Journal of Nuts 6(2):143-153, 2015
ISSN:2383 - 319x

Effect of Different Concentrations of Brassinosteroid on Physiomorphological Characteristics of Five Pistachio Genotypes (*Pistacia vera*. L)

*E. Farazi[1], H. Afshari[*1], H. Hokm Abadi[2]*

[1]Department of Horticulture, Damghan Branch, Islamic Azad University, Damghan, Iran

[2]Damghan Pistachio Research Station, Iran

Received: 17 June 2015 Accepted: 26 September 2015

Abstract

Brassinosteroid as one of steroid hormones has an integral role in controlling plants physiological process especially in response to biological and non-biological stress. This research has been done in Damghan Islamic Azad University Greenhouse. The influence of four concentrations of plant growth regulator -24, homobrassinolide with concentration of 0,10^{-10}, 10^{-8},10^{-6} molar on null seedlings of pistachio genotype sort based on factorials experiments on the basis of completely randomized block design with four replications was performed. After foliar spraying in six - leaves stage of pistachio seedlings and after passing six weeks, somephysio-morphplogical characteristics such as the size of photosynthesis pigments, leaf area index, seedlings height, biomass (the wet and dry weight of root and shoot), and lipoxygenase enzymes were scrutinized.The result indicated that foliar spraying experiment with brassinosteroid hormones had significant effect on some evaluated traits (P). Maximum leaf area index with concentration of 10^{-10} brassinosteroid and genotype G1, and the minimum leave area index with untreated (control) and genotype G5 were achieved. In biomass scrutiny, the wet weigh of aerial organs in level 1% was significant (5% level).The maximum weight of the wet weigh in concentration, 10^{-10} and the minimum weight in concentration of control and 10^{-6} were observed. Besides, the fresh weight of root in level 1% was significant, and the maximum weight with concentration of $10^{-10}$and genotype G (22%) and minimum weight with control concentration and genotype G1 were observed. The maximum amount of lipoxygenase was related to concentration of 10^{-10}, and genotype G2 and the minimum amount was related to null concentration of brassinosteroid hormones and genotype G1.

Keywords: Brassinosteroids, Chlorophyll florescence, Genotype, Lipoxygenase, Pistachio.

Introduction

Brassinosteroids are a group of herbal hormones with considerable impacts on development of plants, e.g., they increase resistance against environmental stresses. They are synthesized naturally and involve a wide range of biological activities. They control a number of major agricultural activities such as time for blooming, seed yield and resistance to stresses. Considerable increase in the crop's yield can be both the result of plant metabolism and plant conservation against environmental stresses (Eskandary, 2011).

Brassinosteroids are almost found in all parts of the plants, seen mostly in genital organs (pollens and unripe seeds) (Clous Steven and Sasse, 1998). Brassinosteroid cause increase in plants, and resistance against different non-living stresses. These substances cause change in gene expression and change in metabolism as well as biosynthesis of nucleic acids and proteins (Khripach *et al.*, 1999).

***Corresponding author:** E-mail**:** h_afshari@ymail.com

Brassinosteroid increase plants, tolerance against a wide range of environmental stresses including drought, salinity, cold and heat.

This increase is generally dependent to generation and duplication of anti-stress genes including heat stress protein that is indicator of increase in the duplication of the genes responsible for stress to enhance tolerance to stress within plants treated with brassinosteroid. (Clouse *et al*., 1992).

The present study aimed to verify and select the genotype tolerant against undesired environmental conditions. One of the most significant reasons for decrease in pistachio yield is environmental stresses of salinity, drought and pests. Brassinosteroidcan create balance in these stresses by its physiological stresses in the plant.

Materials and Methods

This research was done during the year 2013 by producing sampling in "Beram" farm and doing experiments in Islamic Azad University, Damghan Branch, Damghan, Iran. The effect of four concentrations of homobrassinolide -24, 0-, 10^{-10}, 10^{-8} and 10^{-6} molar was done on 5 genotypes of pistachio based on factorial tests on complete random design with four replications.

The seeds of 5 genotypes of pistachio were soaked in water for 24 h after peeling and then were disinfected with sodium hypochlorite 3% for 10min. Then they were washed and placed on a piece of cloth to be planted in prepared pots after germinating. Irrigation was done immediately after planting the seeds. After the seeds grew, the traits of Leaf Area Index (LAI), root fresh weight, seedling height, shoot fresh weight, lipoxygenase enzyme and chlorophyll fluorescence in five types of genotypes were measured.

