Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2018, 10(4):508-515. DOI: 10.25835/nsb10410356
Original Article

Effect of Exogenous Application of Several Plant Growth Regulators on Photosynthetic Pigments of Fennel Plants

Ghasem PARMOON[1]*, Ali EBADI[1], Soodabe JAHANBAKHSH[1], Masoud HASHEMI[2], Seyed Amir MOOSAVI[3]

[1]*University of Mohaghegh Ardabili, Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, Ardabil, Iran; ghasem.parmoon@gmail.com (*corresponding author); ebadi@uma.ac.ir, Jahanbakhsh@uma.ac.ir*

[2]*University of Massachusetts Amherst, Stockbridge School of Agriculture, Massachusetts, USA; masoud@umass.edu*

[3]*Agricultural Sciences and Natural Resources University of Khuzestan, Department of Plant production and genetics, Faculty of Agriculture, Khuzestan, Iran; amir.msa@gmail.com; amirmoosavi@ramin.ac.ir*

Abstract

In order to investigate the effects of some plant growth regulators on photosynthetic pigments and growth of fennel plants, a greenhouse experiment was conducted based on the randomized complete block design with three replicates in 2017. Treatments were the application of methyl jasmonate (25, 50, 100 and 200 µM), putrescine (0.25, 0.5, 1 and 2 mM) and 24-Epibrassinolide at 0.001, 0.01, 0.1 and 1 µM and distilled water as a control. The results indicated that application of 0.5 Mm putrescine, exhibited significant effects on the chlorophyll a (62%), b (104%), total chlorophyll (72%), carotenoids (51%), flavonoids (51%), anthocyanin content (-14%), phenolic compounds (13%) and maximum quantum efficiency (17%) in dark condition and in light condition. Application of 24-Epibrassinolide resulted in a significant increase of chlorophyll a and total chlorophyll, carotenoids, phenol content, maximum quantum efficiency in the dark condition and photochemical quenching of fluorescence. The highest chlorophyll content and carotenoids were observed in treated plants with 0.1 µM 24-Epibrassinolide, while the maximum phenol content was obtained by application of 0.01 µM 24-Epibrassinolide. The application of methyl jasmonate significantly affected the major chlorophyll and accessory pigments (except phenol) of fennel. Plants treated with 50 µM methyl jasmonate exhibited higher concentrations of chlorophyll a (3.25 mg per g FW^{-1}), total chlorophyll (4.35 mg per g FW^{-1}), carotenoids (0.87 mg per g FW^{-1}) and flavonoids (4.75 µg per g FW^{-1}). A significant dry weight increased after the application of methyl jasmonate and it can be concluded that the most effective treatment in this regard for fennel plants was 50 µM methyl jasmonate.

Keywords: anthocyanin; chlorophyll; fennel; jasmonate; phytohormone

Introduction

Foeniculum vulgare Mill or fennel is an important medicinal plant belongs to the *Apiaceae* family. Fennel is originated from the Mediterranean area and widely been cultivated around the world (Mahfouz and Sharaf-Eldin, 2007; Aprotosoaie *et al*., 2010). Fruits and essential oil of fennel are used as flavouring agents in food, pharmaceutical products and cosmetic industry (Diao *et al*., 2014). Essential oil constituent of fennel contained mainly: estragole, trans-anethole, cis-anethole, fenchone and limonene (Díaz-Maroto *et al*., 2006; Rather *et al*., 2016). Fennel is recognized as well as an important plant in pharmaceutical and used as a stimulant, diuretic, sedative, digestive, carminative, antispasmodic and expectorant (Hashmi *et al*., 2012).

Application of plant growth regulators (PGRs) is a management method in modern agriculture and production systems, which have significantly enhance the agricultural inputs use efficiency (Berry *et al*., 2007). The PGRs could regulate many of the physiologic processes in plants, furthermore, enhance resistance to various environmental stresses (Akram and Ashraf, 2013; Asgher *et al*., 2015). Methyl jasmonate (MJs), 24-epibrassinolide (BRs) and Putrescine (Put) are PGRs that in the last years have been successfully incorporated into crop production (Eyidogan *et al*., 2012) of cucumber (Shu *et al*., 2012), soybean (Cevahir *et al*., 2008), Broad bean (Piñol and Simón 2009), and Runner bean (Hanaka *et al*., 2015).

Polyamines (PAs) are small aliphatic amines, which behaved as a promoter of many plant physiological processes

Received: 31 Aug 2018. Received in revised form: 10 Dec 2018. Accepted: 14 Dec 2018. Published online: 21 Dec 2018.

such as cell division and tissue growth and development (Hasanuzzaman *et al*., 2014). Currently, putrescine, spermidine, and spermine are most well-known PAs. These compounds are organic polycations and exist in all organs of the plant, which have been associated with plant response to environmental stress (Alcázar *et al*., 2010). The PAs have substantial roles in DNA, RNA and protein synthesis (Igarashi and Kashiwagi, 2000). The PAs affect different plant developmental processes. For instance, Mahros *et al.* (2011), reported that the application of Put led to decline of pedicle and flower stalk length. However, Put significantly increased flower yield, chlorophyll a, b and the total carbohydrates. Likewise, photosynthetic pigments of African violet (*Saintpaulia ionantha*) were significantly increased by application of putrescine (Nanvakenary *et al*., 2013). In cucumber, exogenous application spermidine significantly increased chlorophyll contents and net photosynthetic rate (P_N) and decreased constitutive loss processes (ΦNO) and enhanced regulated non-photochemical energy loss (ΦNPQ) in the salt-stressed plants (Shu *et al*., 2012).

