Assessing photosynthetic performance of fennel (*Foeniculum vulgare* Mill) influenced by plant growth regulators and drought stress imposed at vegetative and reproductive stages

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Abstract

Maintaining crop productivity under limited irrigation water requires some new strategies. This study investigated the influence of drought stress and the application of plant growth regulators (PGRs) including jasmonic acid, brassinosteroids, and putrescine on photosynthetic performance of fennel (*Foeniculum vulgare* Mill). The results indicated that fennel exhibited active osmoregulation which prevented a sharp decrease in relative water content. Fennel successfully maintained high leaf chlorophyll index, Net photosynthesis rate, and transpiration under mild stress, however, severe stress reduced the photosynthetic parameters by 22%, 24%, and 50%, respectively. Drought stress increased chlorophyll *a* fluorescence when fennel plants exposed to the stress condition. V_k and W_k parameters related to the donor side of photoinhibition of photosystem II (PSII) increased by 44% when severe drought stress imposed at the vegetative stage and 34% when occurred during the flowering. The elevation of V_k and W_k indicated a failure in water splitting in PSII. The V_J and V_I parameters of acceptor sides increased by 16% and 22%, respectively when drought stress imposed at the vegetative phase and to 19% and 30%, when drought stress occurred during reproductive phase. Using PGRs resulted in reduced V_J, V_I, V_k, and W_k, suggesting that some degree of recovery of damages occurred. All three PGRs stimulated biomass production and on average, plants yielded roughly 1.6 fold higher than the control plants. The influences of PGRs were mainly independent of drought stress level.

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Introduction

Fennel cultivated for its tasty stalks and the seeds which contain a considerable amount of medicinal and herbal essence currently used in pharmaceutical and food industry (Ody, 2017). In many regions of the world, drought has been recognised as the most critical stress which hindered crops productivity and threatens the cultivation and yield of many agronomic crops and products (Zhu, 2016; Zandalinas *et al.,* 2018). In addition to genetic improvements, new cultural approaches and strategies are required to remediate the negative impact of drought conditions (Fahad *et al.,* 2015). Application of specific plant growth regulators (PGRs) has been used to efficiently increase plants' tolerance to various biotic and abiotic stresses through modifying their physiological characteristics (Souza *et al.,* 2017). Among commercially available PGRs, jasmonic acid (JA), brassinosteroids (BRs) and putrescine (Put) have been successfully incorporated into crop production in areas with limited access to irrigation water (Ahmed *et al.,* 2017).

Application of JA can maintain several physiological processes of plants experiencing drought stress (Creelman and Mullet, 1995). Anjum *et al.* (2011) reported that the use of methyl jasmonate resulted in higher expressions of stress-related genes, gas exchange, and chlorophyll content of soybean under drought stress condition.

The BRs are chemically analogous to animal steroid hormones (Tang *et al.,* 2016). The BRs can regulate various crucial physiological processes including cell division, stomata and vascular differentiation in plants under biotic and abiotic stresses (Gill *et al.,* 2017). A report indicated that the application of BRs improved antioxidative defence mechanism of *Xanthoceras* under drought stress (Li and Feng, 2011).

The Put is an aliphatic amine natural compounds with aliphat-

ic nitrogen structure that belongs to polyamine family and can be found in most living organisms (Gill and Tuteja, 2010). Exogenous application of Put and BRs significantly increased the tolerance of cotton to drought stress (Ahmed *et al.,* 2017).

This study aimed to investigate the applicability of spraying fennel with different PGRs during the vegetative and reproductive growth stages to alleviate drought stress damages.

Materials and methods

Plant material and growth condition

A greenhouse experiment conducted at the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran in 2016. Greenhouse condition was comprised of a day temperature of 24±2°C, a night temperature of 16±2°C, and a relative humidity of 50±5%. Treatments were arranged as a factorial in a randomised complete block design with three replicates. Three levels of drought stress including non-stressed (control), moderate, and severe stress and four PGRs including JA, BRs, Put and distilled water as control (C) were factorially combined with three PGRs *viz* BRs (24-Epibrassinolide at 0.1 µM) (Liu *et al.,* 2017), put (0.5 mM) (Sheokand *et al.,* 2008), and JA (methyl jasmonate 50 µM) (Abdelgawad *et al.,* 2014). Each pot filled with 20 kg sieved field soil. Soil analysis before planting indicated that soil pH (1:1, soil/H_2O) was 7.9, cation exchange capacity was 0.625 ds. m^{-2}, and available P, K were 8.5 and 0.6 mg kg^{-1}, respectively.

