Effects of Paclobutrazol and 24-Epibrassinolide on some morphological and biochemical characteristics of *Salvia Officinalis* under different moisture conditions

Elham Maghsoudi[1], Hossein Abbaspour[2], Abdollah Ghasemi Pirbalouti [3], Sakine Saeidi-Sar[4]

1. Department of Biology, Islamic Azad University, Damghan Branch, Damghan, Iran
2. Department of Biology, Islamic Azad University, Tehran North Branch, Tehran, Iran
3. Research for Medicinal Plants, Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran
4. Department of Agriculture, Factually of Shariaty, Tehran Branch, Technical and Vocational University (TVU), Tehran, Iran

---

Abstract

Sage (*Salvia officinalis* L.) is cultivated worldwide especially in Mediterranean regions where the leaves of this herb are widely used in pharmaceutical and food industries. Brassinolides and paclobutrazol are naturally occurring substances which modulate plant growth and development and have been known to improve the crops' tolerance to biotic and abiotic stresses. The effects of spraying 24-epibrassinolide (BRs: 0.0, 2.5, 3.0, and 3.5 µM), and paclobutrazol (PBZ: 17, 25, and 34 µM), different moisture conditions (I: normal irrigation or non-stress, reduced irrigation or non-stress, reduced irrigation or slight drought, and deficit irrigation or mild drought stress), and interaction effects of BRs × I and PBZ × I on the morphological and biochemical parameters of the *S. officinalis* were investigated. Reduction in irrigation level led to reduced plant height, the number of branches, shoot fresh and dry weights, root fresh and dry weight while it improved anthocyanin and chlorophyll contents. Spraying BRs enhanced plant height, the number of branches, and anthocyanin and chlorophyll contents with 3 µmolar concentration proving the best treatment. The maximum root fresh and dry weights were associated with combination of the highest irrigation (I3) and applying 17 µM PBZ. The maximum chlorophyll a and total contents were obtained under I3×P3 while the maximum anthocyanin content was observed in BRs2×I3.

Key words: sage; spraying; growth; irrigation

Maghsoudi, E., H. Abbaspour, A. Ghasemi Pirbalouti and S. Saeidi-Sar. 2020. 'Effects of Paclobutrazol and 24-Epibrassinolide on some morphological and biochemical characteristics of *Salvia Officinalis* under different moisture conditions'. *Iranian Journal of Plant Physiology* 11(1),3523-3532.

Introduction

Sage (*Salvia officinalis)* is a native plant to the Mediterranean region and has been used for a long time in traditional medicine for various diseases (Ben Khedher et al, 2018). *Salvia* plants' essential oils exhibit broad-spectrum pharmacological activities and are of great interest in food industry as potential natural preservative products.

Brassinosteroids are a new class of phytohormones that modulate plant growth, development, and responses to biotic and abiotic stresses (Peres et al, 2019) and play a

*Corresponding author
E-mail address: abbaspour75@yahoo.com

Received: January, 2020
Accepted: June, 2020

crucial role in response to various stresses (Krishna, 2003; Bajguz and Hayat, 2009). Swamy and Rao (2009) reported that 28-homobrassinolide enhanced the growth and metabolite content in geranium (*Pelarginium graveolens* L.).

Paclobutrazol is a synthetic plant regulator which has been used to mitigate environmental stress in some crops. PBZ-mediated stress protection is often explained in terms of hormonal changes (Manivannan et al, 2008). Many studies have focused on the protective effects of PBZ on plants against drought stress and on water-related physiological and biochemical conditions (Xiu et al, 2018). PBZ application remarkably decreased dry matter and leaf area in *Ocimum basilicum L.* (Keramati et al, 2016). Limited information is available about application of plant growth regulators on *S. officinalis* under irrigation regime. Therefore, the objective of this study was to determine the response of the effects of PBZ, 24-Epibrassinolide application and irrigation regimes on morphological and physiological factors of *S. officinalis*.

Materials and Methods

Plant mater and experimental Design

Sage seedlings with 6-10 leaves were prepared from Isfahan Center for Research on Agricultural Science and Natural Resources. The study was carried out in the Research Filed of Islamic Azad University, Shahrekord Branch, Iran with 25°/15 °C (day/night) air temperatures, 65-70% humidity, and 12/12 hours (light/lightless) for six weeks. Pots were filled with lay loam soils, pH 7.63, containing 0.27% organic carbon, 0.04% total N, 6.3 ppm P (available), 247 ppm K (available), and salinity level E.C.: 1.81 dS/m. Thereafter, the pots of the sage seedlings were transferred from the greenhouse to the farm condition at the Research Filed of Islamic Azad University, Shahrekord Branch (32° 20′ N; 50° 51′ E; altitude. 2070 m ASL). The climate of the study area is classified as cold, semiarid and semi humid.

Treatments and experimental design

Experimental treatments were arranged as factorial with two factors in a completely randomized design with three replications. Each replication involved five pots. The foliar applications of BRs and PBZ as Factor A were 0.0 (solvent + water), 2.5, 3.0, and 3.5 µmolar BRs (24-epibrassinolide), and 17, 25, and 34 µmolar PBZ. BRs and PBZ were sprayed at the vegetation stage of the plant. They were dissolved in ethanol and diluted in distilled water with various concentrations. These solutions were then sprayed at dew point (approximately 100 mL per plant) with a hand sprayer (Ghasemi Pirbalouti et al., 2019). Distilled water spray was given to control plants.

