS 19-36) of sugar beet plants in imparting the WD tolerance under field conditions. The specific purposes of this study therefore were to elucidate the possible roles of JA in WD tolerance, with special reference to the antioxidant defense as well as to some biochemical parameters in sugar beet exposed to WD. We hypothesized that enhanced enzymatic antioxidant system due to foliar JA applications could result in better crop growth, root yield and white sugar content (WSC) in sugar beet.

Material and methods

This research was performed in Chaharmahal-Bakhtiari province in Iran at two different locations (please see Ghaffari *et al.*, 2019 for complete details). The climatic growth conditions at the experimental locations during whole crop growth season are presented in Table 1. Monogerm Castile sugar beet rhizomania resistant seeds (SesVanderHave, Tienen, Belgium) were planted in late May 2015 on a clay loam soil (Ghaffari *et al.*, 2019). A completely randomized block design with three replicates was arranged as split-plot. Factor one was kept in main plots comprising of normal irrigation (control: 100% water requirement of plant), mild WD (75% water requirement of plant) and severe WD (50% water requirement of plant), whereas the second factor included no JA foliage applications as control, low JA (5 μM) and high JA (10 μM) applications which were maintained in the subplots. The planting density was 10 plants m^{-2} with 50 cm apart rows, whereas the subplots were 3 m × 4 m in width and length. WD was imposed 75 days after sowing when crop attained about 16-20 leaves (BGS 19-35). JA foliage spray treatments were applied three times in two-week intervals on foliage parts of sugar beet plants in respective plots and first spray was carried out after 45 days after planting. Three JA applications were applied at BGS 16-32, BGS 19-34 and BGS 19-36, respectively (BBCH Scale: https://www.politicheagricole.it/flex/AppData/WebLive/Agrometeo/MIEPFY800/BBCHengl2001.pdf). Plots related to control treatments were sprayed with water at time of JA foliar applications. WD treatments were determined based on maximum allowable depletion by adopting the method developed by Allen *et al.* (2000) and explained in our previous report (Ghaffari *et al.*, 2019).

Table 1. Seasonal trends of the mean monthly temperature, moisture, evaporation, and rainfall sugar beet crop growth season (2015) at both study sites.

	May	June	July	August	September	October
				Shahrekord		
Temperature (°C)	15.1	21.2	22.9	21	17.4	13.5
Relative humidity (%)	47	32	33	29.9	41.1	42
Evaporation (mm)	8.5	11	10.9	10.3	8	5.7
Rainfall (mm)	0.4	0	0.7	0	0	1.2
				Shalamzar		
Temperature (°C)	16.4	22.5	23.8	22.8	19	15.5
Relative humidity (%)	33.1	25.2	23.4	21.1	27	38.2
Evaporation (mm)	9.8	13.2	12.5	10.9	9.5	7.1
Rainfall (°C)	0.4	0	0.4	0	0	1.2

Enzyme assays

CAT, APX and POX activities were measured from leaf extracts prepared according to the methods of Aebi (1984), Nakano & Asada (1981), and Herzog & Fahimi (1973) respectively, with some modifications as reported in our previous work (Ghaffari *et al.*, 2019).

Lipid peroxidation and hydrogen peroxide

MDA contents were measured to assess lipid peroxidation using thiobarbituric acid (TBA) according to De Vos *et al.* (1991); fresh leaf samples (0.1 g) were macerated in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 ×g for 5 min. For every 1 mL of the aliquot of the supernatant, 4 mL of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then cooled quickly on ice bath and centrifuged at 10,000 ×g for 15 min and the absorbance of the supernatant was recorded at 532 nm by a spectrophotometer (Model U-1800, Hitachi High-Technologies Corporation, Tokyo, Japan). Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1} and the results expressed as nanomol MDA g^{-1} fresh weight.

Hydrogen peroxide (H_2O_2) was analyzed following the method developed by Yu *et al.* (2003). The leaves were extracted in potassium-phosphate buffer (pH 7.0) (centrifuging at 12,000 ×g for 15 min), then reacting with the mixture of $TiCl_4$ in 20% H_2SO_4 (v/v) and measured spectrophotometer (Model U-1800, Hitachi High-Technologies Corporation, Tokyo, Japan) at 410 nm.

Sugar beet yield potential

Sugar beet crop was harvested 160 days after sowing (BGS-49) when crop reached the physiological maturity at both study sites. For this purpose, the plant shoots were cut at ground level from each experimental plot. The sugar beet roots were then taken out from ground by using a harvesting machine (Beet digger, model TAKA 623, Iran). To assess the root yield, ten sugar beet roots were chosen randomly from the each harvested plot in each treatment.