The MJs are found naturally in plants and they are responsible for regulation of many physiological and metabolic processes. Application of MJs improved plant resistance to various abiotic stresses (Bari and Jones, 2009). The MJs may influence Rubisco activity as well as the synthesis of other chloroplast proteins responsible for photosynthesis (Marín-Navarro *et al*., 2007). It was reported that MJs treatment of soybean appeared to arrest the growth, impaired leaf gas-exchange attributes and caused the loss of chlorophyll contents under water deficit conditions (Anjum *et al*., 2011). In another experiment, MJs increased leaf area, photosynthetic pigments and decreased chlorophyll fluorescence in *Phaseolus coccineus* plants (Hanaka *et al*., 2015).

The BRs have been recognized as polyhydroxysteroids, which play a fundamental role in many of the physiological and developmental processes of plants. It is reported that the BRs regulate seed germination, time of flowering, and maturation (Vardhini *et al*., 2006; Tang *et al*., 2016), Moreover, they are one of determining factor which confer plants resistance to environmental stresses (Fariduddin *et al*., 2014). Furthermore, BRs plays important roles in cellular responses such as stem elongation, pollen tube growth, leaf bending and epinasty, ethylene biosynthesis, xylem differentiation, and regulation of gene expression (Houimli *et al*., 2008; Hayat *et al*., 2012). Cevahir *et al*. (2008) reported that the application of exogenous BRs significantly affected the production of plant pigments such as chlorophyll, carotenoids, and anthocyanin in soybean. However, this effect was depended on the light condition.

Application of various plant growth regulators for modifications of plant physiological responses and alleviate abiotic stresses damages to plants has been well documented (Gururani *et al*., 2015; Mo *et al*., 2016; Awan *et al*., 2017). However, the time of PGRs application and eventual impact on photosynthesis behaviours of fennel plants is still elusive. This study was therefore conducted to study the photosynthesis potential and relative performance of fennel pigments obtained from PGRs treated plants.

Materials and Methods

Plant material and growth condition

A greenhouse experiment was conducted at the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran, in 2017.

Greenhouse condition was related to a day temperature of 24 ± 2 °C, a night temperature of 16 ± 2 °C and a relative humidity of 50 ± 5%.

Each pot was filled with 20 kg sieved field soil. A soil sample taken prior to planting indicated soil pH (1:1, soil/H_2O) was 7.9, cation exchange capacity (CEC) was 0.625 ds/m, and available P, K were 8.5 and 0.6 mg/kg, respectively. Ten seeds were planted in each pot and then thinned to five seedlings per pot once plants were well-established.

Treatments were arranged as a randomized complete block design with three replicates. Treatments application MJs include 25, 50, 100 and 200 µM, Put include 0.25, 0.5, 1 and 2 mM and BRs at 0.001, 0.01, 0.1 and 1 µM and distilled water as control were randomly assigned to the experimental pots. Treatments were imposed prior to the before flowering stage (65 days after planting) and 10 days after treatments imposed sampling for measured all parameters.

Determination of photosynthetic pigment

Chlorophyll a, b, total chlorophyll and carotenoids content were measured using the method described by Arnon (1967). For the extraction of pigments, 0.1 g of fresh tissue leaves were homogenized with acetone 80%, then the volume of the solution was reached to 20 ml by acetone 80%. Final solution centrifuged at 4,000 rpm at 10 min and optical absorption of the supernatant was performed in wavelengths 470, 645 and 663 nm. Chlorophyll content and carotenoids according to mg per g fresh weight were determined by following equations:

Chlorophyll a = $(19.3\times A_{663}-0.86\times A_{645})$ V/100W

Chlorophyll b = $(19.3\times A_{645}-3.6\times A_{663})$ V/100W

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoids = $(1000A_{470}-1.82\times Ca-85.02\times Cb)$ / 198

Determination of anthocyanin and total phenol

For detriment anthocyanin content of fresh tissue, 0.1 g of leaves was homogenized with 10 ml ethanol acid (ethanol: HCl 99:1 v/v) and the solution was kept at in dark and 25 °C condition. Then solution centrifuged in 4,000 rpm at 10 min and absorbance was measured in wavelengths 550 nm. Anthocyanin content according to mg /g fresh weight (Wagner, 1979).

Total phenol content determined using Folin-Ciocalteu method described by Fletcher and Kott (1999). In brief, 0.1 g of leaves was homogenized with 10 ml ethanol 96%, then crude extract was allowed to stand for a further 24 hours in the dark. In finally, 1 ml ethanol 95%, 0.5 ml folin 50% and 1 ml sodium carbonate 5% addition to extract and the mixture was kept for 1 hour in dark condition. Absorbance was measured at 725 nm and total phenolic was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g fresh weight.

Determination of flavonoids

The flavonoid was determined according to Krizek *et al.* (1998) methods, in order to 0.1 g from leaves extract in ethanol acid (ethanol: glacial acetic 99:1 v/v) and solution centrifuged in 3,600 rpm at 10 min. The supernatant was separate and allowed to stand for a further 10 min 80 °C. Finally, the absorbance was measured in three wavelengths 270, 300 and 330 nm.

Determination of chlorophyll fluorescence

Chlorophyll fluorescence was measured on the uppermost fully expanded leaf by using a fluorometer (model: OS-30P+ chlorophyll Fluorometer, Optic Science, USA). In this method, plants were adapted to darkness using dark adaptation clip for 15 minutes and then the fluorescence was measured in 1,000 µM photon $m^{-2}s^{-1}$ by illumination of far-red light and calculated using the following equations (Arnon, 1949): In these equations, F′v/F′m is maximum quantum efficiency of PSII photochemistry in the light-adapted state, PSII equivalent maximum quantum efficiency of PSII photochemistry in the dark-adapted state, qP or photochemical quenching of fluorescence and NPQ is non-photochemical quenching.