Chlorophyll florescence on leaf area was measured using Opti Sciences Inc.(LAI) indicates leaf area (only one side) to the ground area occupied by the crop.For this purpose, leaf area meter was used. Lipoxygenase activity enzyme was done by measuring increase in absorption in 234 nanometer wavelengths in room temperature (Hessler *et al*., 2002).

Statistical analysis was done by Mstatc software. Excel software was used to draw diagrams and charts.

Results

Leaf Area Index

The findings of variance analysis indicate the significant effect of different concentrations of brassinostreiods hormone on LAI in 1% level. The average of the highest LAI was 10^{-10}M and the lowest degree was zero in control (Table 1).

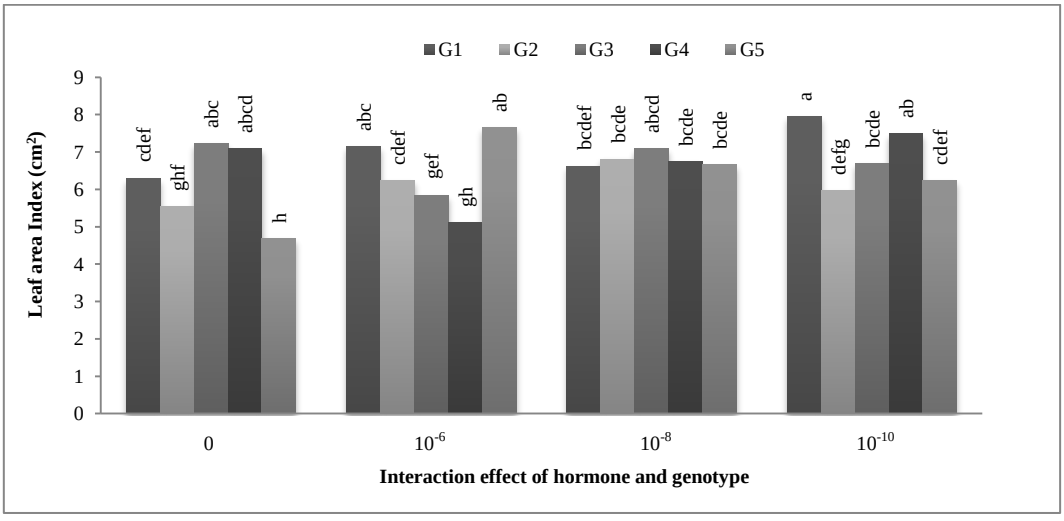

Fig.1. Interaction effect of hormone and genotype (G1-G5) on LAI.

Fresh Root Weight

The findings of variance analysis showed that the effect of different concentrations of brassinosteroid hormone and investigated genotypes as well as their interaction effect and the figures gained by the table for average comparison in the level of 1% was significant. Insert Table of variance analysis.

Seedling height

The effect of spraying and brassinosteroid on the height of plant was significant in level of 5%. Comparing averages showed that the highest sidling was for applying brassinosteroid hormone with 10^{-10} and 8^{-10} concentrations and lowest was for applying brassinosteroid hormone with 6^{-10} concentration. Interaction effect of spraying brassinosteroid on the height of pistachio seedling, the findings of variance analysis indicate significance of interaction effect in the level of 5%. The most height was for G1 and zero concentration of brassinosteroidand the least height was for G2 and zero concentration of brassinosteroid.

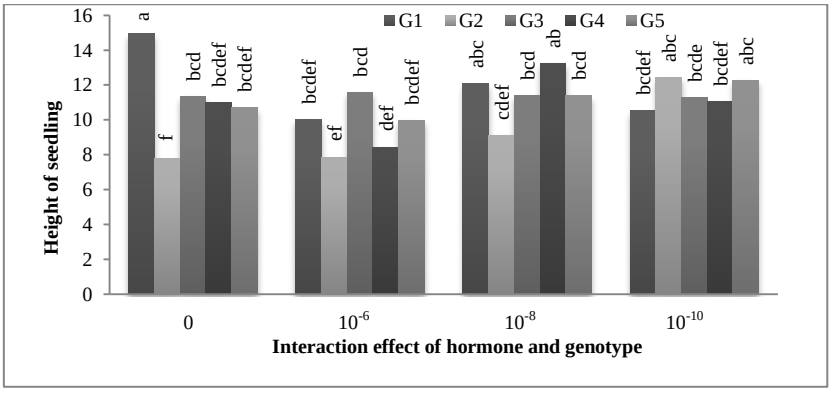

Fig.2. Interaction effect of hormone and genotype (G1-G5) on seedling height.