Ten seeds planted in each pot and later thinned to five seedlings when plants well established. Soil moisture holding characteristics, including the volumetric soil moisture content at –0.03 MPa (FC) and –1.5 MPa (PWP) determined, using a pressure plate apparatus. Volumetric soil moisture content at FC and PWP were 0.41 and 0.09 g cm^{-3}, respectively. The difference between FC and PWP considered as the available soil water content. Soil moisture of pots monitored twice a day using a time-domain reflectometer fitted with 20 cm probe rods. Before imposing drought stress, all pots maintained at 80% field capacity by daily irrigation. Watering of control pots (non-stress) based on maintaining soil water content at 80% FC (0.32±0.005 g cm^{-3}). Pots in moderate and severe drought stress received 60% and 40% of the amount of irrigation water used in the control pots, respectively. Drought stress treatments imposed at two stages of growth *viz* vegetative (65 days after planting) and at 50% flowering (110 days after planting). PGRs sprayed three days before the onset of drought stress treatments. All parameters measured 20 days after the implementation of the stress treatments. Plants harvested 160 days after planting and their biomass measured after dried in an air-forced oven at 60°C for 48 h.

Osmotic parameter

Proline content measured using the method described by (Bates *et al.,* 1973). Total soluble sugars measured by the method described by Irigoyen *et al*. (1992). Relative water content (RWC) of leaves determined by floating leaves on distilled water for four hours and turgid weight recorded. The leaf tissues dried in an oven at 65°C for 24 h and the RWC calculated using the following formula:

RWC = (Fresh Weight – Dry Weight) / (Turgid Weight – Dry Weight) ×100. (1)

The osmotic potential (OP) measured using Janardhan *et al*. (1975) formula:

OP = – (electrical conductivity × 0.36 × ((sample weight× 25) / moisture content)) / 0.987 (2)

Photosynthetic parameters and chlorophyll *a* fluorescence transient

Net photosynthesis rate (P_N), transpiration (E), and leaf chlorophyll index (SPAD) measured 20 days after drought stress imposed. P_N and E measured on two upper fully expanded leaves using a portable photosynthesis meter (model: LCi-SD, ADC Co. Bio Scientific Ltd., England). Photosynthetic photon flux density maintained at 470-560 µmol m^{-2} s^{-1} using an internal red/blue LED light source and the CO_2 concentration set at 400 µmol mol^{-1} by mixing external air with CO_2 from a source attached to the unit. SPAD, that represents the chlorophyll content of the leaves, measured using a chlorophyll meter (SPAD model 502, Minolta, Japan). Chlorophyll a fluorescence transient obtained from the leaves, which dark-adapted for at least 30 min before measurements. Measurements were made using fluorometer (model: OS-30P^+ chlorophyll Fluorometer, Optic Science, USA) and analysed with the JIP-test (Wang *et al*., 2017). The description and calculation formula of parameters listed in Table 1 (Bussotti, 2004; Wang *et al*., 2017).

Statistical analysis

Analysis of variance (ANOVA) performed using SAS (SAS Inc., Cary, NC, USA). The least significant difference (LSD) used for mean separation. Sigma plot v. 11 used for presenting data in charts.

Results and discussion

Osmotic parameters

Drought stress at both stages of growth significantly increased proline and total soluble sugars (Table 2). However, the extent of changes in proline and total soluble sugars was higher at the vegetative stage.

Compared with the normal condition, fennel leaves accumulated 2.7 fold and 1.6 fold more proline under severe stress during the vegetative and reproductive phase, respectively (Table 2). Similarly, changes in total soluble sugar in fennel leaves due to the drought stress were higher during earlier stages of growth. The total soluble sugar in control plants was 0.97 mg g^{-1} fresh weight, which increased to 1.60 mg g^{-1} fresh weight under severe stress (Table 2).

In the current study, the osmotic adjustment in fennel as a defensive strategy to remediate the drought condition exercised primarily through proline accumulation rather than total soluble sugars. Other reports also indicated that the levels of soluble sugar and proline increased when plant species exposed to drought condition (Patakas and Noitsakis, 2001; Li *et al.,* 2017).

Changes in RWC during both stages of growth followed a trend similar to osmolytes. The RWC of control plants during the vegetative and flowering stages were 84.0% and 81.5%, respectively. However, RWC declined to 79.0% and 78.0% under drought stress conditions. The active osmotic adjustment which

exercised by fennel leaves prevented a sharper decrease in RWC which otherwise could have been detrimental to the plants. The results obtained in this study are not in full agreement with Askari and Ehsanzadeh (2015) who reported that drought stress significantly decreased the RWC in fennel.