Factor B or different moisture conditions included I_1 (unstressed or control; irrigation at 85-90% F.C.), I_2 (slight drought or irrigation at 65-70% F.C.), and I_3 (mild drought or irrigation at 30-35% F.C.). Irrigation treatments began 30 days after transplanting the seedlings to pots in the field.

Measurement of growth and morphological characteristics

The growth and morphological characteristics of all plants under different treatments were investigated, including plant height and number of branches. For the determination of fresh and dry weight, the samples were separated into two parts, including roots and aerial parts. The fresh weight of each sample was measured immediately, and the dry weight was measured after drying in the shade at room temperature.

Photosynthetic pigment assay

Photosynthetic pigment assay of plant samples was carried out by the modified method of Porra et al. (2002). Fresh leaves were collected from the plants and washed with tape water to remove the soil particles. Leaf samples (100 mg) were crushed with mortar and pestle (ice cold condition) with 1 ml of 80% acetone (v/v).

Table 1
The simple effect of 24-epibrassinolide, paclobutrazol, and irrigation regimes on morphological and biochemical characteristics of *S. officinalis*.

	morphophysiological characteristics	I1	I2	I3	ANOVA	BR1	BR2	BR3	PAC1	PAC2	PAC3
1.	No. of branches	18.25^{a}	16.49^{ab}	14.78^{b}		18.38^{b}	20.74^{a}	18.52^{b}	19.83^{a}	19.41^{a}	18.4
2.	Height (cm)	20.70^{a}	18.90^{b}	18.03^{b}	$p < 0.01$	18.38^{b}	20.74^{a}	18.52^{b}	19.83^{a}	19.41^{a}	18.4
3.	Dry aerial parts Weight (g)	16.47^{a}	13.91^{a}	10.42^{b}	$p < 0.01$	12.58^{a}	14.34^{a}	13.88^{a}	15.50^{a}	12.57^{b}	12.72
4.	Fresh aerial parts Weight (g)	60.28^{a}	51.99^{ba}	42.27^{b}		50.92^{a}	52.15^{a}	51.47^{a}	56.88^{a}	52.94^{ba}	44.71
5.	Root dry weight (g)	26.47^{a}	13.09^{b}	8.30^{c}		17.42^{a}	15.17^{a}	15.27^{a}	19.64^{a}	11.83^{c}	16.39
6.	Root fresh Weight (g)	82.07^{a}	39.81^{b}	29.43^{c}	$p < 0.05$	54.41^{a}	48.69^{a}	48.20^{a}	56.72^{a}	43.56^{b}	51.03
7.	Chlorophyll a	19.42^{c}	19.62^{b}	21.29^{a}		19.06^{c}	20.28^{b}	20.99^{a}	19.32^{c}	20.76^{a}	20.26
8.	Chlorophyll b	12.62^{b}	12.65^{b}	16.15^{a}		12.57^{c}	14.99^{a}	13.87^{b}	12.82^{b}	16.03^{a}	12.58
9.	Total Chlorophyll	32.36^{b}	32.29^{c}	37.42^{a}		31.64^{b}	35.24^{a}	35.19^{a}	32.12^{c}	36.82^{a}	32.36
10.	Anthocyanin (µ mol g^{-1} fw.)	^{c} 4.31*10^5	^{b} 5.04*10^5	6.23*10^5 ^{a}		^{b} 5.04*10^5	^{a} 5.57*10^5	^{c} 4.97*10^5	^{b} 4.99*10^5	^{a} 5.71*10^5	4.88*10^5

Different letter in a row for each treatment (24-epibrassinolide, paclobutrazol, and irrigation regimes) are statistically significant at 5% level of probability by DMRT method.

Homogenized leaf samples were centrifuged at 5000 rpm for 10 minutes. After centrifugation, the absorbance of extract was measured at 645, and 663 nm in the UV – Visible spectrophotometer. The following equations were used for computing the photosynthetic pigments:

Chlorophyll (a) = 12.7 (ab663) – 2.69 (ab645) ×v/1000 × w

Chlorophyll (b) = 22.9 (ab645) – 4.68 (ab663) ×v/1000× w

Total chlorophyll (a) and (b) = 20.2 (ab645) + 8.02 (ab663) × v/1000× w

Determination of anthocyanin content

Determination of anthocyanin contents was carried out using the method of Wagner (1979). Plant leaf tissues (0.1 g fresh weight) were homogenized with a pestle and mortar in 10 ml of methanol (99% methanol, 1% HCL). The solution was centrifuged at 6000 g for 10 min. The absorbance for each sample was read at 550 nm. For calculation of the amount of anthocyanins, the extinction coefficient 33,000 mol.cm was used and anthocyanin content was expressed as µMg^{-1} fw.