Sugar beet root quality

After thorough washing with water, the sugar beet roots (25 kg for each sample) were sent out to the Sugar Technology Laboratory (Sugar Beet Research Center, Esfahan, Iran) to assess the root quality traits. Sugar contents (SC) were assessed as sugar weight (g per 100 g of root pulp) by adopting the Betalyzer polarimetrically method (Reinfeld *et al.*, 1974). The root Na and K concentrations were analyzed in the digested solution using flame-photometry (Model 405, Corning, Halstead, Essex, UK). Alpha-amino-nitrogen (α-N) in sugar beet roots was measured using double beam photometer based on the method developed by Reinfeld *et al.* (1974). Sugar beet root molasses contents (MS), WSC and white sugar yield (WSY) were calculated following Märländer *et al.* (2003) as described in equations (1), (2) and (3).

$$MS = 0.343\,(K + Na) + 0.094\,(\alpha\text{-}N) - 0.31 \quad (1)$$

$$\%\,WSC = (\%\,SC - \%\,MS) - 0.6 \quad (2)$$

$$WSY = WSC \times RY \quad (3)$$

Statistical analysis

Collected data set was analyzed using vers. 9.2 (SAS Inst. Inc. Cary, NC, USA) to evaluate the effects of WD, foliar applications of JA and/or two experimental locations as fixed effects by general linear model as described in Table 2. Differences between the treatment means were calculated by slicing location method (WD×JA). However, due to prime interests, the results of two-way analysis of variance WD×JA on each location are presented here. The figures were prepared using SigmaPlot software (vers. 12.1 from Systat Software, Inc., San Jose, CA, USA).

Results

Quantification of enzymatic activities

Analysis of variance revealed significant ($p<0.05$) effects of WD, JA foliar applications, locations (L), and their interactions on sugar beet leaf enzyme activities and root quality with few exceptions (Table 2). The two-way analysis of variance (WD × JA) revealed significantly ($p<0.05$) higher CAT (1.5 ± 0.1 and 1.3 ± 0.0 nmol H_2O_2 mg^{-1} protein min^{-1}), APX (0.2 ± 0.0 and 0.2 ± 0.0 nmol H_2O_2 mg^{-1} protein min^{-1}), and POX (3.9 ± 0.1 and 3.6 ± 0.1 nmol H_2O_2 mg^{-1} protein min^{-1}) activities in sugar beet leaves under severe WD and higher JA foliage applications (10 μM) at Shahrekord (Fig. 1a-c) and Shalamzar (Fig. 1d-f) sites, respectively. However, the CAT, APX and POX activities were significantly ($p<0.05$) lower under control treatment without WD or JA foliage applications at both study sites (Fig. 1a-f). Generally the CAT, APX and POX enzymes were significantly ($p<0.05$) upregulated in the sugar beet leaves in WD plants in the order of 100%, <75%, and <50% WD treatments and 10 μM, >5 μM, and >0 μM JA applications at both study sites. Study results further demonstrated that WD increased APX (150% and 290%), CAT (119% and 80%) and POX (94% and 94%) activities when compared with control treatment at Shahrekord and Shalamzar locations, respectively (Fig. 1a-f).

Cell membrane stability and reactive oxygen species

WD × JA applications expressed significant ($p<0.05$) effects on MDA as well as H_2O_2 contents at both study sites (Table 2). Significantly ($p<0.05$) higher MDA contents (64.4 ± 1.7 and 64.2 ± 2.6 nmol g FW^{-1}) were recorded in sugar beet leaves when grown under severe WD with no JA foliage application at Shahrekord and Shalamzar sites, respectively; whereas lower values at Shahrekord (24.5 ± 0.6; 25.0 ± 0.4 and 24.7 ± 0.5 nmol g FW^{-1}) and Shalamzar (24.6 ± 1.1, 24.6 ± 0.4 and 24.7 ± 0.5 nmol g FW^{-1}) sites were noticed in normal irrigation with no JA, 5 and 10 μM JA foliage application, respectively (Fig. 2a-c). Apparently, the MDA contents were increased up to 105% under mild WD and up to 163% under severe WD compared to normal irrigation

Table 2. Analysis of variance to evaluate the effects of water deficit (WD) and jasmonic acid (JA) application on leaf catalase (CAT), ascorbate peroxidase (APX), peroxidase (POX), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), root sodium (Na), potassium (K), α-amino nitrogen (α-N), molasses (MS), white sugar content (WSC), white sugar yield (WSY) and root yield (RY) at two study sites during 2015. L: location.

	Source of variation							
	L	**WD**	**JA**	**L×WD**	**L×JA**	**WD×JA**	**L×WD×JA**	**Error**
CAT	0.11***	1.56***	2.10***	0.32***	0.10***	0.25***	0.09***	0.003
APX	0.003***	0.01***	0.05***	0.002***	0.001**	0.01***	0.001**	0.000
POX	0.49**	7.75***	14.31***	0.01^{ns}	0.22**	1.83***	1.07***	0.03
MDA	3.81^{ns}	4855***	317.10***	2.06^{ns}	2.10^{ns}	91.01***	1.45^{ns}	9.27
H_2O_2	0.03^{ns}	21.13***	1.32***	0.06^{ns}	0.04^{ns}	0.45***	0.04^{ns}	0.03
Na	1.19***	1.42***	0.18***	0.02*	0.03**	0.44***	0.01***	0.01
K	390.60***	4.06***	1.39**	1.25***	0.17*	0.26**	0.28**	0.05
α-N	27.36***	4.68***	1.45***	0.38*	0.14^{ns}	0.83**	0.47**	0.09
MS	58.43***	0.02*	0.07**	0.09**	0.01^{ns}	0.01^{ns}	0.02*	0.01
WSC	113.40***	5.78**	1.97*	1.52^{ns}	0.18^{ns}	1.15^{ns}	0.42^{ns}	0.62
WSY	36.26***	44.75***	7.17***	2.49^{ns}	0.51^{ns}	1.12*	0.09^{ns}	0.44
RY	5.16^{ns}	1864***	99.42***	2.03^{ns}	13.95^{ns}	27.49*	1.86^{ns}	7.53

^{ns}, *,**,***: non-significance and significance at 5, 1 and 0.01% level, respectively.