Drought stress alters many physiological and metabolic processes of plants which in general hinders plant growth and developments (Farooq *et al.,* 2012). Accumulation of proline and other osmolytes in plants that exposed to drought stress may act as a protective strategy to alleviate cell damages caused by scavenging reactive oxygen species (ROS) thus acting as osmoprotectant (Cuin and Shabala, 2007; Das and Roychoudhury, 2014). However, this defence mechanism costs plants a significant amount of energy and resources which may ultimately compromise plants' productivity (Huot *et al.,* 2014).

Compared with drought stress, application of PGRs had a minimal influence on the accumulation of proline and total soluble sugars (Table 2). Plants sprayed with BRs during the vegetative stage, and those treated with JA during reproductive phase accumulated slightly more proline in their leaves than control plants. As Li *et al*. (2017) reported for *Agrostis stolonifera*, we hypothesised that application of PGRs on fennel might alleviate the negative impact of drought stress on RWC. However, fennel plants responded differently, and we concluded that overall, use of PGRs, especially during the reproductive stage, had no or little influence on osmotic adjustment and RWC. The non-responsiveness of RWC and osmosis in fennel to the application of PGRs could be partially due to the higher biomass production in the treated plants, which consequently diluted the proline and total soluble concentration per gram fresh weight of plants. Thus, the RWC and OP did not change considerably when plants sprayed with PGRs (Table 2). Since the interaction between PGRs and drought stress was not significant, we concluded that osmolytes are regulated independently of PGRs (Table 2).

Photosynthetic parameters

All measured photosynthetic parameters including SPAD, P_N, and E influenced by drought stress (Table 3). During the vegetative stage, the photosynthetic parameters of plants grown under moderate drought stress remained almost unchanged, however, when drought stress intensified, all three photosynthetic parameters reduced significantly where SPAD, P_N, and E reduced as much as 22%, 24%, and 50%, respectively (Table 3). Photosynthetic parameters measured during reproduction stage were lower than the vegetative stage, presumably due to a declining trend in photosynthesis in the aging leaves. Photosynthesis rate usually decreases when plants exposed to drought stress. However, drought tolerant plants are capable of maintaining their photosynthesis at an equal rate under drought conditions (Moshelion *et al.,* 2015). The primary effects of drought stress often include a reduction in photosynthesis and gas exchange (Rouhi *et al.,* 2007). Consequently, the stomatal closure during drought stress incident is the primary physiological response that aims to maintain the RWC of leaves. However, reduced stomatal aperture also restricts the gas exchange

Table 1. Formulae and definitions of the selected JIP-test fluorescence parameters used in the present study.

Parameter and formula	Description
Technical fluorescence parameters	
$V_J = (F_J - F_0)/(F_m - F_0)$	Relative variable fluorescence at 2 ms. F_M; Maximal fluorescence intensity, F_0; F_{50ms}, fluorescence intensity at 50 ms, F_J= Fluorescence intensity at 2 ms
$V_I = (F_I - F_0)/(F_m - F_0)$	Relative variable fluorescence at 30 ms. FI= Fluorescence intensity at 30 ms
$V_K = (F_K - F_0) / (F_M - F_0)$	Relative variable fluorescence at phase K of the fluorescence induction curve
$W_K = (F_K - F_0) / (F_J - F_0)$	Represent the damage to oxygen evolving complex OEC
Quantum efficiency or flux ratios	
$M_0 = 4(F_{300} - F_0)/(F_m - F_0)$	The maximum rate of QA reduction
$TR_0/ABS = (F_m - F_0)/F_m$	The maximum quantum yield of primary photochemistry
$ET_0/ABS = [1 - (F_0 - F_m)]\ ET_0/TR_0$	The quantum yield of electron transport
$D_0/ABS = 1/\ ET_0/ABS$	Thermal dissipation quantum yield
$ET_0/TR_0 = 1 - V_J$	The efficiency with which a trapped exaction can move an electron into the electron transport chain further than QA
Specific fluxes or specific activities	
$ABS/R_C = M_0\ (1/V_J)\ (1/TR_0/ABS)$	Effective antenna size of an active reaction centre (RC). Expresses the total number of photons absorbed by Chl molecules of all RC divided by the total number of active RCs.
$TR0/RC = M0(1/VJ)$	Trapped (maximum) energy flux (leading to QA reduction) per reaction centre (RC)
$ET0/RC = M0(1/VJ)\ ET0/TR0$	Maximum electron transport flux (further than QA–) per photoinhibition of photosystem II (PSII) reaction centre (RC)
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipation energy flux per PSII reaction centre (RC)
The density of reaction centres and performance index	
$RC/CS_0 = TR_0/ABS\ (V_J/M_0)F_0$	Gives the number of active RCs to one inactive RC for a PSII cross-section
$PI_{ABS} = (RC/ABS)\ [TR_0/ABS\ /\ (1 - TR_0/ABS]\ [ET_0/TR_0/(1 - TR_0/ABS)]$	

of the leaves, resulting in a considerable reduction in E and P_N parameters.