Statistical Analysis

The simple and interaction effects of experimental treatments were derived from two-way analysis of variance (ANOVA) based on the GLM procedure of the SAS statistical package (SAS/STAT® v.9.2. SAS Institute Inc., Cary, NC). The significance of differences among treatment means was tested using LSD. The means of characteristics were presented as the mean± standard deviation of the mean.

Results

Plant height

The effects of experimental treatments on the morphological characteristics are summarized in Tables 1, 2 and 3. Data presented in Tables 1, 2, and 3 clearly show that the plants height was significantly affected by BRs, PBZ, and Irrigation (I) factors as well as their interaction effects. Plant height significantly decreased with reduced irrigation. Application of 3 µM BRs under severe water stress treatment (I3×BRs2) enhanced plant height (20/43cm) and the largest plant height (21/89 cm) was observed in the foliar spray of 3 µM BRs under optimum irrigation (I1×BRs2).

Table 2
The interaction effects of 24-epibrassinolide × irrigation regimes on morphological and biochemical characteristics of *S. officinalis*

	morphophysiological characteristics	I1×BR1	I1×BR2	I1×BR3	I2×BR1	I2×BR2	I2×BR3	I3×BR1	I3×BR2	I3×BR3
1.	No. of branches	14.41	20.39	19.74	16.00	17.00	16.76	13.63	17.26	13.44
2.	Height (cm)	19.07	21.89	21.15	19.07	19.89	17.74	17.00	20.43	16.67
3.	Dry aerial parts weight (g)	13.79	17.99	17.63	13.96	13.16	14.60	9.98	11.88	9.40
4.	Fresh aerial parts weight (g)	56.33	59.43	63.06	38.39	44.88	52.50	37.81	52.13	36.83
5.	Root dry weight (g)	32.69	24.83	21.89	12.94	12.46	13.87	6.62	8.21	10.06
6.	Root fresh weight (g)	84.41	79.22	72.56	41.01	37.80	40.64	27.81	29.06	31.41
7.	Chlorophyll a	18.06	20.35	19.87	18.93	19.13	20.79	20.23	21.32	22.32
8.	Chlorophyll b	12.46	15.34	9.86	9.21	13.43	15.29	16.03	15.98	16.45
9.	Total Chlorophyll	30.50	35.81	30.72	28.18	32.60	36.08	36.23	37.28	38.77
10	Anthocyanin (µ mol g-1 fw.)	3.52*10^-5	4.67*10^-5	4.38*10^-5	3.64*10^-5	4.59*10^-5	4.89*10^-5	5.94*10^-5	7.47*10^-5	3.27*10^-5

Different letter in a row for each treatment (24-epibrassinolide and irrigation regimes) are statistically significant at 5% level of probability by DMRT method

The Numbers of branches

In this study, decreased soil moisture resulted in the reduced numbers of branches per plant (Table 1). The application of three concentrations of BRs during irrigation regimes increased the number of branches and the highest number of branches was obtained from foliar spray of 3 µM BRs under optimum irrigation (I1×BRs2) (Table 2). Foliar applications of PBZ under three irrigation levels enhanced the number of branches though it was not significant.

Shoot fresh and dry weight

The results presented in Table 1 illustrated that shoot fresh and dry weights (g plant^{-1}) were significantly affected by irrigation factor. Shoot fresh and dry weights were reduced by reduction of irrigation water rate. In this study, the highest fresh and dry weights of plants were obtained from full irrigation regime (I1) while the treatment (I3) recorded the shortest plants. Foliar applications of BRs under different irrigation compared to decreased irrigation regimes without spraying increased shoot fresh and dry weight but not statistically significant ($p<0.05$) (Table 2). Also, other treatments did not have a significant effect on fresh and dry weight of plants.

Root fresh and dry weights

Fresh and dry weights of roots were significantly affected by irrigation, PBZ, and their interaction effects (Table 1). Application of PBZ under different irrigation regimes increased the fresh and dry weights of roots compared to decreased irrigation regimes without spraying and the highest values of fresh and dry root weights (99.01 g plant^{-1}) were associated with the combination of the highest irrigation level and applying 17µM PBZ (I1×P1).

Chlorophyll content

Chlorophyll contents increased significantly ($p<0.05$) by reduction in irrigation water rate (Table 1). The highest values of chlorophyll a and total chlorophyll were observed under application of PBZ (34 µmolar) and severe water stress treatment (P3× I3) (Table 3). Also, the data in Table 1 indicates positive effects of BRs on chlorophyll a, b, and total.

Anthocyanin content

The results of this study showed water deficiency significantly increased anthocyanin ($P<0.05$) (Table 1). Maximum content of anthocyanin (7.47×10^{-5} µM g^{-1} fw) was related to spraying 3 µM BRs under the lowest level of