Shoot Fresh Weight

The findings of variance analysis table indicated the significant effect of different concentrations of brassinosteroid on shoots fresh weight in the level of 1% (Table 1). The highest fresh weight was for 10^{-8} and 10^{-10} concentrations and the least was for 10^{-6} concentration and the control. The findings of variance analysis table also showed that the effect of different concentrations of brassinosteroid hormone and genotypes on dry weight of shoots was not significant.

Undesired effects of stress on plant's growth and physiology including growth, development, photosynthesis system, food elements absorption and nitrogen metabolism are among most important reasons for weight reduction of the plant during the period, A plant's growth and development depends on cell division , growth and distinction of the cells.

Table 1. Mean comparison the effect of genotypes on some characteristics in pistachio

	Index of leaf level		fo		fm		fv		fv/m		Chlorophyll a		Chlorophyll b	
Geno-type	Average and standard devia-tion	Grouping	Average and standard devia-tion	Grouping	Average and standard devia-tion	Grouping	Average and standard devia-tion	Grouping	Average and standard deviation	Grouping	Average and stand-ard deviation	Grouping	Average and standard deviation	grouping
G1	7.003±0.9342	a	219.35±25.3487	a	1007.55±185.018	a	787.6±169.795	a	0.77485±0.041	a	0.01727±0.008742	b	0.047365±0.020866	a
G2	6.14±0.72553	c	217.6±45.2611	a	938.6±289.525	a	717.8±263.45	a	0.73895±0.094876	a	0.021±0.01344	b	0.054275±0.038615	a
G3	6.7135±1.25467	ab	237.45±28.6218	a	1046±209.257	a	808.55±203.596	a	0.7707±0.045694	a	0.04886±0.04554	a	0.055765±0.085068	a
G4	6.6115±1.11381	abc	240.45±36.3817	a	1060.65±67.041	a	820.5±71.699	a	0.7725±0.03586	a	0.014875±0.007924	b	0.04155±0.012955	a
G5	6.312±1.12652	bc	230.1±37.8986	a	959.75±206.047	a	731.1±187.637	a	0.7531±0.051698	a	0.0269±0.015022	b	0.04435±0.013374	a

Treatments with similar letters based on Duncan test now in the level of five percent have no significant difference.

The findings of variance analysis indicate significant effect of the genotypes under experiment in the level of 1%.The most weight of shoot was for 8^{-10} concentration and 5gr. And the least was for zero concentration of Brassinosteroid and 3 and 4 g, 46% higher than control plants (Fig.3).

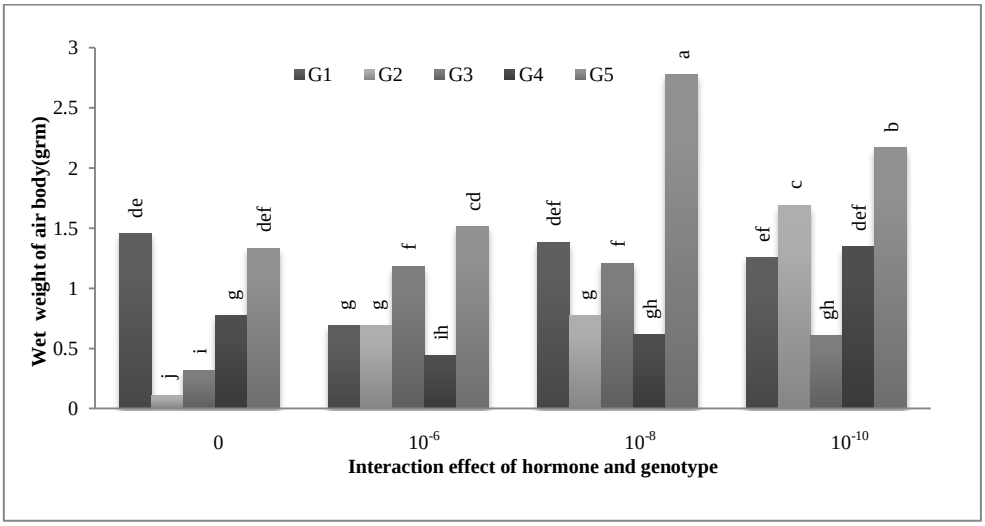

Fig.3. Interaction effect of genotype and spraying on fresh weight of shoot in pistachio

Lypoxygenasis enzyme

The findings of variance analysis showed significant effect of different concentrations of brassinosteroid on lypoxygenasis enzyme in level of 1%.