Results of the current study revealed that the application of PGRs in both stages of growth generally reduced the SPAD (Table 3) which confirmed some earlier reports (Ahmad and Murali, 2015; Bistgani *et al.,* 2017). However, unlike SPAD, both P_N and E improved when plants treated with PGRs. For example, spraying fennel by JA during the vegetative and reproductive stages, increased P_N by 34% and 33%, respectively. On the other hand, plants sprayed with Put during the vegetative and reproductive stages, exhibited 78% and 51% higher E values than control plants, respectively. Earlier reports indicated that the photosynthesis in cotton and onion enhanced by the application of different PGRs (Kumar *et al.,* 2001; Ahmad and Murali, 2015).

Chlorophyll *a* fluorescence transient

Drought stress significantly affected chlorophyll *a* fluorescence when fennel plants imposed to the stress condition in both stages of growth. Both the donor and the acceptor sides of the photoinhibition of photosystem II (PSII) have been investigated for insight information of the electron transport system in PSII. The V_k and W_k parameters are defined to characterise the photosynthetic performance at the donor side of PSII (Wang *et al*., 2016). In the current study, drought stress significantly influenced the V_k and W_k, in both vegetative and reproductive stages. V_k increased by 44% when severe drought stress imposed at vegetative stage and 34% when the stress occurred during flowering (Table 4). The response trend of the W_k was similar to V_k and increased by 23.0% and 26.8% when severe drought stress imposed during the vegeta-

Table 2. Effect of drought stress and application of exogenous plant growth regulators on proline (µg/g fresh weight), total soluble sugar (mg/g fresh weight), relative water content (%) and osmotic potential (MPa) of fennel leaves during the vegetative and flowering stage.

Treatments	Proline		Soluble sugar		RWC		OP	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
Drought stress								
Non	0.55^{b}	1.32^{b}	0.97^{b}	0.36^{b}	84.0^{a}	81.5^{a}	–0.17^{b}	–0.14^{b}
Moderate	1.39^{a}	2.11^{a}	1.55^{a}	0.55^{a}	80.1^{b}	79.4^{b}	–0.28^{a}	–0.24^{a}
Severe	1.58^{a}	2.19^{a}	1.60^{a}	0.41^{ab}	78.9^{b}	77.9^{b}	–0.30^{a}	–0.25^{a}
ANOVA	P≤0.01	P≤0.01	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.05	P≤0.05
PGRs								
C	1.28^{ab}	1.84^{b}	1.64^{a}	0.50^{a}	79.9^{a}	77.9^{a}	–0.26^{a}	–0.22^{a}
JA	0.74^{b}	2.32^{a}	1.46^{b}	0.30^{c}	80.8^{a}	78.4^{a}	–0.22^{a}	–0.18^{a}
BRs	1.59^{a}	2.03^{ab}	0.95^{c}	0.42^{b}	82.8^{a}	81.4^{a}	–0.29^{a}	–0.24^{a}
Put	1.09^{ab}	1.30^{c}	1.45^{b}	0.54^{a}	83.0^{a}	80.7^{a}	–0.24^{a}	–0.19^{a}
ANOVA	P≤0.05	P≤0.05	P≤0.01	P≤0.05	ns	ns	ns	ns
Drought×PGRs	ns	ns	ns	ns	ns	ns	ns	ns

RWC, relative water content; OP, osmotic potential; PGRs, plant growth regulators; C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine; ns, not significant. $^{a-c}$Numbers followed by different letter within each column in a set are significantly different at P≤0.05 by the least square means test.

Table 3. Effect of drought stress and application of exogenous plant growth regulators on relative water content, net photosynthetic rate (µmol CO_2 m/s) and transpiration (mmol H_2O/m/s) of fennel leaves during the vegetative and flowering stage.

