



## Study the physiological and biochemical properties of peppermint (*Mentha pipertis* L.) in response to salt stress and coexistence with *Piriformospora indica* fungi

M. Khalvandi<sup>1</sup>, M.R. Amerian<sup>2</sup>, \*H. Pirdashti<sup>3</sup>, M. Baradaran Firouzabadi<sup>2</sup> and A. Gholami<sup>2</sup>

<sup>1</sup>Ph.D. Student, Dept. of Agronomy, Shahroud University of Technology, Iran,

<sup>2</sup>Associate Prof., Dept. of Agronomy, Shahroud University of Technology, Iran,

<sup>3</sup>Associate Prof., Dept. of Agronomy, Genetic and Agricultural Biotechnology Institute Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

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### Abstract

**Background and Objectives:** In Iran, extension of soils salinity along with high levels of water salinity could limit product quality and productivity. Furthermore, in the recent years global demand for the medicinal herbs such as peppermint is increasing, the use of microorganisms that have a symbiotic relationship with plants is a new strategy to improve the environmental performance of the plants in adverse conditions such as saline water and soil. In this study, therefore, the symbiotic effect of mycorrhiza like fungi, *Piriformospora indica*, on the growth improvement of peppermint was designed and implemented under salt conditions.

**Materials and Methods:** The experiment was conducted in pot, arranged in factorial based on randomized complete blocks design with three replications in 2015. Two factors were studied including *P. indica* symbiosis and uninoculated control and four salinity levels (0, 3, 6 and 9 dSm<sup>-1</sup>). The measured parameters were leaf dry weight, root colonization, root length, lipid peroxidation, ion leakage, total phenol content of leaves, flavonoids, anthocyanins and the antioxidant enzymes activity (catalase, superoxide dismutase and polyphenol oxidase, radical scavenging DPPH).

**Results:** Results showed that the root colonization, leaf dry weight and root length decreased by increasing salinity, while catalase, superoxide dismutase and polyphenol oxidase, radical scavenging DPPH, total phenol content of leaves, flavonoids, anthocyanins, lipid peroxidation and ion leakage increased. There was a significant correlation ( $r=0.95^{**}$ ) between antioxidant activity and phenol content. Therefore, the high antiradical activity has been attributed to the high levels of phenolic compounds. The symbiotic fungi improved plant dry weight, root length, phenolic compounds and antioxidant enzymes. Biological treatment, also, ameliorated the negative effects of salinity on membrane peroxidation, cell membrane stability and activated the antioxidant enzymes activity in saline conditions. In general, the *P. indica* inoculated plants performed better than the non-inoculated plants in terms of growth performance, however, this increase was different under different salinity levels.

**Conclusion:** In conclusion, the utilization of *P. indica*, not only stimulate the plant to increase the synthesis of phenolic compounds, but also developed the root system by fungal hyphae resulted in alleviating the effect of salinity in the peppermint. Therefore, it seems that *P. indica* could induce salt tolerance and roughly improve growth parameters of peppermint plants faced salt stress conditions.

**Keywords:** Anthocyanins, Antioxidants, Growth, Peppermint, Total flavonoids, Total phenol

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\* Corresponding author; h.pirdashti@sanru.ac.ir



## Induction of parthenogenetic haploid embryos and production of haploid plants in cucumber (*Cucumis sativus* L.)

H. Ebrahimzadeh<sup>1</sup>, H. Soltanloo<sup>2</sup>, \*M. Enayati Shariatpanahi<sup>3</sup>,  
A. Eskandari<sup>4</sup> and S.S. Ramezanpour<sup>2</sup>

<sup>1</sup>Ph.D. Student of Nuclear Agriculture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, <sup>2</sup>Associate Prof., Dept. of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, <sup>3</sup>Associate Prof., Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran, <sup>4</sup>Assistant Prof., Nuclear Agriculture Research School- Nuclear Science and Technology Research Institute, Karaj, Iran