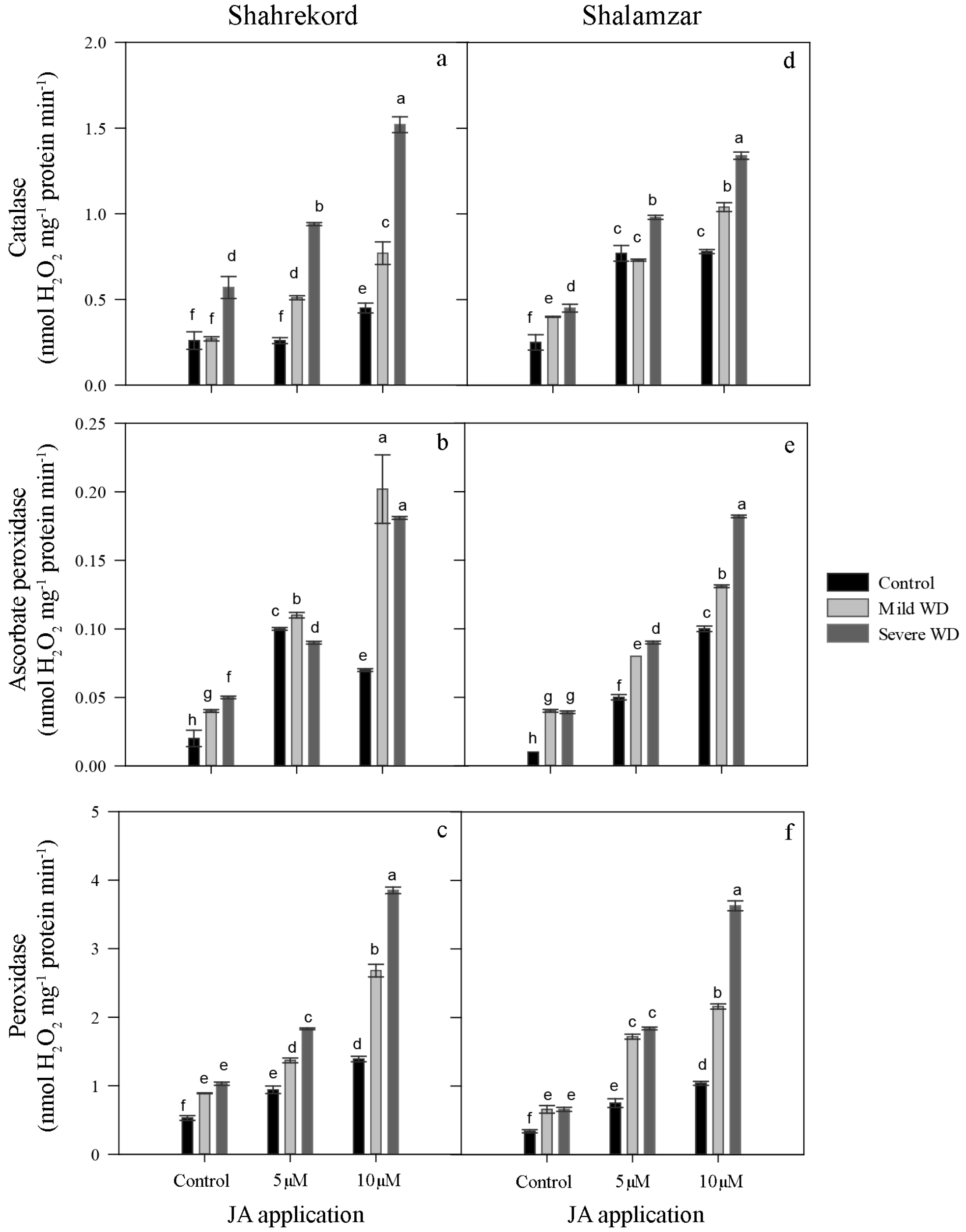

Figure 1. Effects of water deficit (WD) and jasmonic acid (JA) foliar applications on leaf antioxidant enzymes at Shahrekord (a-c) and Shalamzar (d-f) on slicing method by location (Slicing, $p<0.05$). Each bar represents the mean of three replications ± SE. Different letters in each figure represent significant ($p<0.05$) differences among treatments (WD×JA).

(Fig. 2a). JA applications caused a prominent decrease in MDA contents under WD treatments in order of 10 µM > 5 µM > 0 µM (Fig. 2a); however, the JA treatment effects were more pronounced under WD compared to normal irrigation.