Treatments	SPAD		P_N		E	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
Drought stress						
Non	41.31^{a}	18.30^{a}	10.64^{a}	9.11^{a}	1.13^{a}	0.91^{a}
Moder^{a}te	42.85^{a}	17.81^{a}	10.21^{a}	9.70^{a}	1.02^{a}	0.86^{a}
Severe	32.41^{b}	16.78^{a}	8.14^{b}	7.73^{b}	0.57^{b}	0.49^{b}
ANOVA	P≤0.01	ns	P≤0.01	P≤0.01	P≤0.01	P≤0.01
PGRs						
C	43.88^{a}	22.88^{a}	7.91^{b}	7.51^{b}	0.63^{c}	0.63^{b}
JA	46.42^{a}	19.40^{a}	10.61^{a}	10.07^{a}	0.99^{ab}	0.84^{a}
BRs	33.82^{b}	13.92^{a}	9.73^{a}	9.24^{a}	0.88^{b}	0.85^{a}
Put	31.30^{b}	14.32^{a}	10.42^{a}	9.09^{a}	1.12^{a}	0.95^{a}
ANOVA	P≤0.01	ns	P≤0.01	P≤0.01	P≤0.05	P≤0.05
Drought×PGRs	ns	ns	P≤0.05	ns	ns	ns

SPAD, relative water content; PN, net photosynthetic rate; E, transpiration; RWC, relative water content; OP, osmotic potential; PGRs, plant growth regulators; C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine; ns, not significant. $^{a-c}$Numbers followed by different letter within each column in a set are significantly different at P≤0.05 by the least square means test.

tive and flowering stages, respectively. According to Oukarroum *et al*. (2007), elevation of V_k and W_k are the indication of failure in water splitting in PSII. The V_J and V_I parameters (acceptor sides) also increased by about 16% and 22%, respectively when drought stress imposed at the vegetative phase and to 19% and 30%, when drought stress occurred during reproductive phase (Table 4).

Despite statistically significant, we did not detect a dramatic change in donor and acceptor sites of electron transport system in response to the hormonal application (Table 4). The use of PGRs at both stages of growth enhanced donor and acceptor sites. Reduction of V_J, V_I, V_k, and W_k in hormone-treated plants suggests that the use of PGRs performed some degree of recovery of damages caused by drought stress. Among the PGRs, JA was the most active hormone on these parameters at both stages of growth. Plants treated with JA at the vegetative stage demonstrated a reduction in W_k, V_J, and V_I by approximately 17%, 16%, and 26%, respectively, and 24%, 8%, and 15% when used during flowering stage (Table 4).

The imposed severe drought stress, and to a lesser degree moderate stress, interrupted the defence mechanisms of fennel plants against ROS damages, mainly due to the excess excitation energy and negatively altered chlorophyll a fluorescence. Zivcak *et al*. (2014) reported that drought stress negatively influenced the linear electron transport (LET) and electron transport system. The increased donor and acceptor sides in stressed plants could have been due to termination of the oxygen-evolving complex (OEC) at

Table 4. Effect of drought stress and application exogenous plant growth regulator in relative variable fluorescence at 2 ms, relative variable fluorescence at 30 ms, relative variable fluorescence at phase K, and represent the damage to oxygen evolving complex OEC, of fennel leaves during the vegetative and flowering stage.

Treatments	V_J		V_I		V_k		W_k	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
Drought stress								
Non	0.56^{b}	0.64^{b}	0.99^{c}	1.10^{c}	0.14^{b}	0.15^{b}	0.39^{b}	0.41^{b}
Moderate	0.59^{a}	0.72^{ab}	1.15^{a}	1.23^{b}	0.16^{b}	0.17^{b}	0.41^{b}	0.46^{b}
Severe	0.65^{a}	0.76^{a}	1.21^{a}	1.43^{a}	0.20^{a}	0.20^{a}	0.48^{a}	0.52^{a}
ANOVA	P≤0.05	P≤0.05	P≤0.01	P≤0.05	P≤0.01	P≤0.05	P≤0.05	P≤0.05
PGRs								
C	0.67^{a}	0.77^{a}	1.24^{a}	1.37^{a}	0.20^{a}	0.19^{a}	0.47^{a}	0.55^{a}
JA	0.56^{b}	0.71^{a}	0.92^{b}	1.17^{b}	0.14^{a}	0.15^{a}	0.39^{b}	0.42^{b}
BRs	0.62^{ab}	0.66^{a}	1.19^{a}	1.16^{b}	0.15^{a}	0.16^{a}	0.40^{b}	0.42^{b}
Put	0.56^{b}	0.69^{a}	1.12^{a}	1.29^{a}	0.17^{a}	0.18^{a}	0.45^{a}	0.46^{b}
ANOVA	P≤0.05	ns	P≤0.01	P≤0.05	ns	ns	P≤0.05	P≤0.05
Drought×PGRs	ns	ns	ns	ns	ns	ns	ns	ns

VJ, relative variable fluorescence at 2 ms; VI, relative variable fluorescence at 30 ms; Vk, relative variable fluorescence at phase K; Wk, represent the damage to oxygen evolving complex OEC; PGRs, plant growth regulators; C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine; ns, not significant. ^{a,c}Numbers followed by different letter within each column in a set are significantly different at P≤0.05 by the least square means test.