The highest lypoxygenasis enzyme was for 10^{-10} mole concentration and the least was of lypoxygenasis enzyme was for control and 10^{-6} concentration (Table 2).

Table 2. Mean comparison the effect of genotypes on some characteristics in pistachio

	Total chlorophyll		Height of seedling		Wet weight of shoot		Dry weight of shoot		Wet weight of root		Wet weight of root		Enzyme lipoxigenase	
Genotype	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping	Mean and standard deviation	Grouping
G1	0.06464±0.023109	b	11.9±2.90299	a	1.1975±0.34892	b	0.4385±0.091	a	0.89425±0.55431	d	16.9173±72.516	a	24.119±7.3973	a
G2	0.07528±0.036845	ab	9.3±2.53896	b	0.8176±0.58413	c	0.4479±0.269	a	1.3379±0.79194	b	0.619±0.491	a	24.642±5.4528	a
G3	0.10463±0.095815	a	11.4±2.53336	a	0.8315±0.43325	c	0.3546±0.121	a	1.09475±0.39351	c	0.3846±0.211	a	13.268±13.5505	b
G4	0.05643±0.015591	b	10.93±3.08257	a	0.79305±0.36796	c	44.0669±195.111	a	1.0524±0.45172	c	38.5187±170.525	a	11.543±3.9074	b
G5	0.07125±0.021494	b	11.09±1.82148	a	1.94985±0.59791	a	0.4121±0.225	a	1.4541±0.6522	a	0.739±0.114	a	14.706±4.1238	b

Treatments with similar letters based on Duncan test now in the level of five percent have no significant difference.

Florescence chlorophyll: The mutual effect of brassinosteroid hormone and genotype on the amount of FO Florescence chlorophyll was significant in the level of 1%. The highest amount of FO was in 10^{-10} mole concentration and genotype G4 and the least FO Florescence chlorophyll was in 10^{-10} concentration and genotype G2 (Fig.4).

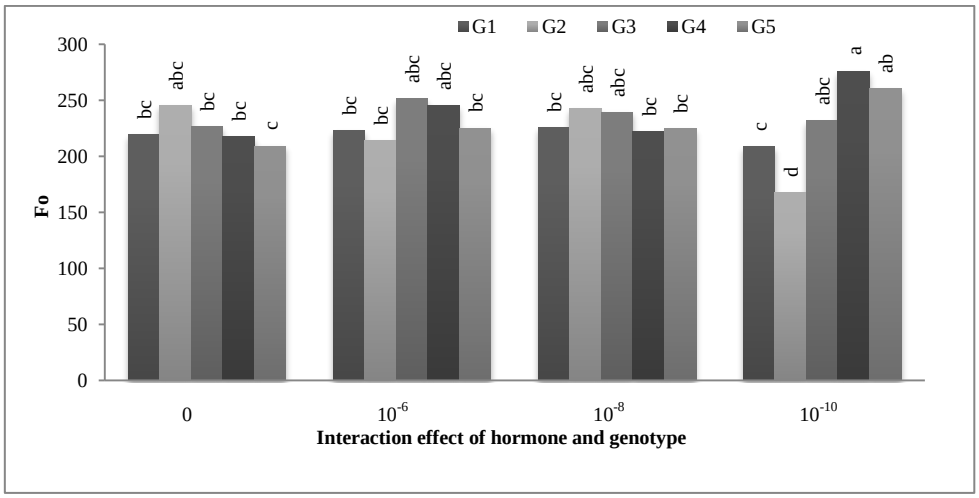

Fig. 4. Interaction effect of genotypes and brassinosteroid concentration on primary Florescence chlorophyll in pistachio

Discussion

Swamy *et al*., 2010 showed that growth increase in *Pelargonium graveolens* as the result of applying A P-24 brassinolid caused increase in leaves photosynthesis and finally increase in biomass accumulation of shoots. Increase in photosynthesis speed was for increase in chlorophyll content of leaves as the result of using hormones. The findings showed that LAI had increased in the treatments under study. By using brassinosteroid hormones, leaves area can be increased and in this way, photosynthesis increases and gain a higher yield (Daneshvar and Kiani, 2004).