Experimental treatments expressed similar trends on H_2O_2 contents (Fig. 2b). Significantly ($p<0.05$) higher H_2O_2 contents were noticed under severe WD treatment with control JA foliage applications (3.4 ± 0.2 and 3.3 ± 0.1 µmol g FW^{-1}) at Shahrekord and Shalamzar sites, respectively. However, the minimum H_2O_2 contents were recorded under normal irrigation at all levels of JA applications at both experimental sites (Fig. 2b-d). Mild and severe WD resulted up to 100% and 202% H_2O_2 increase in sugar beet leaves compared to normal irrigation (Fig. 2b-d).

Sugar beet root sodium, potassium and α-amino nitrogen

WD × JA interaction expressed significant ($p<0.05$) effects on sugar beet root K, Na, and α-amino nitrogen

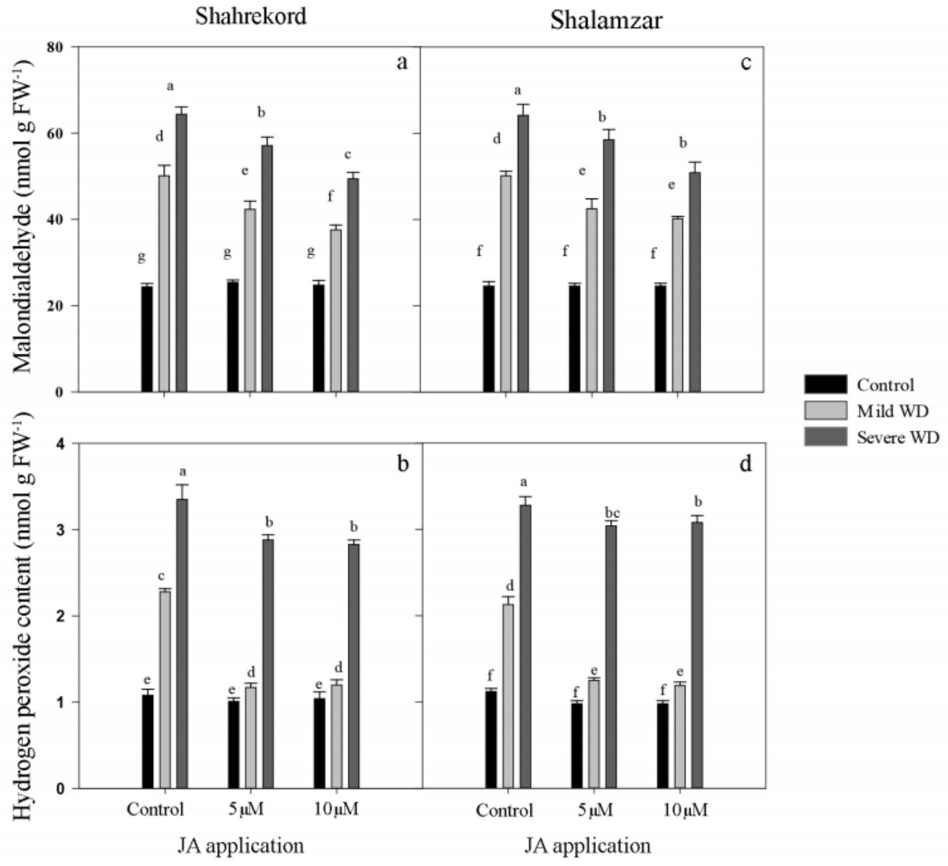

Figure 2. Effects of water deficit (WD) and jasmonic acid (JA) application on leaf malondialdehyde and hydrogen peroxide content of sugar beet ($p<0.05$) at Shahrekord (a-b) and Shalamzar (c-d) sites based on slicing method by location. Each bar represents the mean of three replications ±SE. Different letters in each figure represent significant ($p<0.05$) differences among treatments (WD×JA).

(α-N) at both study sites (Table 2; Fig. 3a-f). WD significantly ($p<0.05$) enhanced the Na and α-N contents in sugar beet roots at both study sites (Fig. 3a-f). Significantly higher Na was recorded under severe WD and control JA foliar applications (1.4 ± 0.1 and 1.2 ± 0.0 meq 100 g beet^{-1}) at Shahrekord (Fig. 3a) and Shalamzar growing sites (Fig. 3d). However, the minimum sugar beet Na contents were noticed under normal irrigation when 10 μM JA foliage was applied (0.6 ± 0.0 and 0.5 ± 0.0 meq 100 g beet^{-1}) at Shahrekord (Fig. 3a) and Shalamzar growing sites (Fig. 3d). Higher increase in α-N was recorded under severe WD and control JA foliar applications (5.3 ± 0.2 and 4.0 ± 0.1 meq 100 g beet^{-1}) at Shahrekord and Shalamzar sites, respectively (Fig. 3b, 3e). The lowest α-N values (3.3 ± 0.1 meq 100 g beet^{-1} and 2.2 ± 0.1 meq 100 g beet^{-1}) were recorded under normal irrigation and 10 μM JA foliar applications at same sites as depicted in Fig. 3b and 3e.