Table 3
The interaction effects of paclobutrazol × irrigation regimes on morphological and biochemical characteristics of *S. officinalis*

	morphophysiological characteristics	I1× P1	I1× P2	I1×P3	I2×P1	I2×P2	I2×P3	I3×P1	I3×P2	I3×P3
1.	No. of branches	18.15^{bc}	16.07^{bc}	20.32^{a}	16.19^{bc}	17.19^{bc}	16.11^{bc}	13.44^{c}	15.56^{bc}	13.32^{bc}
2.	Height (cm)	20.63^{bc}	21.59^{a}	19.89^{ab}	20.44^{bcd}	18.96^{ab}	17.30^{d}	18.41^{bcd}	17.68^{cd}	18.00^{bcd}
3.	Dry aerial parts weight (g)	19.00^{a}	12.91^{ab}	14.50^{bc}	16.05^{bc}	13.71^{bcd}	11.96^{cde}	11.45^{cde}	11.10^{de}	8.70^{e}
4.	Fresh aerial parts weight (g)	68.65^{a}	56.07^{bc}	56.11^{bc}	39.53^{bc}	51.77^{bcd}	44.67^{bc}	42.47^{bc}	50.99^{bc}	33.34^{c}
5.	Root dry weight (g)	36.28^{a}	14.15^{cd}	28.97^{b}	15.15^{c}	11.92^{cde}	12.20^{cde}	7.49^{e}	9.41^{de}	8.00^{e}
6.	Root fresh weight (g)	99.01^{a}	57.35^{b}	89.84^{a}	42.54^{bc}	38.63^{cd}	38.27^{cd}	28.61^{d}	34.70^{cd}	24.98^{cd}
7.	Chlorophyll a	18.08^{g}	20.92^{b}	19.26^{f}	20.11^{d}	20.66^{c}	18.10^{g}	19.77^{e}	20.69^{c}	23.42^{a}
8.	Chlorophyll b	10.66^{f}	16.52^{bc}	10.68^{f}	12.89^{e}	15.48^{cd}	9.58^{g}	14.90^{d}	16.09^{bc}	17.47^{a}
9.	Total Chlorophyll	28.69^{h}	37.52^{b}	30.87^{g}	33.03^{f}	36.14^{d}	27.69^{i}	34.64^{e}	36.79^{c}	40.83^{a}
10	Anthocyanin (μ mol g^{-1} fw.)	3.31*10^-5	3.14*10^-5	4.49*10^-5	3.32*10^-5	3.22*10^-5	4.58*10^-5	6.32*10^-5	6.78*10^-5	5.58*10^-5

Different letter in a row for each treatment (24-epibrassinolide and irrigation regimes) are statistically significant at 5% level of probability by DMRT method.

irrigation (BRs 2 × I 3) (Tables 1, 2). The interaction effects of PBZ and irrigation also showed that anthocyanin increased with increasing in concentration of PBZ under lower levels of irrigation (Table 1, 3).

Discussion

Plant Height

Results showed that the plants' height was significantly affected by BRs, PBZ, and Irrigation factors and their interaction effects. Plant height was significantly decreased with reduced irrigation. One of the first signs of water shortage is the decrease in turgor which causes a decrease in both growth and cell development, especially in the stem and leaves (Ghasemi Pirbalouti et al., 2017). However, the growth of cells is the most important process that is affected by water stress and the decrease in the growth of cells leads to decrease in the plant height. On the other hand, the depressive effect of water stress on growth parameters may also be attributed to a drop in leaf water content and the results show a general decrease in the content of the different amino acids as a response to water stress (Reddy et al., 2018; Serret et al, 2018) affecting the rate of cell division and enlargement. Application of BRs enhanced the growth in terms of plant height and dry weight of shoots of *Plectranthus forskohlii/ coleus* (Swamy and Rao, 2011) and *Pelargonium graveolens (L.) Herit* (Swamy and Rao, 2009). Supplementation of Brassinolide stimulates stem elongation, leaf formation, and adventitious shoot formation and increases the stress tolerance in sugarcane (Chavan et al, 2018). Stimulation of longitudinal growth of young tissues via cell elongation and cell division is a major biological effect of brassinosteroids. Besides its effects on cell elongation and division, brassinosteroids have also been shown to stimulate vascular differentiation, a developmental process critical for stem elongation (Hayat and Ahmad, 2003). In later experiments with pakchoi (Brassica chinensis), cell elongation of the hypocotyls induced by BRs was found to occur with little or no change in the mechanical properties of cell walls but with an increase in wall relaxation properties and a dilution of the osmotic pressure of the cell sap (Wang et al., 1993). It was found that electrogenic proton secretions in stems were stimulated by the application of BRs. These findings implied that BR cell elongation is dependent on the decrease in pH of the cell wall space inducing loosening of the cell wall (Hayat and Ahmad, 2003). The results indicated that foliar spray of different concentrations of BRs had a significant effect ($p \leq 0.01$) on plants' height and as shown in Table 1 with increasing concentration of BRs, the height did not increase. It is now clear that BRs-induced stress responses are strictly concentration dependent and the optimal concentration for improving the plant adaptability may vary depending on the plant species, developmental stages, and environmental conditions (J Ahammed et al, 2015). Although BRs are known to stimulate elongation of young tissues, these plant growth regulators have also been previously reported to inhibit branch elongation in various plant species such as rice (Oryza sativa) and pea (Pisum sativum). These reductions in elongation have been attributed to enhanced ethylene release and/or enhanced cyanide, a byproduct of ethylene biosynthesis when ethylene is produced from ACC (Pereira-Netto et al, 2006). BRs have been demonstrated to stimulate ACC and ethylene biosynthesis in systems such as the primary roots of maize (Zea mays) in which exogenously applied brassinolide enhanced ethylene release and ACC oxidase activity in a dose-dependent manner (Lim et al. 2002). Thus, a possible way that BRs might inhibit elongation of stem treated with doses of BRs over 3 µM might be through a stimulation of ethylene and/or cyanide biosynthesis, especially if ethylene and/or cyanide biosynthesis is BRs dose-dependent.