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### Abstract

**Background and Objectives:** The objective of haploid plant production is to obtain doubled haploid (DH) lines universally suited to breeding programs. Crop productivity can be greatly improved by using F<sub>1</sub> hybrids, which are made by crossing pure lines with some important traits. These pure lines are very valuable in breeding programs and genetic researches. Production of pure lines in a conventional breeding program via self-fertilization takes a long time and requires high costs, but still may not be 100% homozygous. Alternative biotechnological approaches are by far more efficient and sustainable than traditional methods. The most common and best-known method of obtaining haploid cucurbit plants is via pollination with irradiated pollen, which induces parthenogenetic development of haploid embryos in plants. According to the previous reports, there has not been consensus for superiority of gamma ray doses between 300 and 500 Gy; however these two doses have not been simultaneously compared in any research study published so far. The aim of this study was to investigate the possibility of haploid embryos induction in different cucumber cultivars through pollination with gamma-irradiated pollen and determine the optimal dose of irradiation for cucumber haploid plants production.

**Materials and Methods:** In this study the effects of irradiation doses and maternal genotypes on fruit set, seed production, parthenogenetic embryo induction were investigated after pollination by irradiated pollen and production of haploid plants in cucumber. This research study was performed in a factorial experiment with two factors (gamma irradiation dose and plant genotype) based on a completely randomized design (CRD) with 6 replications. Seven cucumber genotypes including, Extreme F<sub>1</sub>, Negin F<sub>1</sub>, Karim F<sub>1</sub>, Super Dominus F<sub>1</sub>, Beith Alpha OP, Basmenj and Dasgerdi were pollinated with gamma-rays irradiated pollen grains at doses including 300 and 500 Gy. The pollen viability and pollen tube behavior after irradiation were evaluated. The seeds extracted from the fruits were rescued by *in vitro* embryo culture, and the ploidy level of the plantlets obtained was determined by flow cytometry.

**Results:** The impact of different doses of gamma irradiation, genotype, and interaction of dose and genotype on total number of seeds produced were significant. The highest number of parthenogenetic embryo were observed in genotype Extreme irradiated with dose of 300 Gy (2.25) and genotype Karim irradiated with dose of 500 Gy (1.75). No embryo and plant was

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\* Corresponding author; [mehran.shariatpanahi@abrii.ac.ir](mailto:mehran.shariatpanahi@abrii.ac.ir)

obtained in genotypes Negin, Extreme and Beith Alpha irradiated with the dose of 500 Gy. The difference of various doses of irradiation on production of haploid plants was not significant, but genotype and especially interaction effect of dose and genotype were significant on production of haploid plants. Pollination of genotype Extreme by 300 Gy – irradiated pollen grains gave rise to the highest number of haploids (1.25Hs/fruit) produced in this experiment.

**Conclusion:** In this research study similar to previous studies, it was confirmed that production of haploid plants using irradiated pollen grains is an efficient method for producing haploid inbred lines in cucumber. According to our results, different genotypes exhibited different responses to various doses applied and parthenogenic embryogenesis can be induced when optimal dose of irradiation was applied in each genotype.

**Keywords:** Cucumber, Embryo rescue, Haploid, Irradiation, Parthenogenesis



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## Transient expression-based CRISPR/Cas9 system for manipulation of tall fescue *SGR* gene

**\*M. Khoshhal Sarmast**

Assistant Prof., Dept. of Horticultural Science, Faculty of Plant Production, Gorgan University of  
Agricultural Sciences and Natural Resources (GUASNR), Gorgan, Golestan 49189-43464, Iran

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### Abstract

**Background and Objectives:** A clustered regularly interspaced short palindromic repeat (CRISPR) is one of the precise genome editing techniques. This technology is a prokaryotic immune system that confers resistance to foreign viruses. A virus-derived spacer sequence helps on endonuclease enzyme to recognize and cut foreign pathogenic DNA. In present experiment guide RNA associated to the tall fescue *SGR* gene (*FaSGR*) joint to the Cas9 protein in CRISPR system so as to target *SGR* in tall fescue (*Festuca arundinacea* 'Jaguar') leaves.