Significantly ($p<0.05$) higher K contents (9.5 ± 0.2 meq 100 g beet^{-1}) were recorded at Shahrekord site under normal irrigation and higher JA foliage applications (10 μM); however, this treatment was at par with normal irrigation and low JA (5 μM) foliage applications (Fig. 3c). The lowest root K was recorded under severe WD at control JA foliage applications at Shahrekord (Fig. 3c). Significantly ($p<0.05$) higher K (3.7 ± 0.3 meq 100 g beet^{-1}) was found in the roots under mild WD and higher JA foliage applications (10 μM) at Shalamzar site, albeit at par with control and mild WD under 5 μM JA foliage applications (Fig. 3f). However, the minimum sugar beet root K was noticed under severe WD without JA applications (Fig. 3c, 3f).

Sugar beet root molasses and white sugar contents

WD×JA foliage application expressed non-significant ($p>0.05$) effects on sugar beet molasses contents at Shahrekord and Shalamzar locations (Table 2). Although non-significant, higher contents in molasses were observed in sugar beet roots under normal irrigation treatment with 5 μM JA foliage

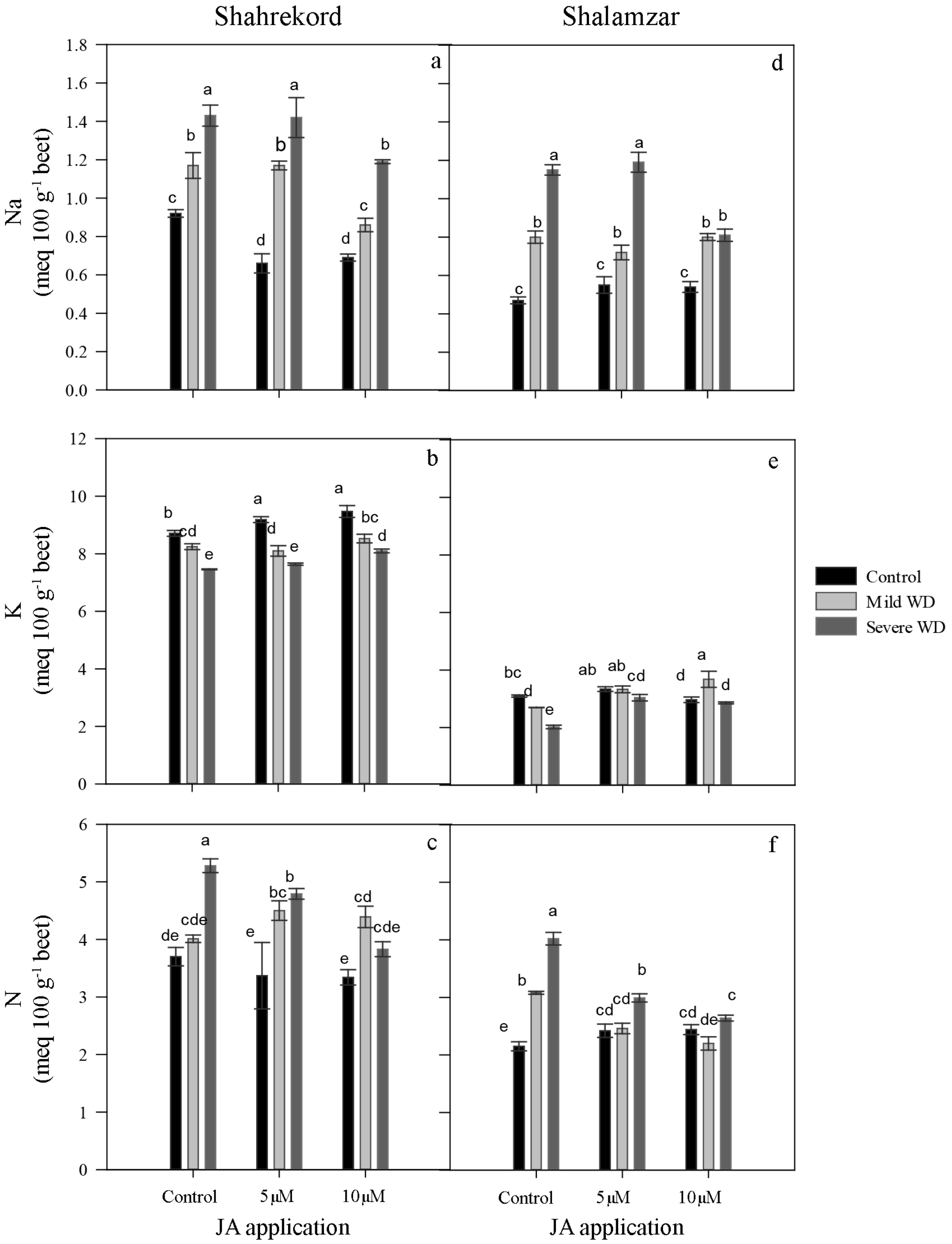

Figure 3. Effects of water deficit (WD) and jasmonic acid (JA) application on root Na (a, d), α-amino nitrogen (b, e) and K (c, f) at Shahrekord and Shalamzar sites on slicing method by location (Slicing, *p*<0.05), respectively. Each bar represents the mean of three replications ± SE. Different letters in each figure represent significant (*p*<0.05) differences among treatments (WD×JA).

application (3.5 ± 0.1%) at Shahrekord (Fig. 4a). The trends of treatment effects on sugar beet root molasses contents were different at Shalamzar site, where a slightly higher value (1.5 ± 0.1%) was observed at mild WD with 10 µM JA foliage applications (Fig. 4b).