F′v /F′m = (F′m –F′o)/Fm′

PSII = (F′m – Fs)/Fm′

qP = (F′m – Fs)/F′m – Fo′

NPQ = (Fm – F′m)/F′m

F_m (maximum fluorescence) after a saturated light pulse on plants adapted to darkness, and F_0 (minimum fluorescence) when all PSII reaction centers (RCs) open. F'_m and F'_o also maximum and minimum fluorescence on plants adapted to light.

Statistical analysis

Analysis of variance (ANOVA) was performed using Minitab, Ver, 16. The least significant difference (LSD) was used for mean separation and data is shown as a mean ± standard error (SE). Sigma plot v. 11 was used to calculate the regression equation. The reliability of models was evaluated using the coefficient of determination (R^2) and root-mean-square error (RMSE). In the present study, the parameters fitted by recon models (Table 1).

Results and Discussion

Effect of Put application on the fennel pigments and photosynthetic parameters

The results of the present study clearly showed that there was a significant effect of Put application on the chlorophyll a, b and total chlorophyll content of fennel leaves (Table 2). The Put application resulted in enhancement of chlorophyll content, which followed the Gaussian function. Leaves of treated plants with 0.5 mM Put showed the highest chlorophyll a, b and total content. Application of 0.5 mM Put increased chlorophyll a, b and total chlorophyll content by approximately 63%, 105%, and 73% respectively compared with control. Model analysis revealed that the maximum chlorophyll a, b and total chlorophyll were estimated by 0.65, 0.72 and 0.68 mM put respectively (Table 2).

Accessory pigments including carotenoids, anthocyanin, flavonoids and phenol content were significantly affected by Put treatments (Table 2). Results showed that the changes of anthocyanin and phenol due to the application of Put is followed a Gaussian function while other carotenoids and flavonoids were following log-normal function. The highest carotenoids content (0.82 mg per FW^{-1}) and flavonoids (5.29 ug per FW^{-1}) content was recorded at 0.5 mM Put, which approximately showed an enhancement about 50% and 51% over control. The maximum anthocyanin (11% higher than control) and phenol content (16% higher than control) was an observation of 0.25 and 1 mM Put respectively (Table 2).

The present results are in agreement with the results of previous studies which reported that there was a significant increase in total carbohydrates, chlorophyll a, b, carotenoids and P_N in *Chrysanthemum indicum* and cucumber (Mahros *et al*., 2011; Shu *et al*., 2012). The Put might be involved in improving heat dissipation capacity and regulating the de-epoxidation state of the xanthophyll cycle (Yuan *et al*., 2015). In addition, Put may improve the photosynthetic capacity of mesophyll cells by enhancing the carbon assimilation capacity (Araújo *et al*., 2011; Yuan *et al*., 2014).

Results of the present study showed that the application Put significantly affected the two parameters of chlorophyll fluorescence including PSII and F'v/F'm (Table 2). PSII and F'v/F'm in leaves of Put treated plants were higher than those of control plants. Maximum of PSII and F'v/F'm by change approximately 24% and 17% compared to control, were recorded when plants treated with 0.5 mM of Put. Log normal and Gaussian functions were provided the best fit to data obtained from PSII F'v/F'm traits of Put treated plants, respectively (Table 2).

Application of Put was not significant for fluorescence parameters of qP, and F_v/F_0. Moreover, plant height and total dry weight were not affecting by the Put application (Table 2). Application of Put declined the ΦNO and enhanced regulated ΦNPQ in stressed plants (Nanvakenary *et al*., 2013). Some studies revealed that Put plays a role in protecting PSII against excessive energy through improving the thermal dissipation of the excitation energy (Rascher *et al*., 2000). The quantum functions of fennel leaves were significantly improved by Put application. The Put may be involved in alleviating the photoinhibition of UPSII and enhancing the photochemical quenching process (Yuan *et al*., 2014).

In addition, PAs can neutralize the negative charges of LHCII that, it is a putative site of qE, a rapid phase of NPQ, so that repulsion between different complexes is minimized (Navakoudis *et al*., 2007).

Table 1. Models used to fit parameters measured of fennel

Model	Formula	Parameters
Gaussian	$Y= y_0+ a\times\exp(-.5\times((x- x_0)/ b)^2)$	a or upper asymptote, b was a slope, x_0 Critical point or the x that reached of a and y_0 is a lower asymptote
Log Normal	$Y= y_0+a\times\exp(-0.5\times(\ln(x/ x_0)/ b)^2)/x)$	
Single	$Y= y_0 + a\times\exp(b\times x)$	
Cubic	$Y= y_0 + c\times x+ b\times x^2+a\times x^3$	y_0 is a lower asymptote, a, b and c are slope

Table 2. Effect application of Put concentration on photosynthetic pigments, chlorophyll fluorescence parameters and biomass of fennel (*Foeniculum vulgar* Mill) leaves