**Materials and Methods:** In order to construct programmable CRISPR/Cas9 system for targeting *FaSGR*, 20 nucleotides of exon originated *FaSGR* gene were selected. This 20 bp firstly integrated into pEn/Chimera vector followed by LR reaction (gateway system) in which aforementioned segment transferred into the *ccdB* frame of expression vector. Sequenced and PCR certified vector by gene specific primers (GSPs) then inserted to the GV 3101 strain of *Agrobacterium tumefaciens*. For construction of overexpression vector (OE) of *SGR* gene, full length of *FaSGR* was isolated from tall fescue leaf through gene specific primers and after cloning in *E-Coli* integrated into pB2WG7 vector using Gateway technology. In second step CRISPR construct and overexpression pB2WG7 vector harboring full length of *FaSGR*, co-transformed into tobacco leaves through Agrobacterium infiltration method. DNA extracted from agro-infected area and sequenced by gene specific primers. In other experiment CRISPR /Cas9 construct transiently and directly expressed in young mature tall fescue leaves subjected to abiotic stress.

**Results:** Polymerase chain reaction and DNA sequencing strongly support guide RNA order in two vectors. Transient co-expression of two separate vectors in tobacco leaves after receiving DNA extraction and sequencing data, vividly showed, although the segment sequence and order of guide RNA is fine but no deletion in *FaSGR* gene was observed by applying CRISPR/Cas9 system. However in second experiment transient expression of CRISPR/CAS9 in tall fescue leaves which having natural *FaSGR* gene under vacuum pressure surprisingly suppress *SGR* expression whereupon improve tall fescue leaf color quality compared to control leaves.

**Conclusion:** Gateway technology which has been used in this experiment greatly improved preciseness of CRISPR/Cas9 construct design and shorten times in contrast to that of restriction enzymes method. On model plant tobacco CRISPR/Cas9 was not successful in order to cut *FaSGR* gene on target site. This likely can be due either to the heterologous expression of this gene in untargeted organisms or different promoter governs genes. However, when we applied this transient expression-based genome editing system on tall fescue leaves under vacuum, we clearly observed *FaSGR* inactivated by CRISPR/Cas9, as a result tall fescue leaves stayed much greener under salt and heat stress than control.

**Keywords:** CRISPR, Gateway technology, Genome editing, Stay green gene

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\* Corresponding author; [mkhsarmast@gau.ac.ir](mailto:mkhsarmast@gau.ac.ir)



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## Variations in phosphorus concentration, accumulation and partitioning in some of crop and weed species of Poaceae family

A. Abidi<sup>1</sup>, \*E. Zeinali<sup>2</sup>, A. Soltani<sup>3</sup> and A.R. Gharanjiki<sup>4</sup>

<sup>1</sup>M.Sc. Student, Dept. of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, <sup>2</sup>Associate Prof., Dept. of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, <sup>3</sup>Professor, Dept. of Agronomy, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, <sup>4</sup>Academic Member, Cotton Research Institute of Iran, AREEO, Gorgan, Iran

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### Abstract

**Background and Objectives:** Phosphorus (P) after nitrogen is the most important nutrient limiting the yield of crops especially in acidic and alkaline soils. Extensive studies indicate considerable genetic variation in the efficiency of uptake and utilization of phosphorus which is related to concentration, accumulation and partitioning of phosphorus to different parts of the plant. The use of this diversity in order to increase the growth and yield of crops, as well as to reduce the need for phosphorus fertilizers, especially in low-phosphorous soils, has attracted the attention of researchers in recent years. In addition, accurate information on this issue can be used in the efficient management of mineral nutrition of crops. Therefore, the present study was conducted to investigate the variations in concentration, accumulation and allocation of P to different parts of the plant in some important crops and weeds species of the Poaceae family.