Analysis of variance revealed significant effects of WD, JA foliar applications and locations on WSC (Table 2). A higher WSC (18.7 ± 0.4% and 18.9 ± 0.3%) was obtained under mild and severe WD, respectively, and with 5 µM JA foliage application (18.7 ± 0.4%) (Fig. 5).

White sugar yield and root yield

Significant (*p*<0.05) effects of WD and JA foliar applications were observed on WSY and RY on both experimental sites (Table 2). Severe WD caused 29% decrease in WSY, whereas 35% reduction in RY compared to normal irrigation (Fig. 6a, 6b). The

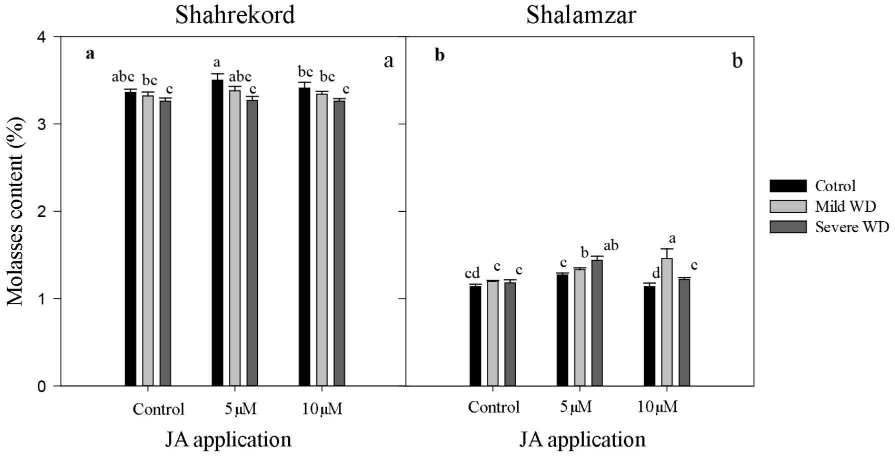

Figure 4. Effect of water deficit (WD) and jasmonic acid (JA) application on molasses content of sugar beet root at Shahrekord (a) and Shalamzar (b) sites on slicing method by location (Slicing, $p<0.05$). Each bar represents the mean of three replications ±SE. Different letters in each figure represent significant ($p<0.05$) differences among treatments (WD×JA).

pronounced positive effects of foliar JA application on WSY and RY were recorded under severe WD compared to mild WD or normal irrigation. Higher JA foliage applications (10 µM) resulted in 24% and 21% increase in WSY and RY under severe DW (Fig. 6a, 6b) compared to controlled JA application. Significantly ($p<0.05$) higher WSY (12.8 ± 0.7 and 12.6 ± 0.6 ton ha^{-1}) were recorded in normal irrigation when 5 and 10 µM JA was foliage applied, respectively (Fig. 6a). Significantly ($p<0.05$) higher RY (70.9 ± 0.7 ton ha^{-1}) was measured under normal irrigation with higher JA applications (10 µM); however, this treatment was at par under normal irrigation with non-JA and low JA (5 µM) foliage applications (Fig. 6b).

Discussion

Growing plants show various symptoms to express drought stress effects; *e.g.*, electrolyte leakage and MDA contents occur commonly due to plant cell membranes damage (Poonam *et al.*, 2013). The estimated MDA value is commonly utilized to measure the lipid peroxidation as an index of the membrane damage caused by drought (Lin & Kao, 2000). The lower membrane stability