Characteristic		Put concentration (mM) 0	0.25	0.5	1	2	F value	Estimated function	R^2	RMSE
Major Chlorophyll										
†Chlorophyll a	means	1.70±0.32	1.99±0.35	2.76±0.58	2.18±0.58	1.49±0.64	10.9**	Y= 1.53+ 1.41×exp(-.5×((x- 0.65)/ 0.27)^2)	0.989	0.10
	%	-	16.9	62.5	28.0	-12.5				
†Chlorophyll b	means	0.53±0.19	0.58±0.21	1.08±0.05	0.92±0.17	0.57±0.12	4.88**	Y= 0.54+ 1.20×exp(-.5×((x- 0.72)/ 0.17)^2)	0.996	0.028
	%	-	10.2	104.6	73.7	7.6				
†Total chlorophyll	means	2.23±0.48	2.57±0.53	3.85±0.54	3.10±0.76	2.06±0.72	14.3**	Y=2.11+ 2.29×exp(-.5×((x- 0.68)/ 0.24)^2)	0.996	0.092
	%	-	15.3	72.5	38.9	-7.8				
Accessory Pigments										
†Carotenoids	means	0.55±0.08	0.61±0.09	0.82±0.06	0.82±0.05	0.71±0.06	18.7**	Y=0.54+0.34×exp(-0.5×(ln(x/1.43)/ 0.75)^2)/x)	0.963	0.047
	%	-	11.1	50.8	50.2	30.1				
†Anthocyanin	means	0.55±0.08	0.61±0.09	0.47±0.11	0.39±0.05	0.19±0.06	12.3*	Y= 0.16+ 0.40×exp(-.5×((x- 0.05)/ 0.84)^2)	0.949	0.073
	%	-	11.4	-14.2	-28.2	-65.3				
††Flavonoids	means	3.49±0.51	3.88±0.56	5.29±0.11	4.85±0.25	4.30±0.31	6.13*	Y= 3.49+1.62×exp(-0.5×(ln(x/ 1.17)/ 0.70)^2)/x)	0.912	0.42
	%	-	11.0	51.4	38.8	23.0				
†Phenol	means	1.02±0.04	1.13±0.04	1.14±0.02	1.18±0.01	1.09±0.01	6.39**	Y= 1.53+ 1.41×exp(-.5×((x- 0.65)/ 0.27)^2)	0.903	0.038
	%	-	11.1	12.8	16.3	7.5				
Chlorophyll fluorescence:										
PSII	means	0.723±0.04	0.903±0.06	0.893±0.01	0.793±0.00	0.787±0.00	5.51*	Y=0.72+0.090×exp(-0.5×(ln(x/1.06)/ 1.22)^2)/x)	0.944	0.036
	%	-	24.90	23.51	9.68	8.85				
qP	means	0.903±0.05	0.940±0.01	1.073±0.06	0.917±0.02	0.910±0.05	2.35ns	Y= 0.90+0.18×exp(-.5×((x-0.57)/ 0.177)^2)	0.998	0.005
	%	-	4.10	18.83	1.55	0.78				
NPQ	means	0.310±0.11	0.277±0.10	0.277±0.04	0.333±0.15	0.373±0.19	0.19ns	Y= 0.310-0.214 ×x+ 0.351 ×x^2 -0.114×x^3	1.000	0.00
	%	-	-10.8	-10.8	7.5	20.4				
F'v/F'm	means	0.827±0.01	0.847±0.02	0.967±0.06	0.853±0.01	0.840±0.00	5.05*	Y =0.83+0.187×exp(-.5×((x-0.63)/ 0.169)^2)	0.993	0.0093
	%		2.42	16.93	3.14	1.57				
Fv/F0	means	2.46±0.47	2.70±0.05	2.71±0.42	3.52±1.72	3.24±2.14	1.46ns	Y =2.49+1.45×exp(-.5×((x-1.42)/ 0.49)^2)	0.975	0.136
	%	-	9.76	10.16	43.09	31.71				
Growth plant										
†††Height plants	means	52.73±5.43	54.20±5.70	55.80±5.57	51.60±1.51	52.40±3.03	0.57ns	Y=52.13+3.76×exp(-.5×((x-0.44)/ 0.18)^2)	0.949	0.751
	%	-	2.8	5.8	-2.1	-0.6				
†Dry weight plants	means	7.21±0.68	7.40±0.68	8.38±1.23	7.88±1.26	6.68±0.64	1.28ns	Y=6.77+1.77×exp(-.5×((x-0.67)/ 0.33)^2)	0.951	0.285
	%	-	2.7	16.2	9.3	-7.3				

† mg per g FW^{-1}, †† µg per g FW^{-1} and ††† cm.

Effect of BRs application on the fennel pigments and photosynthetic parameters

Application of BRs exhibited a significant increase of chlorophyll a and total chlorophyll content compared to control while there was no significant effect on the chlorophyll b content (Table 3). Results showed that the highest chlorophyll content by approximately 133% and 154% of control treatment, obtained by the exogenous application of 0.1 µM BRs on fennel plants. The Log-normal function was capable to provide the best fit for the variations of chlorophyll content in treated plants. The slope of changes in chlorophyll a and total chlorophyll concentrations were estimated approximately 2.38 and 3.09 respectively (Table 3).

Among accessory pigments, only carotenoids and phenol content were influenced by the application of BRs. The accessory pigments of leaves were significantly improved by application of 0.1 and 0.01 µM BRs and the carotenoids and phenol contents increased about 43% and 11% of control treatments, respectively (Table 3). Furthermore, it was found that BRs improved chlorophyll fluorescence parameters in fennel leaves. Results showed that PSII and qP were significantly increased in BRs treated plants. The Log normal function provided the best fit for the PSII and qP parameters. Plants treated with 0.01 and 0.1 µM Put exhibited higher chlorophyll fluorescence parameters such as PSII and qP, which were increased 25% and 11% higher than a control treatment (Table 3).