The Numbers of branches

The findings of this study showed that The number of branches decreased with reducing irrigation. Razmi et al. (2013) stated that different irrigation treatments had significant effects on the numbers of branches of Glycine max. With increasing irrigation round, the number of branches per plant decreased from 1.6 per plant in I1 and reached 1.35 in I4. Drip irrigation with 100 per cent PE recorded significantly a higher number of branches in tomato (Parameshwarareddy et al., 2018). Increase in the number of branches in savory herbs with application of 28-Homobrassinolid has been reported by Eskandari (2011). Exogenous application of BRs on *Jasminum sambac L*. plant at 3 µM concentration resulted in the maximum plant height (66.72 cm) and number of branches per plant (62.15) compared to the other treatments (Akram et al, 2014). In the study by Farazi et al. (2015) applying 10^{-10} and 10^{-8} molar 24- homobrassinolide concentrations in irrigation reduction conditions caused significant increase in the number of secondary branches in stressed plants as compared to the control plants.

Shoot fresh and dry weight

The loss of fresh and dry mass in drought treated Arabidopsis plants confirms that drought stress can result in the inhibition of photosynthetic efficiency leading to the reduction of photoassimilates production (Kalaji et al, 2018). CDK activity reduced under water deficit conditions which increased the duration of cell division and decreased the number of cell divisions per unit time, ultimately reducing the growth of leaves and the plant (Aslam et al. 2015). However, water deficit stress conditions cause a marked suppression in plant photosynthetic efficiency. These changes in growth can be considered as a morphological adaptation of the plant to water and environmental stresses to reduce transpiration and to induce a lower consumption of water (Bañon et al., 2006). Deficit soil water induces limitation of leaf area expansion, temporary leaf wilting or rolling, and early leaf senescence which result in reduced photosynthesis (Earl and Davis, 2003). Growth reduction as a result of water deficit has been widely reported (Nelissen et al, 2018; Mahmoud et al, 2018; Dambreville et al, 2017). Similarly, Said-Al Ahl et al. (2009) in oregano, Jungklang et al. (2017) in *Curcuma alismatifolia*, and Houshmand et al. (2012) in chamomile also reported that drought stress reduced fresh and dry weights and herbage yield of the tested species. Generally, severe reductions in plant growth manifested by vegetative parameters were recorded due to decreased available soil water. Cortes et al. (2003) reported that exogenous BRs applications to cactus plants did not alter cladode weight compared with the control. The morphogenetic responses such as increased number of leaves, leaf area, fresh weight and dry weight of foliage, and the growth of productive branches and tillers have been observed after treatment with brassinosteroids (Kamuro, 1999). Also in the study reported by Amiri et al. (2018) foliar applications of BRs and its interaction with drought stress did not affect ($p \leq 0.05$) the weight of *Coriandrum sativum*.

Root fresh and dry weight

Results indicated that lower supplied irrigation resulted in reduced fresh and dry weights of the roots. Generally, the fresh and dry weights of the roots were significantly reduced due to drought stress (Quiroga et al., 2018). The root weight increased in a dose dependent manner with PBZ, but the highest concentration of PBZ reduced the dry weight of roots in maize (Kamran et al, 2018). Our results are in agreement with an earlier report by Percival and Noviss (2008) who indicated that PBZ foliar leaf of plants under deficit water stress enhanced root weight. It can be explained that rerating the plants with PBZ increased the root mass and dry weight as a result of increased root growth and root system expansion. This may influence the ability of treated plants to make contact with water (Davis et al, 1988).

Chlorophyll content

Under drought stress, due to lower leaf area, chlorophyll accumulation increases at lower leaf level. Chlorophyll concentration per unit leaf area was enhanced by PBZ due to a greater concentration of chlorophyll in a much smaller leaf area and synthesized more cytokinin which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis and prevented chlorophyll degradation (Xia et al., 2018). Triazoles may also have a direct effect on the biosynthesis of chlorophylls through the effect on biosynthesis of isoprenoid (Amoaghaee and Shariat, 2015). Also, as Table 1 shows, positive effects of BRs on were observed on chlorophyll a, b, and total. Net photosynthetic rate and leaf chlorophyll content of *Vigna radiata* L. was enhanced by the HBR treatment (Fariduddin et al, 2003). Siddiqui et al (2018) reported increased leaf chlorophyll content upon treatment with BRs in Brassica juncea L.