**Materials and Methods:** This experiment was conducted in a completely randomized design with a factorial arrangement with three replications in Gorgan University of Agricultural Sciences and Natural Resources using a low available P soil (4.85 mg/kg), during 2016-17 growing season. In this pot study, 6 plant and weed species of Poaceae family including durum wheat, common barley, naked barley, triticale, wild oat and canary grass were studied under two conditions of unfertilized and fertilizerd as amount as recommended. In the physiological maturity, P concentration in different parts of plants separately, and P accumulation and allocation coefficients were calculated using P concentration and dry weight of the organs.

**Results:** The results of analysis of variance showed that the effect of genotype, fertilization and their interactions on the amount of accumulated P in all parts of the plant and total plant were significant. The average of P content of the whole plant in the studied species indicates an increase of 4.2 times the amount of P absorbed by fertilizer application. In fertilization conditions, the lowest P content in plant parts and total plant was observed in two weed species, while under non- fertilization conditions, there was no significant difference between species in the plant parts. Alterations in P content in the studied species were due to changes in its determinant components, i.e. the amount of dry matter and P concentration. Although, both the amount of dry matter and P concentration in all parts of the plant significantly ( $P=0.01$ ) were influenced by the factors of the experiment, and in most of the cases, their interactions, the amount of alterations in the dry matter and as a result, its contribution to changes in the P content was more than the P concentration. The average of P content allocated to grain in all species was 54.3%, in the crop species was 64.1% and in the two weed species was 34.7%, which showed a much higher allocation of P to the grains in the crop species.

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\* Corresponding author; e.zeinali@yahoo.com

**Conclusion:** This study showed that the accumulation of P in different parts of plant in physiological maturity stage were influenced by fertilization and species and the interactions between of these two factors more than P concentration and P allocation coefficients. More alterations in P accumulation can be attributed to its dependence on variations in dry matter and P concentrations. The obtained results showed a very low variation of the studied species in term of P accumulation in low available P conditions compared to the conditions of fertilizer application as amount as recommended which is a result of the similarity of their tolerance to P deficiency as well as different response of species to P fertilizer application.

**Keywords:** Allocation coefficient, Plant organ, Poaceae, Weeds



## Effect of compost on micro elements uptake and relationship with physiological traits of saffron (*Crocus sativus* L.)

\*M.H. Aminifard

Assistant Prof., Dept. of Horticultural Science and Special Plants Regional Research Centre,  
College of Agriculture, University of Birjand, Iran  
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### Abstract

**Background and Objectives:** The most expensive medicinal spicy plant in the world is saffron (*Crocus sativus* L.). Nutritional management one of the main factors affecting chemical properties and yield of saffron. Municipal waste compost is one organic fertilizer which moreover contain micro elements and compost to be increased ability this elements. Therefore the effects of application of municipal waste compost on uptake micro elements (Fe, Cu, Zn and Mg) in saffron plant and relation with arable characteristics and yield of saffron were evaluated under field conditions.

**Materials and Methods:** This experiment was carried out based on a randomized completely blocks design with three replications in research farm of University of Birjand, Iran, during cropping year 2015-2016. Treatments were four levels municipal waste compost (0, 5, 10 and 20 t.ha<sup>-1</sup>). The measured indices were included of concentration of micro elements (in soil, leaf, corm and stigma), pigments photosynthesis (chl a, b and total and carotenoids) and arable characteristics (leaf fresh and dry weight, flower fresh yield and average and yield dry stigma). Finally, data analysis was done using SAS 9.1 and means were compared by Duncan's multiple range test at 5% level of probability.