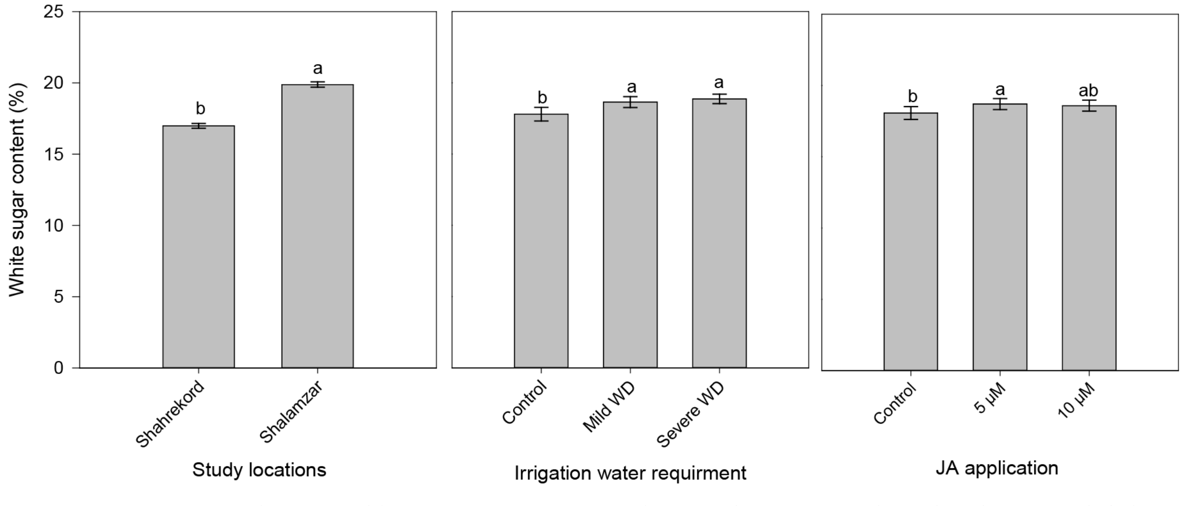

Figure 5. Mean comparison for white sugar content (WSC) in sugar beet crop evaluated under water deficit (WD) and jasmonic acid (JA) foliar applications at both experimental sites (Shahrekord and Shalamzar) at $p<0.05$. Each bar represents the mean of three replications ± SE.

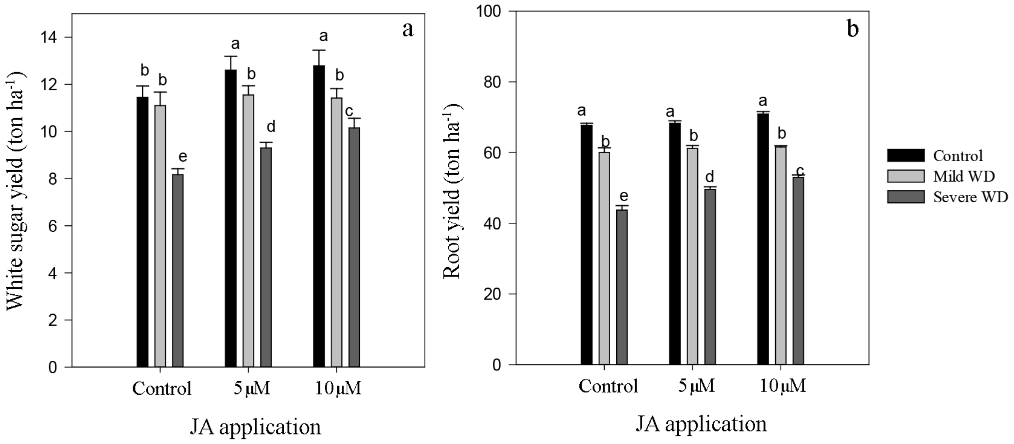

Figure 6. Effect of water deficit (WD) and jasmonic acid (JA) application on white sugar yield (WSY) and root yield (RY) of sugar beet ($p<0.05$). Each bar represents the mean of three replications ±SE. Different letters in each figure represent significant ($p<0.05$) differences among treatments (WD×JA).

index is a consequence of higher oxidative stress under drought stress (Nazarli *et al.*, 2014). Majid & Akbar (2006) reported that MeJA mitigated the ROS effects in maize seedlings under WD; they showed that MeJA lowered the ratio of membrane fatty acids, and targeted free radicals. Imbalance between ROS production and antioxidant concentration resulted from stress conditions in cells, may lead to damage to membranes and increase in membrane permeability. However, ROS are not only produced as a toxic by-product but also plays a role in plant response to stress conditions. These responses to stress include hormone signaling, stomatal regulation, and gene activation (Sharma *et al.*, 2012). Increased ROS levels under drought stress are reported to initiate the protective mechanisms in growing plants; however, such mechanisms are further enhanced due to phytohormones such as JA to mitigate the drought stress effects (Nazarli *et al.*, 2014).

JA foliar applications significantly ($p<0.05$) reduced the cell membrane damage by down regulating the MDA contents and H_2O_2 levels in WD compared to normal irrigation treatment (Fig. 2a, 2b). The effects of JA foliar application were more pronounced under WD conditions compared to normal irrigation where high JA (10 µM) foliar applications resulted in up to 22% reduction in MDA contents compared to controlled JA application under severe WD (Fig. 2a). Similarly, H_2O_2 levels decreased by 45% under mild and by 8% under severe WD compared to normal irrigation sugar beet plants under high JA foliar applications (Fig. 2b). JA may be used in large-scale production systems because of its low cost and high production of secondary metabolites. The present study indicates that JA applications increased antioxidant enzymes activities whether plants were under stressed or non-stressed conditions, by supplementing the ROS scavenging mechanism (Fig. 1-2). However, the effects of JA on enzymes activities were more pronounced under WD conditions. Higher accumulation of antioxidative enzymes in the plants may lead to increased resistance against water stress (Kadkhodaie *et al.*, 2014). Additionally, we have observed an enhanced drought tolerance in growing plants due to JA applications that reduced the ROS production and lipid peroxidation (MDA and H_2O_2) on both study sites (Fig. 2ab, 2cd). Additionally, a reduction in ROS production and lipid peroxidation (MDA and H_2O_2), and enhanced drought tolerance in plants under stress conditions (Fig. 2a, 2b). It appears that JA increases CAT, SOD and POX activities and reduces the toxic effects of free radicals or quenched ROS, thereby it plays an important role in signal transduction pathway in oxidative stress (Norastehnia & Nojavan-Asghari, 2006). These findings are in agreement with those of Shan & Liang (2010), who reported that JA increased the transcript levels of APX and CAT in *Agropyron cristatum* under water stress. It was also observed that foliar application of JA reduced the deleterious effects of WD on the growth of sugar beet crop. Anjum *et al.*, (2011) and Mahmood *et al.* (2012) reported significantly positive effects of JA in reducing the water losses and upregulating the drought tolerance in growing plants. In addition, under drought, JA may act as an inducer of signal that leads to the upregulation of ascorbate and glutathione metabolism leading to water stress tolerance (Shan & Liang, 2010). Many studies have shown the positive effects of JA in mitigating the deleterious effects of drought on growing plants. Exogenous application of JA may cause an increase in compatible osmolites levels, activity of antioxidant enzyme and level of betaine aldehyde dehydrogenase protein in plants grown under drought conditions (Gao *et al.*, 2004).

