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Full Length Research Paper

The effect of 28-Homobrassinolid in reducing the effects of drought in savory herbs

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Drought stress is the most significant factor that reduces crop yield and is used as a drug. Using materials such as a plant growth regulator (Brassinosteroids) is a practical solution to reduce losses. Brassinosteroids steroidal plant compounds are broad biological activities that are able to increase plant performance through changes in plant metabolism and in protecting them against environmental stress. In a greenhouse study that was carried out in Vocational City Center Arsanjan, the impact of three levels of irrigation: field capacity (FC), mild stress (FC2/3) and severe stress (FC1/3) and four concentrations of plant growth regulators: Article 28-Homobrassinolid zero, M 10^{-10}, 10^{-8} M and 10^{-6} M digits on some savory characters of *Satureja bachtiarica* was investigated. The results showed that reducing irrigation to reduce the significant effect it has on growth parameters, including length and root dry weight, stem diameter, branch number, plant height, shoot yield and total yield, was essential. More so, the concentration of 10^{-8} M 28-Homobrassinolid that was significantly used to increase root dry weight, stem diameter, branch number, plant height, total yield and performance was also essential. Percentage oil increased significantly at 1% by reducing irrigation so that 36% oil in full irrigation increased to 87% in severe stress, and 10^{-8} M was recorded for the use of hormone. Essential oil yield at 5% level under the influence of irrigation and the use of hormones were the most essential functions related to irrigation and the use of 10^{-8} M 28-Homobrassinolid and value 32/29 kg/ha, respectively. The relationship of these hormone levels in full irrigation, mild and severe stress significantly increased by 59, 30 and 24% oil yield respectively than the control plants did.

Key words: Drought stress, Brassinosteroids, essential oil percentage, shoot yield.

INTRODUCTION

Drought stress is one of the most important environmental stresses affecting agricultural productivity around the world and may result in considerable yield reductions (Ludlow and Muchow, 1990). In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). The effect of drought stress on medicinal and aromatic plants' growth and development has been studied lowly. The results indicated that water deficit during the vegetative period (before flowering stage) can result in shorter plants and smaller leaf areas of mint (Abbaszadeh et al., 2008), yarrow (Sharifi et al., 2005), chicory (Taheri et al., 2008), reduced water use due to the reduction in plant size of calendula (Rahmani et al., 2008) and decreased vegetative dry matter of balm (Aliabadi et al., 2009).

Basically, water stress reduced the fresh and dry weights of *Satureja hortensis* L. (savory) plants. However, it was observed that severe water stress increased the essential oil content more than the moderate water stress did. The main constituents, such as carvacrol, increased under moderate water stress, while β-terpinene content decreased under moderate and severe water stress of *S. hortensis* L. (Baher et al., 2002). The effect of water stress on essential oil was studied in excised leaves of palmarosa (*Cymbopogon martinii* var. motia) and citronella java (*C. winterianus*). As such, essential oil percentage was increased under water stress, while essential oil content was decreased under this same condition (Fatima et al., 2006).

One of the compounds which have antioxidative characteristics is brassinosteroid (Haubrick and Assmann, 2006). Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds with wide-ranging biological activity that offer the unique possibility of increasing crop yields through both changing plant metabolism and protecting plants from environmental stresses. In recent years, genetic and biochemical studies have established an essential role for BRs in plant development, and on this basis BRs have been given the stature of a phytohormone (Krishna, 2003). Brassionosteroids are common plant-produce compounds that can function as growth regulators (Bishop et al., 2006). In addition, it has been suggested that BRs could be induced in the category of phytohormones (Haubrick and Assman, 2006). Exogenous application of BRs may influence a range of diverse processes of growth and development in plants (Cao et al., 2005; Ozdemir et al., 2004).

The growth and essential oil production of aromatic plants can be altered by the application of plant growth regulators. Exogenous application of triacontanol was found to be beneficial in improving the herbage yield, as well as artemisinin content in *Artemisia annua* (Shukla et al., 1992). Application of indole-3-acetic acid resulted in a 4-fold increase in the geraniol content of *in vitro* developed plantlets and a 2.2-fold increase in 60-day-old plants of *Melissa officinalis* (Silva et al., 2005). A significant increase was reported in essential oil accumulation in Japanese mint (*Mentha Arvensis)* by chlormequat chloride (Farooqi and Farooqi, 1988). A strategy for improving medicinal and aromatic plants' yields is to identify the compounds that are relatively drought resistant and which can result in superior yields under dry land conditions (Popp et al., 2002).