Although application of BRs did not significantly affects fennel growth, the overall trend of dry mater accumulation was followed a log-normal function. There are some reports showing that the application of BRs increases the chlorophyll, carotenoids and anthocyanin production in soybean (Cevahir *et al*., 2008). It has been reported that the exogenous application of BRs increased the pigment content in response to NaCl stress in some plants (Anuradha and Rao, 2003; Cevahir *et al*., 2008; Anuradha and RAO, 2011). Bajguz (2000) suggested that the BRs induced transcription and/or translation of the enzymes involved in chlorophyll biosynthesis linked with a decrease in the level of catabolizing enzymes. Piñol and Simón (2009) reported that the application of BRs led to palliative effects of terbutryn on fluorescence parameters, CO_2 assimilation, and growth *Vicia faba* plants. It is suggested that the application of BRs could affect the terbutryn inhibition of PSII by displacement of QB from its binding site on the D1 protein of PSII. This protein is degraded when the photosynthetic system cannot process the energy of accumulated photons but little is known about the PSII repair process, either at the level of protein synthesis, insertion, and concomitant assembly of the D_1 protein or at later functional post-translational assembly steps (Zhang *et*

al., 2000). Thus, the D_1 protein of PSII must be degraded, resynthesized de novo, and reinserted into the PSII reaction centre to repair the damage and re-establish PSII function (Piñol and Simón, 2009). It is known that BRs could influence the gene expression and protein synthesis. Thus, BRs could be implicated in the control of D_1 damage and repair (Wang *et al*., 2006).

Effect of MJs application on the fennel pigments and photosynthetic parameters

It was cleared that the exogenous application of MJs significantly affected the major chlorophyll and accessory pigments (except phenol) of fennel (Table 4). Lognormal provided the best fit for changes in major chlorophyll, carotenoids and flavonoids, whereas the Gaussian function well applied to describe the changes of anthocyanin in the MJs treated fennel leaves. The result indicated that exogenous MJs affect the chlorophyll concentration in treated plants. The maximum and minimum slope of variations in photosynthetic pigments was obtained 0.78 and 0.53 for chlorophyll a and b, respectively (Table 4). Application of MJs enhanced total concentrations of the chlorophyll and accessory pigments. In comparison with control plants, treated plants with 50 µM MJs exhibited 91%, 95%, 59%, and 36% chlorophyll a, total chlorophyll, carotenoids, and flavonoids, respectively (Table 4).

Marín-Navarro *et al*. (2007) reported that MJs are regulators of rubisco activity as well as the synthesis of other chloroplast proteins responsible in photosynthesis.

Also, MJs treatment appeared to arrest the growth, impaired leaf gas-exchange attributes and caused the loss of chlorophyll contents of soybean plants under water deficit (Anjum *et al*., 2011). It is reported that the MJs increased leaf area, photosynthetic pigments and decreased chlorophyll fluorescence in *Phaseolus coccineus* plants (Hanaka *et al*., 2015). Wierstra and Kloppstech (2000) reported that MJs declined the light-harvesting complex II (LHC II) and small subunit of Rubisco (SSU) transcript and D_1 protein levels. The reduction in D_1 levels of MJs treated leaf-segments might be due in part to the diminished

Table 3. Effect application of BRs concentration on photosynthetic pigments, chlorophyll fluorescence parameters and biomass of fennel (*Foeniculum vulgar* Mill) leaves

Characteristic		BRs concentration (µM) 0	0.001	0.01	0.1	1	F value	Estimated function	R^2	RMSE
Major Chlorophyll										
†Chlorophyll a	means	1.70±0.32	2.49±0.24	2.82±0.11	3.97±0.27	3.04±0.08	11.29**	Y= 1.86+3.55×exp(-0.5×(ln(x/ 31.2)/ 2.38)^2)/x)	0.916	0.47
	%	-	46.5	65.7	133.3	79.0				
†Chlorophyll b	means	0.53±0.19	1.03±0.15	1.20±0.29	1.69±0.42	1.69±0.59	2.80ns	Y =0.66+1.02×x/(0.005+x)	0.913	0.20
	%	-	95.1	126.0	218.5	219.2				
†Total chlorophyll	means	2.23±0.48	3.52±0.19	4.01±0.37	5.65±0.68	4.73±0.66	7.35**	Y=2.33+53.7×exp(-0.5×(ln(x/213)/ 3.09)^2)/x)	0.938	0.64
	%	-	58.0	80.0	153.6	112.2				
Accessory Pigments										
†Carotenoids	means	0.55±0.08	0.40±0.20	0.53±0.17	0.78±0.09	0.62±0.13	4.60*	Y=0.47+0.14×exp(-0.5×(ln(x/1.29)/ 1.44)^2)/x)	0.865	0.10
	%	-	-26.1	-2.9	42.9	13.6				
†Anthocyanin	means	0.55±0.08	0.62±0.12	0.48±0.05	0.29±0.11	0.40±0.03	1.93ns	ns	-	-
	%	-	13.2	-13.0	-47.7	-27.6				
††Flavonoids	means	3.49±0.51	4.42±0.73	4.70±0.55	5.03±0.42	4.98±0.66	1.56ns	Y =3.49+1.44×x/(0.0006+x)	0.976	0.13
	%	-	26.5	34.5	43.9	42.5				
†Phenol	means	1.02±0.04	1.10±0.01	1.13±0.02	1.09±0.03	1.07±0.02	4.71*	ns	-	-
	%	-	8.2	10.8	6.8	15.4				
Chlorophyll fluorescence										
PSII	means	0.723±0.04	0.773±0.01	0.903±0.06	0.790±0.01	0.763±0.02	3.85*	Y=0.74+0.005×exp(-0.5×(ln(x/0.079)/ 1.37)^2)/x)	0.958	0.027
	%	-	6.92	24.90	9.27	5.53				
qP	means	0.903±0.05	0.947±0.05	0.998±0.06	1.003±0.06	0.943±0.10	9.93**	Y= 0.90+0.079×exp(-0.5×(ln(x/17.12)/ 2.5)^2)/x)	0.997	0.004
	%	-	4.87	10.52	11.07	4.43				
NPQ	means	0.310±0.11	0.157±0.10	0.270±0.12	0.387±0.11	0.583±0.26	1.11ns	Y= 0.237+0.405×x/(0.17+x)	0.878	0.0784
	%	-	-49.5	-12.9	24.7	88.2				
F'v/F'm	means	0.827±0.01	0.880±0.04	0.967±0.06	0.800±0.04	0.787±0.04	2.54ns	Y=0.8+0.038×exp(-0.5×(ln(x/0.003)/ 0.38)^2)/x)	0.961	0.028
	%	-	6.41	16.93	-3.26	-4.84				
F_v/F_0	means	2.46±0.47	3.03±0.44	3.30±0.68	3.24±2.14	2.96±0.32	1.61ns	Y= 2.46+11.6×exp(-0.5×(ln(x/76.5)/ 3.5)^2)/x)	0.999	0.015
	%	-	23.17	34.15	31.71	20.33				
Growth plant										
†††Height plants	means	52.73±5.43	53.00±3.67	54.60±5.57	57.40±8.70	52.80±7.45	0.65ns	Y=50.9+1.56×exp(-0.5×(ln(x/0.78)/ 1.56)^2)/x)	0.787	2.82
	%	-	0.5	3.5	8.9	0.1				
†Dry weight plants	means	7.21±0.68	7.50±0.62	8.51±1.21	8.37±0.90	7.35±0.83	0.26ns	Y=7.2+0.191×exp(-0.5×(ln(x/0.59)/ 1.76)^2)/x)	0.998	0.046
	%	-	4.1	18.0	16.0	1.9				