There is also a positive relationship between external use of brassinosteroid and root growth in *Arabidopsis thaliana* and applying castasterone 24-Ap and brassinolide 24-Ap stimulated root growth in this plant.Similarly,applying BRZ2001 that is a brassinosteroid inhibitor, completely stopped root growth. However, brassinolide 24-Ap reversed the inhibitive effects and revived the root's normal growth (Mozorra *et al*., 2004). Such responses are related to the ability to release internal potential (genetic potential) of the plant for rooting (Swamy *et al*., 2010).

Using homobrassinolide-24 in the conditions of using hormone caused significant improvement in dry weight of the root.

One of the main reasons of decrease in dry weight of the plant's shoot during treatment is related to producing active varieties of oxygen or ROS. During drought stress, active varieties of oxygen increase which cause disorder in electron transmission system and cause antioxidant activities in chloroplasts, mitochondrial and micro -bodies (Sofo *et al*., 2005).In normal conditions, plants inhibit ROS production through producing anti-oxidant enzymes (Inze and Montagu, 2000). Meanwhile during severe water stress, producing ROS varieties goes farther than antioxidant system capacity to omit these varieties that leads to oxidative stress (Sofo *et al*., 2005).In this experiment, drought stress caused significant decrease in parameters of development including root length, root dry and wet weight, stem diameter, number of secondary branches, plant's height and eventually wet and dry weight of savory shoot.

The result of decrease in cell size in relation with the pattern of plant growth depends on the time of occurring water shortage based on plants physiology.

If water stress occurs in the beginning of plant's cycle of growth, leaf area decreases and as a result, carbon fixation will decrease in the season of plant's growth. Other secondary effects caused by decrease in leaf area include change in the pattern of water and nitrogen consumption (Miri, 2008). Mirza *et al*., 2004 announced that in stressed samples of savory, blooming occurs sooner than others do and stress causes decrease in wet and dry weight of root, stem and stem height in stressed samples.

Abd, Krima, 2004 stated that using spirimidine and stigmasterol caused increase and improvement in the parameters of chamomile growth (including height, number of secondary branches, fresh weight and dry weight) during vegetative stage. Using 10^{-10} and 10^{-8} molar hormone concentrations in irrigation decrease conditions cause significant increase of stem diameter and the number of secondary branches in under-stress plants as compared to the control plants (Fig. 2)

It was revealed in this experiment that using homobrassinolide -24 in 10^{-8} and 10^{-10} molar concentrations caused significant increase and improvement in parameters of development and yield of the crop such as sidling height, LAI and the amount of chlorophyll .The findings indicated that using homobrassinolide -24 plant -growth regulator with its anti-oxidant properties is essential for maintaining economic yield of the plants under stress and is justified. On the other hand, gaining the point of crossing between different genotypes and homobrassinolide -24 in order to determine genotypes that are more tolerant is not something to ignore. Anti-oxidant increase is a defensive activity that decreases peroxidation of membrane lipids and its permeability and prevents leakage of electrolytes. Thus increase in lypoxygenas, which is in 10^{-10} concentration, indicates seedling reaction toward hormone. This confirms the findings of Khavarinejad *et al*., 2010 that showed salicylic acid in nickel-treated plants significantly increased the level of activity of lypoxygenasis in the plant. According to the findings, one of the reasons for decrease in chlorophyll b and its insignificance is increase in lypoxygenasis. One of the properties of lypoxygenasis enzyme is its power to decolorize and this confirms with the findings of Moharami *et al*., 2009.

The findings show that using 10^{-10} molar homobrassinolide increases production of dry matter by stimulating parameters of growth and as this increase is significant in comparison with control treatment it is justified economically. The findings of this test proves that in mild and severe stress conditions, using this substance causes significant increase in LAI and sidling height against control treatment. Considering climatic conditions and environmental stresses in planting pistachio, choosing suitable genotype, tolerate to unsuitable environmental conditions can be effective in reducing costs of production.