The results further revealed that the WSC increased under both water stress and foliar application of JA, which are in line with the results reported by Ghamarina *et al.* (2012), who found 4.45%, 17.32% and 37.63% higher WSC at 25%, 50% and 75% drought stress treatments, respectively. Some studies have reported that higher applications of K result in higher sugar beet yield, sucrose percentage, water use efficiency, higher recoverable sugar yield and lowest impurities under drought stress (Abdel-Motagally & Attia, 2009; Neseim *et al.*, 2014). Therefore, the increased WSC in our study with JA foliage application was related to the high K contents and reduced impurities as Na and α-N (Fig. 3), and increase in sugar recovery as water stress increased. In addition, JA probably increases stress-tolerance by increasing K absorption under stress conditions; as a result, more K accumulation occurs in the sugar beet root (Fig. 3). Reduction of RY and WSY under WD could be a result of the negative effect of WD on sugar beet growth parameters such as dry weight of plant and leaf area index, which were reflected on the lower relative growth rate and net assimilation rate (Mahmoud *et al.*, 2018). Severe water stress causes a photosynthesis reduction, and in result would tend to reduce RY (Ghaffari *et al.*, 2019). Despite the high WSC in severe water stress treatment, RY could not recover the low WSY as compared to the control. Therefore, the plants were not able to increase the potential of producing WSY. In this regard, the 50% water stress had the least WSY and decreased by 35% compared to the normal irrigation. In conclusion, it can be seen that the RY has a greater impact than sugar content on sugar yield (Masri *et al.*, 2015). Chołuj *et al.* (2014) indicated that the RY and WSY were reduced 22% and 35%, respectively under drought, but there were significant differences between genotypes. It has been reported that water stress can adversely influence tap-root and WSY of sugar beet by about 15–50% depending on the severity and duration of drought during the vegetation season. Additionally, Chołuj *et al.* (2008) reported that long-term water shortage resulted in significant changes in sugar content, and slightly increase K, Na, and α-N contents in the tap-roots of sugar beet. In contrast, Hoffmann (2010) showed that sucrose accumulation in the beet tap-root was markedly reduced, whereas ions contents and compatible solutes were increased under drought. However, sucrose and other compounds contents were not affected by water supply, indicating a negative relationship between the content of sucrose and compatible solutes (Hoffmann, 2010). Also, Topak *et al.* (2011) showed that sucrose, K, and Na contents were not significantly affected by drought stress. Whereas α-N was accumulated in beet root only under severe drought.

Application of JA may enhance the fruit quality that may be due to metabolites acting as signal transduction under drought stress. Nitric oxide, ROS, calcium, abscisic acid (ABA), ethylene, and salicylic acid have also been reported to be important mediators of plant growth and development during JA signal transduction and synthesis (Ahmad *et al.*, 2016). Moreover, Rakwal & Komatsu (2005) demonstrated that treating leaf sheath of rice with foliar application of JA caused an increase in endogenous level of ABA. In addition, ABA accumulation trigger ABA-inducible gene expression, leading to closure of stomata and organ drop, therefore reducing water loss in the aerial part (De Ollas *et al.*, 2013). These findings suggest the possible existence of a positive feedback regulatory system for JA biosynthesis and the possibility of cross-talk between JA signaling and other signaling pathways stresses.

In conclusion, the results of the present study indicate that JA could be an effective compound for enhancing drought tolerance in sugar beet crop. The studies regarding the effects of JA in conferring drought as well as abiotic stress tolerances on quality of sugar beet are scarce. JA has been reported as an important signaling molecule that also interacts with other hormones or signaling molecules known to be important in stress signaling pathways. Our results showed that JA application reduced the oxidative stress and improved the biochemical adaptation in sugar beet under water stress. JA applications also resulted in higher K content that caused lower impurities and increased white sugar contents. In addition, JA increased the activity of antioxidant enzymes and tolerance under water stress conditions, as well as increased root yield and white sugar yield. It is worth noting that the reduction in root yield accompanying water deficit was not compensated by the increase in sucrose, sugar recovery percentage, and finally WSY decreased.

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