† mg per g FW^{-1}, †† µg per g FW^{-1} and ††† cm.

carotenoids content as β-carotene is required for the assembly of D_1 protein into functional PSII reaction centres. It is suggested that MJs in developing leaves might play a role to prevent the formation of premature photosynthetic structures and accumulation of nutritive storage proteins for further leaf development.

Based on the obtained results, it is noted that among all of chlorophyll fluorescence parameters, only PSII, and qP were significantly affected by MJs (Table 4). The MJs increased these parameters, while the highest amount PSII, and qP by was observed in 50 µM MJs (increased by 24% and 8% compared to control). The log-normal function was appropriate to describe the variation of PSII, and qP in treated plants with MJs (Table 4). Jin *et al.* (2011) showed that the foliar spraying of 0.2 and 0.5 mmol MJs exhibited the positive effects on mitigating the decrease of Fv/Fm, PSII, and qP and the increase of qN under drought stress. It is suggested that foliar application of MJs could alleviate the degradation of chlorophyll and play a definite role in protecting the PSII under drought stress, decrease the damage of drought stress on the seedlings, promote the rapid MJs recovery of chlorophyll fluorescence parameters after re-watering, and thus, ensure the regrowth of flue-cured tobacco seedlings. Wierstra and Kloppstech (2000) stated that the increase of photochemical efficiency of PSII by MJs leads to an equilibrium between D_1 destruction and repair which depends on the light intensity and a restoration phase during which PSII activity is recovered due to the integration of the newly formed D_1 protein.

Application of MJs significantly increased the total dry weight of fennel plants, while this was not significant for plant height (Table 4). Change in plant dry weight was well explained by Gaussian function, with the slop of 17.8 and in 61 µM MJs reached to maximum dry weight content.

Table 4. Effect application of MJs concentration on photosynthetic pigments, chlorophyll fluorescence parameters and biomass of fennel (*Foeniculum vulgar* Mill) leaves