Anthocyanin content

Bucchetti et al (2011) reported on high concentrations of anthocyanins under water shortage in Merlot *grape*. Increase in anthocyanin in plants treated with BRs under low irrigation regimes revealed the activation of the plant΄s antioxidant system by BRs and reduced oxidative damage. Increasing of phenylpropanoid compound (anthocyanin) in stress condition and treatment with 24-epibrasinolid indicated the activation of phenylpropanoid biosynthesis pathway in this condition and the role of anthocyanin in suppressing free radicals (Rezanezhad et al., 2010). Treatment with BRs (at 0.4 mg·L^{-1}) increased the levels of anthocyanins in *Vitis vinifera* L. (Vergara et al, 2018). Also, Triazole treatment increased the anthocyanin content in both *Plectranthus aromaticus* and *Plectranthus vettiveroides* (Rajalekshmi et al, 2009). Finally, in Stevia rebaudiana B. irrigation with PBZ induced a significant increase in anthocyanin (Hajihashemi, 2018).

References

Akram, A., M. A. Khan, A. Younis and **M. Ashfaq**.2014. 'Exogenous application of 24-epibrassinolide on morphophysiological, biochemical attributes and essential oil contents of *Jasminum sambac* L'. *Pak. J. Agri. Sci, 51*(4): 881-886.

Amiri, N.,N. Zeinali and **I. Tavassolian** .2018. 'Effect of 24-epibrassinolide on some physiology and yield (*Coriandrum sativum* L.)' under drought stress conditions. *Iranian Journal of Horticultural Science*.48(4): 899-907.

Amoaghaee, R. and **E. Shariat**. 2015. 'Effect of cultivar, cold and paclobutrazol on growth, chlorophyll content and cell membrane injury in bean seedlings'. *Journal of Plant Biology*, 22 (4): 77-90.

Aslam, M., M. A. Maqbool and **R.Cengiz**. 2015. *Drought stress in maize (Zea mays* L.*): Effects, resistance mechanisms, global achievements and biological strategies* for improvement Mazie: Springer Briefs Agric, Switzerland, 1-74.

Bajguz, A., and **S. Hayat.** 2009. 'Effects of brassinosteroids on the plant responses to environmental stresses'. *Plant physiology and biochemistry, 47*(1): 1-8.

Bañon, S., J.Ochoa, J.Franco, J.Alarcón and **M. J. Sánchez-Blanco.** 2006. 'Hardening of oleander seedlings by deficit irrigation and low air humidity'. *Environmental and Experimental Botany, 56*(1): 36-43.

Ben Khedher, M. R. M.Hammami, J. R. Arch, D. C.Hislop, D. A.Eze, E. T.Wargent, M. A. Kępczyńska and **M. S. Zaibi.** 2018. 'Preventive effects of *salvia officinalis* leaf extract on insulin resistance and inflammation, in high fat diet-induced-obesity mice model'. AIP Conference Proceedings, 1-6.

Bucchetti, B., M. A.Matthews, L.Falginella, E. Peterlunger, and **S. D.Castellarin.** 2011. 'Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons'. *Scientia Horticulturae, 128*(3): 297-305.

Cramer, C. S. and **M. P.Bridgen.** 1998. Growth regulator effects on plant height of potted *Mussaenda*'Queen Sirikit'. HortScience: 33(1): 78-81.

Chavan, C., S.Chavan, R. Velhal, A. Patil, L. Deshmukh and **V. Bapat.** 2018. 'Effect of Brassinolide on invitro growth of sugarcane Co 86032 *(Saccharum spp.)'.* Paper presented at the AIP Conference Proceedings, 1989(1): 1-6.

Cortes, P. A., T. Terrazas, T. C. León and **A. Larqué-Saavedra.** 2003. 'Brassinosteroid effects on the precocity and yield of cladodes of cactus pear (*Opuntia ficus-indica* (L) Mill.)'. *Scientia horticulturae, 97*(1): 65-73.

Dambreville, A., M. Griolet, G. Rolland, M. Dauzat, A. Bédiée, C. Balsera, B. Muller, D. Vile and **C. Granier.** 2017. 'Phenotyping oilseed rape growth-related traits and their responses to water deficit: the disturbing pot size effect'. *Functional Plant Biology,* 44(1): 35-45.

Davis, T. D., G. L. Steffens and **N. Sankhla.** 1988. 'Triazole plant growth regulators. Triazole plant growth regulators'. In: *Janick J (ed) Horticultural reviews*, PP:63-105

Earl, H. J. and **R. F.Davis.** 2003. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize'. *Agronomy journal,* 95(3): 688-696.

Eskandari, M. 2011. 'The effect of 28-Homobrassinolid in reducing the effects of drought in savory herbs'. *International Journal of Plant Physiology and Biochemistry,* 3(11): 183-187.

Farazi, E., H. Afshari and **H. Hokm Abadi.** 2015. 'Effect of Different Concentrations of Brassinosteroid on Physiomorphological Charac-teristics of Five Pistachio Genotypes (*Pistacia vera.* L)'. *Journal of Nuts,* 6(02): 143-153.

Fariduddin, Q., A. Ahmad and **S. Hayat.,** 2003. 'Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide'. *Photosynthetica,* 41(2): 307-310.

Ghasemi Pirbalouti, A. 2019. 'Phytochemical and bioactivity diversity in the extracts from bulbs and leaves of different populations of Allium jesdianum, a valuable underutilized vegetable'. Acta Sci Pol Hortorum Cultus, 18(2): 115-122.