The present study was an attempt to carry out investigations on the effect of 28-Homobrassinolid on savory plants under drought stress, with the aim to: (1) characterize the variation in the growth parameters of different concentrations of BR under mild and severe drought stress conditions; and (2) investigate the essential oil content as affected by drought stress and 28-Homobrassinol in plants treated with four concentrations of BR.

MATERIALS AND METHODS

Experiments were carried out at the greenhouse of the Technical and Vocational Center during 2009 and 2010. Before testing, the physical and chemical properties of the soil used in this study were determined according to the method of Jackson (1973) and Cottenie et al. (1982), and are presented in Table 1.

A factorial experiment in randomized complete block design with four replications and 12 treatments was conducted. The first treatment includes three levels of irrigation in drought factor, based on the values of field capacity, mild stress and severe stress, such that in steps 6 to 8, the sub-branches of drought treatment were applied. The second treatment includes a steroid hormone factor (Article 28-Homobrassinolid) with four levels: 0, 10^{-6}, 10^{-8}, 10^{-10} M solutions, witnessed in the 3 to 5 leaf stage, before the drought stress treatment was performed. Seeds of Satureja hortensis were sown into plastic pots (20 cm diameter and 40 cm in height), after which the pots were transferred to greenhouse adjusted to 22/35 C, 70/50% RH day/night and light intensity of approximately 3600 lux. Each pot was filled with 10 kg of air-dried soil. In this experiment, savory varieties (S. bakhtiarica) were used. Before stress treatment, all plants were regularly irrigated in field capacity.

On the 9th of April, 2010, during the distinguished savory flowering, plants with roots were carefully removed from the pots, and the desired characteristics include: plant height, stem diameter, dry weight of roots and shoots, and number of branches. Also, dry weight production per hectare was measured and calculated. Fresh weight of plants was collected from each treatment during the full flowering stage and was dried by air and weighed to extract essential oil from it.

Essential oil extraction

For this experiment, ten gram of the aerial organs' dry matter was immediately transferred to a round bottom flask of the Clevenger apparatus. Water was added till the plant material was completely submerged and was subjected to hydro distillation for 3 h. The volume of the oil collected in the collecting tube of the apparatus was recorded. In addition, total essential oil as kg per hectare was calculated by using the dry weight of the herb.

Statistical analysis

All experiments were performed in 4 replicates, using randomized complete block design. Data were statistically analyzed by one-way analysis of variance using SPSS and the means were separated by Duncan's multiple range tests at 0.05 probability level.

RESULTS AND DISCUSSION

Growth parameters

The total dry weights of the plants (kg/ha), root (g per pot), number of sub-branch, plant height and diameter of shoot (mm) were significantly affected by changes in soil moisture (Tables 2 and 3) and were significantly decreased with decreasing irrigation (1%). Exogenous application of 28-homobrassinolid substantially improved the growth of the savory plants as reflected in the increase of all growth parameters recorded (Table 3). The economic yield of the plant (herbage yield) as reflected in the leaf number, dry shoot weight, plant height, diameter of shoot and number of sub-branch was also increased due to 28-homobrassinolid treatment (Table 3). However, an increase was also observed in the leaf biomass and leaf area in *Cucumis sativus* by exogenous application of brassinosteroids (Yu et al., 2004).

The interaction among treatments was significant during the experiment, and it was observed that the total dry weights of root, diameter of shoot and number of sub-branch of savory were greater than the control. More so, the 28-Homobrassinolid employed in the study caused a significant increase in the growth parameters of savory plants when applied at 10^{-8} M concentration (Table 2). Zhang et al. (2008) found that application of BRs could partially alleviate the detrimental effect of water stress

Table 1. Physical and chemical properties of the tested soil.

Field capacity moisture content (dry weight)	24.60%
Bulk density (grams per cubic centimeter)	1.3
Electrical conductivity (EC (S/m^2)	1. 1
Soil pH	7.62
Soil texture	Loam clay

Table 2. Some features savory different levels of irrigation and different concentrations of 28-homobrassinolid (M, Molar).