Characteristic		MJs concentration (µM) 0	25	50	100	200	F value	Estimated function	R^2	RMSE
Major Chlorophyll										
†Chlorophyll a	means	1.70±0.32	1.89±0.44	3.25±0.41	2.80±0.52	2.59±0.50	24.53**	Y=1.6+174.7×exp (-0.5× (ln(x/152.)/ 0.78)^2)/x)	0.926	0.34
	%	-	11.0	91.0	64.7	52.5				
†Chlorophyll b	means	0.53±0.19	0.46±0.23	1.10±0.17	1.12±0.20	0.70±0.07	6.92**	Y=0.49+66.9×exp (-0.5×(ln(x/100.7)/ .53)^2)/x)	0.939	0.15
	%	-	-12.5	108.4	110.9	32.8				
†Total chlorophyll	means	2.23±0.48	2.35±0.64	4.35±0.49	3.92±0.72	3.30±0.52	23.1**	Y=2.14+222×exp (-0.5×(ln(x/126)/ 0.69)^2)/x)	0.865	0.68
	%	-	5.3	95.1	75.7	47.9				
Accessory Pigments										
†Carotenoids	means	0.55±0.08	0.59±0.02	0.87±0.05	0.80±0.09	0.54±0.07	4.26*	Y=0.54+28.6×exp (-0.5×(ln(x/80.4)/ 0.45)^2)/x)	0.996	0.017
	%	-	7.5	58.8	45.9	-0.5				
†Anthocyanin	means	0.55±0.08	0.57±0.09	0.49±0.05	0.38±0.09	0.41±0.12	4.03*	Y = 0.39+ 0.18×exp(-.5×((x- 17.8)/ 28.1)^2)	0.984	0.02
	%	-	4.7	-10.9	-30.6	-25.8				
††Flavonoids	means	3.49±0.51	3.57±0.35	4.75±0.42	4.23±0.51	4.09±0.54	3.88*	Y=3.49+100×exp(-0.5×(ln(x/109)/ 0.66)^2)/x)	0.747	0.51
	%	-	2.3	35.9	21.1	17.0				
†Phenol	means	1.02±0.04	1.13±0.05	1.15±0.01	1.15±0.03	1.15±0.04	3.51ns	Y=1.01+0.13×x/(3.99+x)	0.996	0.005
	%	-	11.4	12.7	13.4	12.7				
Chlorophyll fluorescence:										
PSII	means	0.723±0.04	0.797±0.01	0.893±0.06	0.793±0.00	0.763±0.02	3.87*	Y=0.74+0.175*exp(-.5*((x-62.7)/ 23.23)^2)	0.933	0.032
	%	-	10.24	23.51	9.68	5.53				
qP	means	0.903±0.05	0.933±0.02	0.970±0.02	0.967±0.02	0.910±0.02	4.90*	Y =0.90+0.089×exp(-.5×((x-73.8)/ 30.09)^2)	0.979	0.009
	%		3.32	7.42	7.09	0.78				
NPQ	means	0.310±0.11	0.257±0.06	0.400±0.02	0.363±0.08	0.327±0.03	1.10ns	ns	-	-
	%	-	-17.2	29.0	17.2	5.4				
F'v/F'm	means	0.827±0.01	0.833±0.00	0.967±0.06	0.823±0.01	0.820±0.02	3.49ns	Y =0.82+0.03×exp(-0.5×(ln(x/0.004)/ 0.35)^2)/x)	0.998	0.005
	%	-	0.73	16.93	-0.48	-0.85				
Fv/F0	means	2.46±0.47	2.51±0.22	3.06±0.23	2.37±0.10	2.29±0.22	1.49ns	Y =2.3+0.16×exp(-0.5×(ln(x/0.004)/ 0.36)^2)/x)	0.960	0.12
	%	-	2.03	24.39	-3.66	-6.91				
Growth plant										
†††Height plants	means	52.73±5.43	52.82±2.71	53.00±4.16	49.80±4.89	48.40±3.75	1.01ns	Y =43.4+9.7×exp(-.5×((x-26.9)/ 80.48)^2)	0.996	0.42
	%	-	0.17	0.51	-5.56	-8.2				
†Dry weight plants	means	7.21±0.68	7.53±0.71	10.48±1.25	7.38±0.25	6.81±0.67	6.49*	Y =6.99+4.21×exp(-.5×((x-61.03)/ 17.8)^2)	0.991	0.27
	%	-	4.4	45.4	2.3	-5.5				

† mg per g FW^{-1}, †† µg per g FW^{-1} and ††† cm.

Conclusions

The study proved that the effects of PGRs on major chlorophyll was more pronounced compared to accessory pigments, although among all the chlorophyll fluorescence parameters, only PSII was influenced by all of the PGRs. The present findings showed that the exogenous application of 0.5 mM Put, 0.1 µM BRs and 50 µM MJs provided the optimum concentrations of PGRs to improve photosynthetic indices of fennel plants. Among PGRs, only MJs exhibited the significant effects on the growth and development of fennel.

References

Akram NA, Ashraf M (2013). Regulation in plant stress tolerance by a potential plant growth regulator, 5-aminolevulinic acid. Journal of Plant Growth Regulation 32(3):663-679.

Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Tiburcio AF (2010). Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231(6):1237-1249.

Anjum SA, Xie XY, Farooq M, Wang LC, Xue LI, Shahbaz M, Salhab J (2011). Effect of exogenous methyl jasmonate on growth, gas exchange and chlorophyll contents of soybean subjected to drought. African Journal of Biotechnology 10(47):9647-9656.

Anuradha S, Rao SER (2011). Amelioration of lead toxicity in radish (*Raphanus sativus* L) plants by brassinolide. Journal of Applied Biological Sciences 3:43-48.

Anuradha S, Rao SSR (2003). Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity. Plant Growth Regulation 40(1):29-32.

Aprotosoaie AC, Spac A, Hancianu M, Miron A, Tanasescu VF, Dorneanu V, Stanescu U (2010). The chemical profile of essential oils obtained from fennel fruits (*Foeniculum vulgare* Mill.). Farmacia 58(1):46-53.

Araújo WL, Nunes-Nesi A, Osorio S, Usadel B, Fuentes D, Nagy R, Martinoia E (2011). Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid–mediated effect on stomatal aperture. The Plant Cell tpc-110.

Arnon AN (1967). Method of extraction of chlorophyll in the plants. Agronomy Journal 23:112-121.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24(1):1-15.

Asgher M, Khan MIR, Anjum NA, Khan NA (2015). Minimising toxicity of cadmium in plants-role of plant growth regulators. Protoplasma 252(2):399-413.

Bajguz A (2000). Blockade of heavy metals accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. Plant Physiology and Biochemistry 38(10):797-801.

Bari R, Jones JDG (2009). Role of plant hormones in plant defence responses. Plant Molecular Biology 69(4):473-488.

Berry PM, Sylvester-Bradley R, Berry S (2007). Ideotype design for lodging-resistant wheat. Euphytica 154(1-2):165-179.

Cevahir G, Yentür S, Eryilmaz F, Yilmazer N (2008). Influence of brassinosteroids on pigment content of *Glycine max* L. (soybean) grown in dark and light. Journal of Applied Biological Sciences 1:23-28.

Diao W-R, Hu Q-P, Zhang H, Xu J-G (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). Food Control 35(1):109-116.

Díaz-Maroto MC, Pérez-Coello MS, Esteban J, Sanz J (2006). Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. Journal of Agricultural and Food Chemistry 54(18):6814-6818.