Increase in root biomass as the result of using brassinosteroids is related to increase in activity of invertase acid (an enzyme changing glucose to fructose) in young leaves, which causes more assimilation in plants and as a result bigger roots in the plants (Schilling *et al*., 1991).The ability of polyamines in stimulating rooting is well known. In a study on Coleus, the role of brassinosteroids in rooting has been proved. Not only brassinosteroids are effective in rooting, they also play a part in improving root growth. Applying 100 micro-molar brassinosteroid in coleus had a great impact in increasing root growth (Swamy and Rao, 2010).

Chlorophyll florescence induction is a rapid and non-destructive technique for measuring photosynthesis electron transmission in plants.

One of the important enzymatic systems in relation with change in cell membrane lipids is the enzymatic system of lypoxygenasis. Lypoxygenasis enzyme controls combination reaction between oxygen molecule and non-saturated fatty acids and producing non-saturated hydroproxides. Damage to cell membrane, increase in ion leakage and cellular fluid waste is related to the changes in lypoxygenasis enzyme activity (Hodges *et al*., 2003).

Using hormone in severe and mild tension causes significant increase in shoot wet weight as compared

to control plants under treatment. Janke *et al.*, 2004 found that using brassinosteroids decreased to some extent the damage of water stress in soya growth through improvement in antioxidant system and stimulating accumulation of dry weight. Brassinosteroids also cause significant increase in plant biomass (Li *et al*., 2008). Even though brassinosteroids and ROS act as the second messenger in reviving defense system in under stress plants, the relationship between brassinosteroids and ROS in transmitting stress message is not known yet (Cao *et al.*, 2005). Behnamnia *et al*., 2009 showed that water shortage causes decrease in wet and dry weight of stem. Using Brassinolide -24 AP significantly, increased wet weight of stems and dry matter of the plant under stress condition and control. The plant's dry matter weight under average stress condition 27% and under severe conditions of stress 70% showed increase in brassinolide-24 Ap treatment. Using brassinosteroids causes physiological and biochemical changes in tomato sidling including root volume increase, antioxidant content (scorbates and carotenoids) and content of free prolenebeside increase in SOD, CAT, POD and APX activity. In the contrary, in brassinosteroids treatments, the contents of MDA and H_2O_2 significantly decreased. The manner brassinosteroids effect on reducing the results of severe stress needs more studies. However, the findings show that treatment by brassinosteroids can reduce water stresses in savory. Thus, this method can act as a managing tool in improving yield in dry and semi-dry regions.

Conclusions

The effect of four concentrations of plant-growth regulators of homobrassinolide-24 on the traits of LAI, wet root weight ,sidling height, shoot wet weight, lypoxygenasis enzyme and chlorophyll florescence in fine genotypes were studied. Using brassinosteroids-24 in 8^{-10} and 10^{-10} molar concentrations caused improvement and significant increase in growth parameters and yield of the produced material such as sidling height, LAI and the amount of chlorophyll. The genotype containing G5 and G1 had the best-measured indexes and parameters.

Using plant-growth regulators of homobrassinolide-24 that have antioxidant properties is essential and justified for maintaining economic yield of the plants. Findings show that using 10^{-10} molar concentrations of homobrassinolide-24 increases the production of dry matter by stimulating growth parameters by stimulating growth parameters and as this increase is significant as compared with control treatment, it is justified economically. The findings of this study proves that in mild and severe stress conditions using this substance causes significant increase in LAI and sidling height as compared to control treatment. Considering climatic conditions and environmental stresses in planting pistachio, choosing suitable genotype tolerant to unfavorable environmental conditions can be effective in reducing costs of production.

Acknowledgements

The authors thank Mr. Evazabadian and Mr. Mohammadi moghaddam for their generous technical assistance.

References

Abd E, Krima M (2004) Stimulation of growth، flowering، biochemical constituents and essential oil of chamomile plant (*Chamomilla recutita*l. rausch) with spermidine and stigmasterol application. Plant Physiology. 30(1-2), 89-102.

Behnamnia M, Kalantari Kh M, Rezanejad F (2009) Exogenous application of brassinosteroid alleviates drought-induced oxidatives tress in *Lycopersicon esculentum* L. General and Applied Plant Physiology. 35 (1–2), 22–34.

Cao S, Xu Q, Cao Y, Qian K, An K, Zhu Y, Binzeng H, Zhao H, Kuai B (2005) Loss of function mutation in Det2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. Plant Physiology. 123, 57-66.