Ghasemi Pirbalouti, A., F. Malekpoor, A. Salimi and **A. Golparvar.** 2017. 'Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of basil (Ocimum ciliatum and Ocimum basilicum) under reduced irrigation'. *Scientia horticulturae*, 217: 114-122.

Hajihashemi, S. 2018. 'Physiological, biochemical, antioxidant and growth characterizations of gibberellin and paclobutrazol-treated sweet leaf (*Stevia rebaudiana B*.) herb'. *Journal of Plant Biochemistry and Biotechnology, 27*(2): 237-240.

Hayat, S. and **A. Ahmad.** 2003. *Brassinosteroids: bioactivity and crop productivity*: Springer Science and Business Media.

Hojjati, M., N. Etemadi and **B. Baninasab.** 2011. 'Effect of paclobutrazol and cycocel on vegetative growth and flowering of *Rudbeckia hirta* '. *Journal of Horticultural Sciences, 24*(2): 122-127.

Houshmand, S., H. Abasalipour, A.Tadayyon and **H. Zinali.** 2012. 'Evaluation of four chamomile species under late season drought stress'. *International Journal of Plant Production, 5*(1): 9-24.

J Ahammed, G., X.-J. Xia, X. Li, K. Shi, J.-Q. Yu, and **Y.-H. Zhou.** 2015. 'Role of brassinosteroid in plant adaptation to abiotic stresses and its interplay with other hormones'. *Current Protein and Peptide Science, 16*(5): 462-473.

Jungklang, J., K. Saengnil and **J. Uthaibutra.** 2017. 'Effects of water-deficit stress and paclobutrazol on growth, relative water content, electrolyte leakage, proline content and some antioxidant changes in *Curcuma alismatifolia* Gagnep. cv. Chiang Mai Pink'. *Saudi Journal of Biological Sciences, 24*(7): 1505-1512.

Kalaji, H. M., W. Pathom-Aree, R. Lotfi, P. Balaji, N. Elshery, E. B. Grska, M. Swiatek , T. Horaczek, J. Mojski, H. Kociel, M.M. Rytel and **S. Yoosathaporn.** 2018. 'Effect of Microbial Consortia on Photosynthetic Efficiency of *Arabidopsis thaliana* under Drought Stress'. *Chiang Mai J. Sci, 45*: 1-10.

Kamran, M., S. Wennan, I. Ahmad, M. Xiangping , C. Wenwen, Z. Xudong, S. Siwei, A. Khan, H. Qingfang and **L. Tiening.** 2018. 'Application of paclobutrazol affect maize grain yield by regulating root morphological and physiological characteristics under a semi-arid region'. *Scientific Reports, 8*(1): 1-15.

Kamuro, Y. 1999. 'Practical applications of brassinosteroids in agricultural fields. *Brassinosteroids:* steroidal plant hormones', In: *Brassinosteriods: steroidal plant hormones*. Sakurai, A. Yokota, T. and SD. Clouse (EDs). Tokyo: Springer Verlag,PP: 223-241.

Keramati, S., H. Pirdashti, V. Babaeizad and **A. Dehestani.** 2016. 'Essential oil composition of sweet basil *(Ocimum basilicum* L.) in symbiotic relationship with Piriformospora indica and paclobutrazol application under salt stress'. *Acta Biologica Hungarica,* 67(4): 412-423.

Krishna, P. 2003. 'Brassinosteroid-mediated stress responses'. *Journal of Plant Growth Regulation, 22*(4): 289-297.

Lim, S. H., S. C. Chang, J. S. Lee, S.-K. Kim and **S. Y. Kim.** 2002. 'Brassinosteroids affect ethylene production in the primary roots of maize (*Zea mays* L.). *Journal of Plant Biology, 45*(3): 148-153.

Mahmoud, A. A., A. Gendy, H. T. Said-Al Ahl, D. Grulova, T. Astatkie and **T. Abdelrazik.** 2018. 'Impacts of harvest time and water stress on the growth and essential oil components of horehound (*Marrubium vulgare*)'. *Scientia Horticulturae, 232*: 139-144.

Manivannan, P., C. A. Jaleel, A. Kishorekumar, B. Sankar, R. Somasundaram and **R. Panneerselvam.** 2008. 'Protection of *Vigna unguiculata (*L.) Walp. plants from salt stress by paclobutrazol'. *Colloids and surfaces B: Biointerfaces, 61*(2): 315-318.

Nelissen, H., X. H. Sun, B. Rymen, Y. Jikumaru, M. Kojima, Y. Takebayashi, R. Abbeloos, K. Demuynck, V. Storme, M. Vuylsteke, J. D. Block, D. Herman, F. Coppens, S. Maere, Y. Kamiya, H. akakibara, G. T. S. Beemster and **D. Inze.** 2018. 'The reduction in maize leaf growth under mild drought affects the transition between cell division and cell expansion and cannot be restored by elevated gibberellic acid levels'. *Plant biotechnology journal:* 16(2), 615-627.

Parameshwarareddy, R., S. Angadi, R. Patil and **M. Biradar.** 2018. 'Influence of Drip Irrigation Levels and Soilless Media on the Growth, Productivity and Economics of Greenhouse Grown Tomato'. *Int. J. Curr. Microbiol. App. Sci, 7*(4): 302-308.