28 -homobrassinolid	Stress (FC)		Root dry weight (g)	Plant height (cm)	Shoot diameter (mm)	Number of sub-shoot	Dry shoot weight (kg/ha)	Essential oil (%)	Essential oil yield (Kg/ha)
H1	I1	FC	2.610^{b}	58.25^{a}	4.33^{a}	17.99^{a}	5059^{c}	0.365^{c}	18.37^{e}
0 M	I2	2/3FC	1.210^{f}	$51.00b^{cd}$	2.838^{d}	16.00^{c}	2871^{hi}	0.475^{bc}	13.61^{g}
	I3	1/3FC	0.6800^{g}	48.00^{cde}	2.122^{e}	11.83^{e}	1093^{i}	0.780^{a}	8.510^{h}
H2	I1	FC	2.410^{b}	52.75^{abc}	4.16^{ab}	16.66^{bc}	5324^{b}	0.450^{bc}	23.45^{bc}
10^{-10} M	I2	2/3FC	1.840^{cde}	52.25^{bcd}	3.84^{ab}	16.00^{c}	4787^{de}	0.510^{b}	23.61^{bc}
	I3	1/3FC	1.570^{e}	45.00^{e}	2.92^{d}	14.79^{d}	2799^{i}	0.820^{a}	22.92^{c}
H3	I1	FC	3.380^{a}	56.75^{ab}	4.298^{a}	17.91^{a}	7049^{a}	0.4175^{bc}	29.32^{a}
10^{-8} M	I2	2/3FC	1.69^{de}	56.75^{ab}	3.697^{bc}	16.99^{abc}	4648^{e}	0.5275^{b}	23.94^{b}
	I3	1/3FC	1.270^{f}	48.50^{cde}	2.730^{d}	14.41^{d}	2757^{j}	0.8775^{a}	21.56^{d}
H4	I1	FC	2.080^{c}	48.50^{cde}	3.680^{bc}	17.33^{ab}	4203^{f}	0.4125^{bc}	17.13^{f}
10^{-6} M	I2	2/3FC	1.940^{cd}	52.25^{bcd}	3.215^{cd}	16.33^{bc}	3408^{g}	0.4150^{bc}	13.98^{g}
	I3	1/3FC	0.796^{g}	46.50^{de}	2.705^{d}	12.16^{e}	2294^{k}	0.8425^{a}	18.51^{e}

Means followed by the same letters in column are not significantly different at P = 0.05

on the growth of soybean through improving the antioxidant system and promoting dry weight accumulation. Also, he observed that BRs participate in the processes of gene expression, transcription and translation in normal and stressed plant (Zhang et al., 2008). As such, the promotion of growth was reported in stressed seedlings of *Robinia pseudoacacia* (Li et al., 2008).

Exposure of sugar beet plants to drought stress led to a reduction in taproot mass which was proportional to stress severity. Treatment with BR fully compensated for the reduction in biomass caused by mild drought stress. The increase in root growth in BR-treated plants versus untreated plants was seen only under water stress conditions. Increases in biomass correlated with increases in acid invertase activity in young leaves, which likely provided more assimilates to the plant due to their larger sizes (Schilling et al., 1991).

Table 3. Mean squares of the main effects and interaction between irrigation levels and the different concentrations of 28-homobrassinolid.

				(Mean Squares)				
Source	**Degrees of freedom**	**Root dry weight (g)**	**Shoot diameter (mm)**	**Number of sub-shoot**	**Plant height (cm)**	**Dry shoot weight (kg/ha)**	**Essential oil (%)**	**Essential oil yield (kg/ha)**
Irrigation (I)	2	** 1.006	** 9.005	** 74.460	**233.68	** 2866.877	** 0.804	* 1.114
Hormone (H)	3	** 0.255	** 0.857	**3.706	**60.472	** 617.378	ns 0.012	** 4.913
Hormone× Irrigation	6	* 0.100	** 0.410	**3.964	ns 25.826	ns 106.836	ns 0.005	* 0.579
Error	33	0.033	0.108	0.615	13.042	0.67001	0.004	0.216

Percentage and oil yield

As shown in Table 2, an increase in essential oil percentage was observed under two water stress levels: 66% (mild drought) and 33% (sever stress) of field water capacity. These results are in line with those of Baher et al. (2002). While the highest yield of essential oil (kg/hag plant-1) was obtained with the 100 field water capacity treatments, these results may be due to the increment in herb dry weight of these treatments.