Table 5. Effect of drought stress and application exogenous plant growth regulator in the maximum rate of QA reduction, the maximum quantum yield of primary photochemistry, the quantum yield of electron transport, and thermal dissipation quantum yield of fennel leaves during the vegetative and flowering stage.

Treatments	Mo		TR_0/ABS		ET_0/ABS		D_0/ABS	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
Drought stress								
Non	1.06^{a}	1.27^{a}	0.77^{a}	0.76^{a}	0.44^{a}	0.36^{a}	0.71^{a}	0.51^{a}
Moderate	1.02^{a}	1.15^{a}	0.79^{a}	0.75^{a}	0.41^{a}	0.28^{b}	0.66^{a}	0.40^{b}
Severe	1.00^{a}	0.99^{b}	0.71^{b}	0.72^{b}	0.35^{a}	0.24^{b}	0.51^{b}	0.34^{b}
ANOVA	ns	P≤0.05	P≤0.05	P≤0.05	ns	P≤0.05	P≤0.01	P≤0.05
PGRs								
C	0.99^{a}	0.99^{c}	0.77^{a}	0.72^{b}	0.33^{b}	0.23^{b}	0.53^{b}	0.31^{b}
JA	1.07^{a}	1.20^{b}	0.78^{a}	0.77^{a}	0.38^{b}	0.31^{ab}	0.57^{b}	0.42^{a}
BRs	0.99^{a}	1.32^{a}	0.79^{a}	0.76^{a}	0.44^{a}	0.34^{a}	0.72^{a}	0.48^{a}
Put	1.06^{a}	1.04^{c}	0.77^{a}	0.72^{b}	0.44^{a}	0.29^{ab}	0.70^{a}	0.46^{a}
ANOVA	ns	P≤0.05	ns	P≤0.05	P≤0.05	P≤0.01	P≤0.05	P≤0.01
Drought×PGRs	ns	ns	ns	ns	ns	ns	ns	ns

Mo, maximum rate of QA reduction; TR0/ABS, the maximum quantum yield of primary photochemistry; ET_0/ABS, the quantum yield of electron transport; D_0/ABS, thermal dissipation quantum yield; PGRs, plant growth regulators; C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine; ns, not significant. $^{a-c}$Numbers followed by different letter within each column in a set are significantly different at P≤0.05 by the least square means test.

the donor side of the PSII, followed by the accumulation of reduced QA and plastoquinone due to inhibited reduction reaction at the acceptor side of PSI (Yan *et al.,* 2013; Kalaji *et al.,* 2014).

It has been suggested that Mo index reflects the maximum rate of Q_A reduction (Bussotti, 2004; Wang *et al.,* 2017). Therefore, Mo can be used to quantify the alterations of foliar PSI under drought stress. In the current study, the reduction of M_O was significant when drought stress imposed during flowering (Table 5). ET_0/ABS and TR_0/ABS are known as indices of the electron transport efficiency and maximum quantum yield of primary photochemistry (Bussotti, 2004). Our results revealed that drought stress reduced both indices by about 7% and 20% during the vegetative stage and 7% and 31% during flowering, respectively (Table 5). D_0/ABS that expresses the thermal dissipation quantum yield remained relatively unchanged under moderate stress condition but decreased as much as 39% and 33% when severe drought stress occurred in vegetative and reproductive stages, respectively (Table 5).

Overall, application of PGRs especially BRs, exhibited some improvement in quantum efficiency parameters, more so during reproductive stage (Table 5). For example, application of BRs increased M_0, TR_0/ABS, ET_0/ABS, and D_0/ABS by 34.5%, 6.5%, 48.0%, and 35.8% during flowering (Table 5).