Clous Steven D, Sasse M (1998) Brassinosteroids: essential regulators of plant growth and development. Annual reviews. 49, 427-451.

Clouse SD, Zurek DM, Morris TCM, Baker ME (1992) Effect of brassinolide on gene expression in elongating soybean epicotyls. Plant Physiology. 100, 1377-1383.

Daneshvar HA, kiani B (2004) Effect of salinity on some local cultivars measures in Isfahan province. Research and Development. 65, 83-76.

Eskandary M (2011) The effect of 28 Hemi Brasynvsteroids to reduce the effects of drought savory. Master's thesis-Islamic Azad University, Arsanjan Branch. Pp. 26-85.

Hessler TG, Thomon MJ, Benscher D, Nachit MM, Soorrells ME (2002) Association of a lipoxygenase locus, Lpx-B1, with variation in lipoxygenase activity in durum wheat seeds. Crop Sciences. 42, 1695-1700.

Hodges DM (2003) Postharvest oxidative stress in horticultuer crops food. Product Press. 266p

Inze D, Montagu MV (2000) Oxidative stress in plants. TJ International Ltd, Padstow, Carnawell. Great Britain. Pp. 321.

Janke R (2004) Farming a few acres of herbs: Calendula. Kansas State University Agricultural Eiperiment Station and Cooperative Extension Service, MF-2610.

Khripach VA, Zhabinski VN, de Groot AE (1999) Brassinosteroids a new class of Plant Hormones. Academic Press Publication. 472p

Khavari nejad R, Kazemi N, Fahimi H, Saadatmand S (2010) Effect of exogenous salicylic acid on lipid peroxidation and antioxidant enzyme activities in leaves of plant sunder stress canola nickel. Journal of Biological Sciences. 10(3), 71 -80.

Li KR, Wang HH, Han G, Wang QJ, Fan J (2008) Effects of brassinolide on the survival, growth and drought resistance of Robinia pseudoacacia seedlings under water – stress. New Forests. 35, 255 – 266.

Mazorra LM, Nunez M, Hechavarria M, Coll F, Sanchez- Blanco MJ (2002) Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperatures. Biologia Plantarum. 45, 593–596.

Miri HR (2008) Water use efficiency in plant biology. Arsanjan Azad University Press. 522pages.

Mirza M, Baher Nik Z, Abbaszadeh B (2004) Quantitative and qualitative evaluation of oil Savory (*Satureja hortensis* L.) during thedroughton the farm. Medicinal Plants Research Institute of Forests and Rangelands. 21, 76-88.

Moharrami I, Shahedi M, Kadivar M (2009) To evaluate the activity of amylase, lipase and lipoxygenase in wheat and alter their activity Before and after germination. Journal of Science and Technology of Agriculture and Natural Resources. 13(47), 1-13. [In Persian].

Swamy K, Rao NS, Ram S (2010) Effect of brassinosteroids on rooting and early vegetative growth of Coleus [*Plectranthus forskohlii* (Willd.) Briq.] stem cuttings.

Swamy K, Rao NS, Ram S (2010) Effect of brassinosteroids on rooting and early vegetative growth of Coleus [*Plectranthus forskohlii* (Willd.) Briq.] stem cuttings. Indian Journal of Natural Products and Resources. 1(1), 74-79.

Sofo A, Tuzio AC, Dichio B, Xiloyannis C (2005) Influence of water deficit and rewatering on the components of the ascorbate-glutathione cycle in four interspecific Prunus hybrids. Plant Sciences. 69, 403-412.

Sofo A, Tuzio AC, Dichio B, Xiloyannis C (2005) Influence of water deficit and rewatering on the components of the ascorbate-glutathione cycle in four interspecific Prunus hybrids. Plant Sciences. 69, 403-412.

Schilling G, Schiller C, Otto S (1991) Influence of brassinosteroids on organ relations and enzyme activities of sugar-beet plants. In "Brassinosteroids. Chemistry, Bioactivity and Applications. ACS Symposium Series" (H.G. Cutler. Phytochemistry Research Progress.

Swamy KNS, Rao R, Ram S (2010) Effect of brassinosteroids on rooting and early vegetative growth of Coleus [Plectranthus forskohlii (Willd.) Briq.] stem cuttings. Indian Journal of Natural Products and Resources. 1(1), 68-73.