Drought stress increases the essential oil percentage of more medicinal and aromatic plants, because in case of stress, more metabolites are produced in the plants. However, the substances prevent them from oxidization in the cells, but the essential oil content reduced under drought stress, because the interaction between the amount of the essential oil percentage and shoot yield is considered to be important as the two components of the essential oil content. By exerting stress, the essential oil percentage is increased, but the shoot yield decreased by drought stress; therefore, the essential oil content reduces (Aliabadi et al., 2009). An experiment was carried out to study the influence of water deficit stress on the essential oil of balm. The results of this experiment showed that essential oil yield was reduced under water deficit stress, but essential oil percentage was increased under stress (Aliabadi et al., 2009).

Rahmani et al. (2008) showed that drought stress had a significant effect on oil yield and oil percentage of calendula. Their results showed that the highest oil yield was achieved under non-drought condition and the highest oil percentage was achieved under drought condition. Also, Bettaieb et al. (2008) investigated the effect of water deficit on fatty acids and essential oil yield and composition of *Salvia officinalis* aerial parts. Drought decreased significantly the foliar fatty acid content and the double bond index (DBI) degree. Later, this was provoked mainly by a strong reduction of the linolenic acid proportion and the disappearance of the palmitoleic acid. Besides, moderate water deficit increased the essential oil yield (expressed as g/100 g on the basis of dry weight). The main essential oil constituents were camphor, thujone and 1, 8-cineole which showed an increase in moderate water deficit.

A study in Iran estimated the influence of soil water stress on essential oil content of Iranian *Satureja hortensis* L. The volatile constituents of the aerial parts of cultivated *S. hortensis* L. were isolated by steam distillation and analyzed by GC/MS. The accumulation of oil increased significantly under severe water stress at the flowering stage, when the mean leaf water potential decreased from -0.5 to -1.6 MPa. This treatment affected the quantity of the essential oils more than the moderate water stress during the vegetative and flowering stages. The main oil constituents are carvacrol and _-terpinene. The amount of carvacrol increased under moderate stress, while _-terpinene content decreased under moderate and severe water stress treatments (Baher et al., 2002). Singh-Sangwan et al. (2006) indicated that the level of essential oils was maintained or enhanced under drought condition. The major oil constituents, geraniol and citral, increased substantially in two lemon grasses (*Cymbopogon nardus* and *Cymbopogon pendulus*). The activity of geraniol dehydrogenase was also modulated under moisture stress. Also, water stress had a significant effect on flowering shoot yield, essential oil yield of flowering shoot and essential oil percentage of flowering shoot of coriander, in that the highest characteristics were achieved under water without stress conditions, while the highest oil percentage of flowering shoot was achieved under water stress conditions (Aliabadi et al., 2008). Consequently, drought stress reduces the essential oil content of more medicinal and aromatic plants and increases the essential oil percentage under drought conditions. More so, it reduces the yield of medicinal and aromatic plants by three main mechanisms. First, the incident of the whole canopy absorption of photosynthetically active radiation may be reduced either by drought-induced limitation of the leaf area’s expansion (by temporary leaf wilting or rolling during periods of severe stress), or by early leaf senescence. Secondly, drought stress decreased the efficiency with which the absorbed photosynthetically active radiation was used by the crop to produce new dry matter (the radiation use efficiency). This can be detected as a decrease in the amount of crop dry matter accumulated per unit of the photosynthetically active radiation absorbed over a given period of time, or as a reduction in the instantaneous whole-canopy net CO_2 exchange rate per unit of the absorbed photosynthetically active radiation. Thirdly, drought stress may limit grain yield of medicinal and aromatic plants by reducing the harvest index (HI). This can occur even in the absence of a strong reduction in total medicinal and aromatic plants dry matter accumulation, if a brief period of stress coincides with the critical developmental stage around flowering (Earl and Davis, 2003).

Conclusion

The following conclusions were drawn from this study:

1. A decrease in plant growth was observed under drought stress.
2. Brassinosteroids, especially 10^{-8} M 28-homo-brassinolid, caused an increase in plant biomass. This study indicated that foliar sprayed 10^{-8} M 28-homobrassinolide caused a number of physiological and biochemical changes in the savory plants, including increased dry weights of shoot, root dry weight, number of sub-branch, plant height and diameter of shoot.
3. The results indicated that treatment with 28-homobrassinolide could reduce the effects of water stress in savory plants.
4. 100% field water capacity resulted in the highest yield of herb and essential oil yield of savory.

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