As presented in Figure 1, the fluorescence transient parameters derived from the JIP test were implemented to summarise the impacts of drought stress and application of PGRs on the PSII activity. The graph demonstrated that the drought stress increased

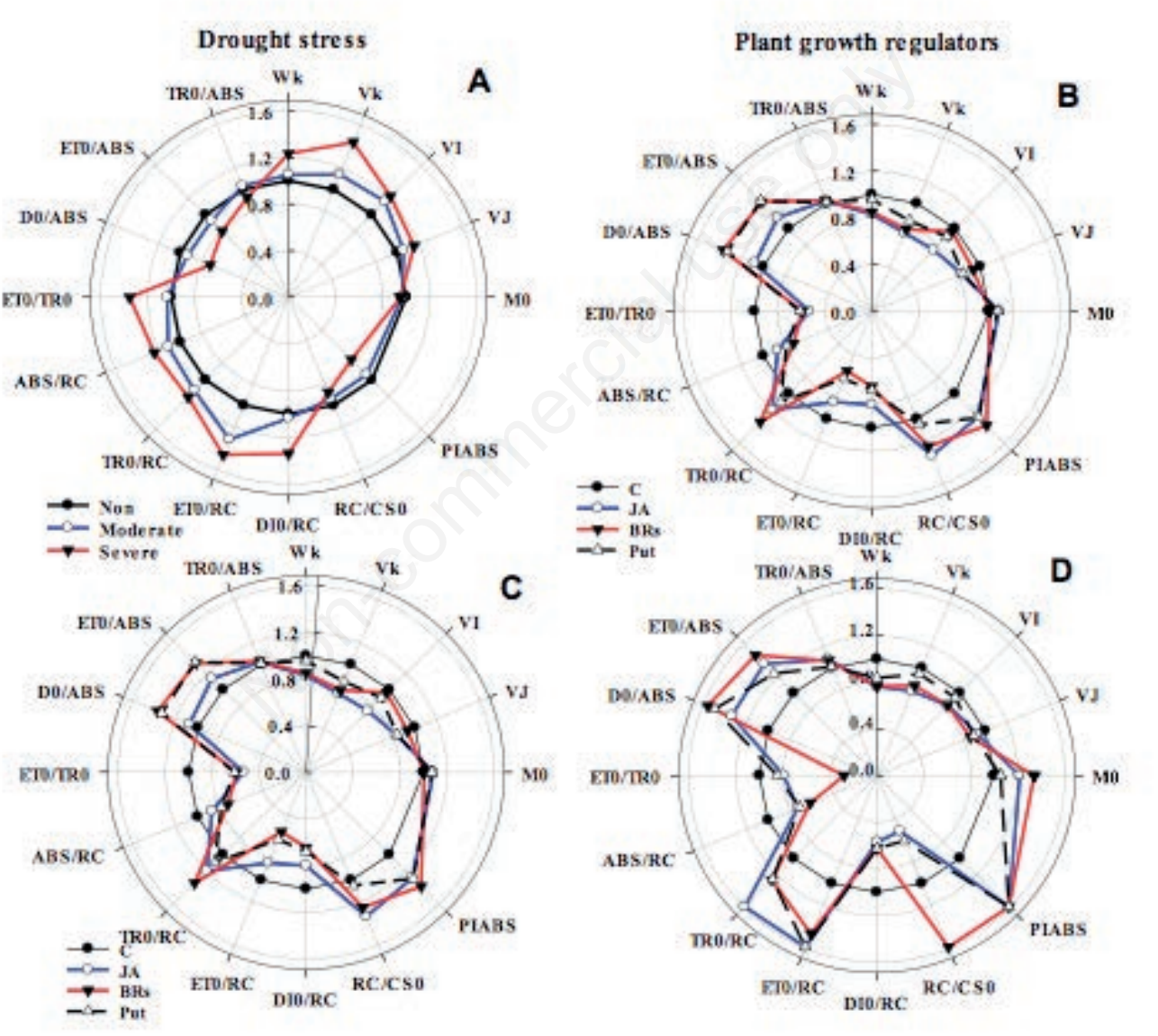

Figure 1. ***Spider plots*** **of select fluorescence transient parameters characterising the behaviour of photoinhibition of photosystem II of fennel leaves. Drought stress during the vegetative stages (A). Exogenous plant growth regulators during the vegetative stages (B). Drought stress during flowering stages (C). Exogenous plant growth regulators during flowering stages (D). All values are shown as percentage of control. C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine.**

the specific energy dissipation (DI_0/RC), a gradually amplified increase in ET_0/TR_0, and a decrease in D_0/ABS. Among the investigated flux ratios parameters, ET_0/TR_0 modified considerably, and V_J and V_I increased under drought conditions. However, the application of JA, Put and BRs resulted in reduced DI_0/RC and an increase in ET_0/TR_0 and D_0/ABS (Figure 1).