Percival, G. C., and **K. Noviss.** 2008. 'Triazole induced drought tolerance in horse chestnut (*Aesculus hippocastanum*)'. *Tree physiology, 28*(11): 1685-1692.

Pereira-Netto, A., C. Cruz-Silva, S. Schaefer, J. Ramírez and **L. Galagovsky.** 2006. 'Brassinosteroid-stimulated branch elongation in the marubakaido apple rootstock'. *Trees, 20*(3): 286-291.

Peres, A. L. G., J. S. Soares, R. G. Tavares, G. Righetto, M. A. Zullo, N. B. Mandava and **M. Menossi.** 2019. 'Brassinosteroids, the sixth class of phytohormones: a molecular view from the discovery to hormonal interactions in plant development and stress adaptation'. *International Journal of Molecular Sciences, 20*(2): 1-33.

Porra, R. J. 2002. 'The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b'. *Photosynthesis research, 73*(1-3): 149-156.

Quiroga, G., G. Erice, R. Aroca, Á. M. Zamarreño, J. M. García-Mina and **J. M. Ruiz-Lozano.**

2018. 'Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and root hydraulic properties in maize plants subjected to drought'. *Agricultural Water Management, 202*: 271-284.

Rajalekshmi, K. M., C. A. Jaleel, M. Azooz and **R. Panneerselvam.** 2009. 'Effect of triazole growth regulators on growth and pigment contents in *Plectranthus aromaticus* and *Plectranthus vettiveroids*'. *Advances in Biological Research, 3*(3-4):117-122.

Razmi, N., J. Iran Nejad, H. Khanzadeh and **Soheili B. Mogddam.** 2013. 'The effects of different irrigation regimes on the morphological and physilogycal characterristics of three soybean cultivars (*Glycine max*)'. Journal of crop ecophysiology (Agriculture science): 7(1 57-70.

Reddy, T., V. Reddy and **V. Anbumozhi.** 2003. 'Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: a critical review'. *Plant growth regulation, 41*(1): 75-88.

Rezanezhad, F., Kh. Manouchehri Kalantari and **M.T. Behnamnia.** 2010. *Study of 24-Epibrassinolide Effects on Reducing Dehydrated Stomata in Tomato (Lycopersicon esculentum* L.*)* . Ph.D. Thesis, Shahid Bahonar University of Kerman., Faculty of Science. (in Persian).

Said-Al, A. H., E. Omer and **N. Naguib.** 2009. 'Effect of water stress and nitrogen fertilizer on herb and essential oil of oregano'. *International Agrophysics, 23*(3): 269-275.

Serret, M. D., S. Yousfi, R. Vicente, M. C. Piñero, G. Otálora-Alcón, F. M. Del Amor and **J. L. Araus.** 2018. 'Interactive effects of CO2 concentration and water regime on stable isotope signatures, nitrogen assimilation and growth in *sweet pepper*'. *Frontiers in plant science, 8*: 1-18.

Siddiqui, H., K. B. M. Ahmed and **S. Hayat.** 2018. 'Comparative effect of 28-homobrassinolide and 24-epibrassinolide on the performance of different components influencing the photosynthetic machinery in *Brassica juncea* L'. *Plant Physiology and Biochemistry, 129*: 198-212.

Suman, V. M. and S. A. John. 2018. 'Study of Biochemical and Yield Traits with Different Growth Retardants in Cowpea cv. Kashi Kanchana'*. Int. J. Curr. Microbiol. App. Sci, 7*(2): 3789-3793.

Swamy, K. and **S. Rao.** 2009. 'Effect of 24-epibrassinolide on growth, photosynthesis, and essential oil content of *Pelargonium graveolens (*L*.)* Herit'. *Russian Journal of Plant Physiology, 56*(5): 616-620.

Swamy, K. and **S. S. R. Rao.** 2011. 'Effect of brassinosteroids on the performance of coleus (*Coleus forskohlii*)'. *Journal of herbs, spices and medicinal plants, 17*(1): 12-20.

Vergara, A. E., K. Díaz, R. Carvajal, L. Espinoza, J. A. Alcalde and **A. G. Pérez-Donoso.** 2018. 'Exogenous applications of brassinosteroids improve color of red table grape (*Vitis vinifera* L. Cv."Redglobe") berries'. *Frontiers in plant science, 9*: 1-11.

Wang, T.-W., D. J Cosgrove and **Arteca R. N.** 1993. 'Brassinosteroid stimulation of hypocotyl elongation and wall relaxation in pakchoi (*Brassica chinensis* cv Lei-Choi)'. *Plant physiology, 101*(3): 965-968.

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

Xia, X., Y. Tang, M. Wei and **D. Zhao.** 2018. 'Effect of paclobutrazol application on plant photosynthetic performance and leaf greenness of herbaceous peony'. *Horticulturae, 4*(1): 1-12.

Xiu, W. Y., Y. Zhu, B. Chen, Y. Hu and **M. M. Dawuda.** 2019. 'Effects of paclobutrazol on the physiological characteristics of *Malus halliana* Koehne Seedlings under drought stress via principal component analysis and membership function analysis'. *Arid Land Research and Management, 33*(1): 97-113.