Eyidogan F, Oz MT, Yucel M, Oktem HA (2012). Signal transduction of phytohormones under abiotic stresses. In: Phytohormones and Abiotic Stress Tolerance in Plants. Springer, pp 1-48.

Fariduddin Q, Yusuf M, Ahmad I, Ahmad A (2014). Brassinosteroids and their role in response of plants to abiotic stresses. Biologia Plantarum 58(1):9-17.

Fletcher R, Kott LS (1999). Phenolics and cold tolerance of *Brassica napus*. Proc 10th Int Rapeseed Congr GCIRC, Canberra, Australia.

Hanaka A, Maksymiec W, Bednarek W (2015). The effect of methyl jasmonate on selected physiological parameters of copper-treated *Phaseolus coccineus* plants. Plant Growth Regulation 77(2):167-177.

Hasanuzzaman M, Nahar K, Fujita M (2014). Regulatory role of polyamines in growth, development and abiotic stress tolerance in plants. Plant Adaptation to Environmental Change: Significance of Amino Acids and Their Derivatives 157-193.

Hashmi N, Khan MMA, Idrees M, Khan ZH, Ali A, Varshney L (2012). Depolymerized carrageenan ameliorates growth, physiological attributes, essential oil yield and active constituents of *Foeniculum vulgare* Mill. Carbohydrate Polymers 90(1):407-412.

Hayat S, Alyemeni MN, Hasan SA (2012). Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. Saudi Journal of Biological Sciences 19(3):325-335.

Houimli SIM, Denden M, El Hadj S Ben (2008). Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide. EurAsian Journal of BioSciences 1(2):83-90.

Igarashi K, Kashiwagi K (2000). Polyamines: mysterious modulators of cellular functions. Biochemical and Biophysical Research Communications 271(3):559-564.

Jin W-W, Wang Y, Zhang H-H, Zhi-Li J, Peng W, Xin L, Guang-Yu S (2011). Effects of foliar spraying methyl jasmonate on leaf chlorophyll fluorescence characteristics of flue-cured tobacco seedlings under drought and re-watering. The Journal of Applied Ecology 22(12):3157-3162.

Krizek DT, Britz SJ, Mirecki RM (1998). Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. 'New Red Fire' lettuce. Physiologia Plantarum 103(1):1-7.

Mahfouz SA, Sharaf-Eldin MA (2007). Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). International Agrophysics 21(4):361-366.

Mahros KM, El-Saady MB, Mahgoub MH, Afaf MH, El-Sayed MI (2011). Effect of putrescine and uniconazole treatments on flower characters and photosynthetic pigments of *Chrysanthemum indicum*. L plant. Journal of American Science 7:399-408.

Marín-Navarro J, Manuell AL, Wu J, Mayfield SP (2007). Chloroplast translation regulation. Photosynthesis Research 94(2-3):359-374.

Nanvakenary R, Moradi H, Ghasemiomran S (2013). Effects of putrescine on morphological and physiological characteristics of ornamental plant African violet (*Saintpaulia ionantha*). Bulletin of Environment, Pharmacology and Life Science 2(10):118-122.

Navakoudis E, Vrentzou K, Kotzabasis K (2007). A polyamine-and LHCII protease activity-based mechanism regulates the plasticity and adaptation status of the photosynthetic apparatus. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1767(4):261-271.

Piñol R, Simón E (2009). Effect of 24-Epibrassinolide on chlorophyll fluorescence and photosynthetic CO2 assimilation in *Vicia faba* plants treated with the photosynthesis-inhibiting herbicide terbutryn. Journal of Plant Growth Regulation 28(2):97-105.

Rascher U, Liebig M, Lüttge U (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant, Cell & Environment 23(12):1397-1405.

Rather MA, Dar BA, Sofi SN, *et al.* (2016). *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian Journal of Chemistry 9:S1574-S1583.

Shu S, Yuan L-Y, Guo S-R, Sun J, Liu CJ (2012). Effects of exogenous spermidine on photosynthesis, xanthophyll cycle and endogenous polyamines in cucumber seedlings exposed to salinity. African Journal of Biotechnology 11(22):6064-6074.

Tang J, Han Z, Chai J (2016). Q&A: what are brassinosteroids and how do they act in plants. BMC Biology 14(1):1-5.

Vardhini BV, Anuradha S, Rao SSR (2006). Brassinosteroids-new class of plant hormone with potential to improve crop productivity. Indian Journal of Plant Physiology 11(1):1-12.

Wagner GJ (1979). Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. Plant Physiology 64(1):88-93.

Wang Z-Y, Wang Q, Chong K, *et al.* (2006). The brassinosteroid signal transduction pathway. Cell Research 16(5):427-434.

Wierstra I, Kloppstech K (2000). Differential effects of methyl jasmonate on the expression of the early light-inducible proteins and other light-regulated genes in barley. Plant Physiology 124(2):833-844.

Yuan Y, Shu S, Li S, *et al.* (2014). Effects of exogenous putrescine on chlorophyll fluorescence imaging and heat dissipation capacity in cucumber (*Cucumis sativus* L.) under salt stress. Journal of Plant Growth Regulation 33(4):798-808.

Yuan Y, Zhong M, Shu S, Du, N, He L, Yuan L, Guo S (2015). Effects of exogenous putrescine on leaf anatomy and carbohydrate metabolism in cucumber (*Cucumis sativus* L.) under salt stress. Journal of Plant Growth Regulation 34(3):451-464.

Zhang L, Paakkarinen V, van Wijk KJ, Aro E-M (2000). Biogenesis of the chloroplast-encoded D_1 protein: regulation of translation elongation, insertion, and assembly into photosystem II. The Plant Cell 12(9):1769-1781.