It seems that decline in RC/CS_0 due to a stressful condition results in falling back of other phenomenological energy fluxes including ABS/RC, TR_0/RC, DR_0/RC, and ET_0/RC. Guan *et al.* (2015) concluded that drought stress resulted in the reduction of many photosynthetic parameters including LHCII, which may interrupt the normal function of PSII, indicated by TR_0/ABS. It is appropriate to refer to some earlier reports including Moshelion *et al.* (2015) and Wang *et al.* (2017), to explain photosynthesis alterations caused by drought stress, who indicated that the fluorescence emission at O phases (F_0) might be due to increment in the proportion of non-Q_B-reducing centres. Drought may interrupt RCs function (RC/CS_0) which may reduce the potential of electron transport beyond QA (ET_0/TR_0), compared with non-stressed plants. In the current study, higher reduction of RC/CS_0 detected when drought stress imposed during the flowering stage, which attributed to the decline in ABS/RC (Figure 1). Reduction of ABS/RC in plants grown under drought stress condition is related, at least to some extent, to deactivation of repair mechanisms in PSII or changes in energy cycle components of RCs (Szabó *et al.,* 2005; Ghotbi-Ravandi *et al.,* 2014). Drought stress imposed at both, vegetative and flowering stages led to significant reduction of the photosynthetic performance index on absorption basis (PI_{abs}) (Figure 1), while application of PGRs, especially BRs exhibited an improvement in PI_{abs} of fennel leaves (Figure 1). It is suggested that the reduction of PI_{abs}, could be an indication of drought stress condition (Wang *et al.,* 2017). Excess of solar radiation and the decline in carbon assimilation due to the stomatal closure may lower the chlorophyll fluorescence under drought stress (Farooq *et al.,* 2012).

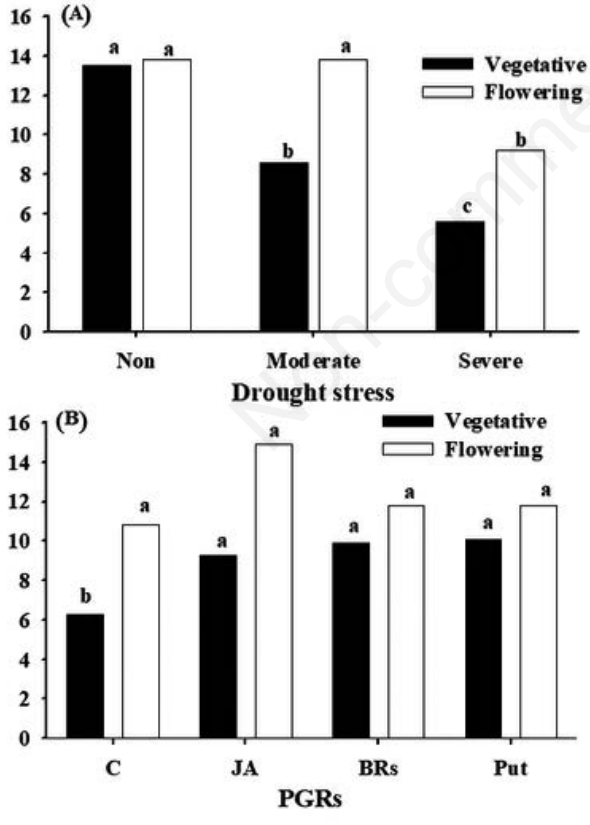

Figure 2. Effect drought stress on during the vegetative and flowering stage in biomass (A) and exogenous plant growth regulators during the vegetative and flowering stage in biomass (B) of fennel. All values are shown as percentage of control. C, control; JA, metel jasmonate; BRs, 24-epbrasinostroid; Put, putrescine.

Biomass

Similar to other medicinal plants (Omobolanle Ade-Ademilua *et al*., 2013; Bahreininejad *et al*., 2014), drought stress resulted in a dramatic reduction in biomass yield. Biomass yield of the stressed plants reduced as much as 37% and 60% when plants experienced mild and severe drought stress, respectively (Figure 2A). However, the negative impact of drought stress that imposed later during reproductive stage was less severe compared with the vegetative stage. Severe drought stress during the reproductive phase reduced the biological yield by 34%. All three PGRs stimulated biomass production at both stages of growth. However, application of PGRs during vegetative stage was more influential and on average, plants yielded roughly 1.6 fold higher than the control plants (Figure 2B).

Conclusions

The present study provided some evidence that the photosynthesis system, thus the biomass production of fennel exhibited acceptable tolerance to mild drought stress. However, when stress condition intensified, almost all photosynthetic parameters impaired. Our findings confirmed that the application of plant growth regulators, especially when used during vegetative stage of growth, enhanced almost all photosynthetic parameters. Consequently, the biomass production of fennel improved. We found no significant interactions between drought stress and PGRs and concluded that the enhancement effects of PGRs were mainly independent of drought stress condition.

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