**Results:** Results showed that municipal waste compost improved the concentration Cu, Fe and Mn of soil, corm and stigma of saffron beside control. The highest concentration Fe (3.038 and 4.334 mg. kg<sup>-1</sup>), Mn (1.980 and 3.116 mg.kg<sup>-1</sup>) and Cu (1.094 and 1.802 mg.kg<sup>-1</sup>) of flower and corm were obtained in plants treated with 20 t.ha<sup>-1</sup> municipal waste compost while the lowest values were recorded in the control. Also results showed that municipal waste compost improved the concentration Zn of soil and leaf beside control. The highest concentration Zn (1.633 and 1.240 mg.kg<sup>-1</sup>) of soil and leaf were obtained in plants treated with 20 and 10 t.ha<sup>-1</sup> municipal waste compost. Leaf carotenoides and total chlorophyll, flower and stigma yield were influenced by municipal waste compost treatments. The highest flower and stigma yield were obtained in plants treated with 10 t.ha<sup>-1</sup> municipal waste compost.

**Conclusion:** Thus, results showed that municipal waste compost has significant impact on uptake micro elements of saffron soil and plant. Municipal waste compost hereby improved the arable and yield characteristics of saffron under field conditions.

**Keywords:** Carotenoides, Chlorophyll, Micro elements, Stigma yield

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\* Corresponding author; mh.aminfard@birjand.ac.ir



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## Callus induction and organogenesis from various explants of *Capparis spinosa* L. plant under *In vitro* conditions

M. Sheikhi Hamuleh<sup>1</sup>, \*L. Fahmideh<sup>2</sup>, F. Benakashani<sup>3</sup> and M. Solouki<sup>4</sup>

<sup>1</sup>M.Sc. Student, Dept. of Plant Breeding and Biotechnology, University of Zabol, Zabol, Iran,

<sup>2</sup>Associate Prof., Dept. of Plant Breeding and Biotechnology, University of Zabol, Zabol, Iran,

<sup>3</sup>Assistant Prof., Dept. of Agronomy and Plant Breeding, University of Tehran, Tehran, Iran,

<sup>4</sup>Associate Prof., Dept. of Plant Breeding and Biotechnology, University of Zabol, Zabol, Iran

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### Abstract

**Background and Objectives:** *Caber (Capparis spinosa)* is the most important species in capparaceae family and is considering as a major medicinal plant. Medicinal usage of this plant due to components such as flavonoids, saponines, pectins, essential oils and specially glycosides and glycosinolates in richness of roots, generative buds and fruits. Considering the importance of *Capparis spinose* as medicinal plant, the present study was performed to optimization of culture conditions for callus induction and regeneration of caber.

**Materials and Methods:** This research was performed at bioengineering and biotechnology research center of Esfahan Industrial University. In order to do research, cotyledon leaf, leaf, bud, flag, hypocotyl, root, petals, sepals and node explants were cultured in MS medium with different plant growth regulator compositions. Two levels of 2,4-D (2.5, 3 mg l<sup>-1</sup>) with kinetin (0.1 mg l<sup>-1</sup>) and 3 levels of NAA (2, 2.5, 3 mg l<sup>-1</sup>) in combination with BA (0.5 mg l<sup>-1</sup>) was used for callus induction. This experiment was carried out in a completely randomized design with 4 replications and 5 explants for each. In order to regenerate shoot from produced calluses, calluses were cultured in MS culture Medium containing hormonal composition of KIN, NAA and IBA with different concentrations.

**Results:** The results of analysis of variance indicated that the effect of explants and interactions of PGR × explants were significant at 1% for dry and fresh callus weight and callus percentage; however, the effect of changes in growth regulator for the percentage of callus was not significant. Additionally, the all PGR combination were appropriate for callus induction in this study, but different explants from different specimens showed different response to callus production, such that the flag explants had the highest percentage of callus (100%) in all PGR compartments. The results of this study demonstrated that the best PGR combination for callus weight, was MS medium containing 0.02 NAA mg.l<sup>-1</sup>+1 KIN mg.l<sup>-1</sup> (with mean 0.27 mg.l<sup>-1</sup>) and for mean dry weight of callus, MS medium containing 3 NAA mg.l<sup>-1</sup> + 0.5 BAP mg.l<sup>-1</sup> and MS containing 0.02 NAA mg.l<sup>-1</sup>+1 KIN mg.l<sup>-1</sup> (with an average of 0.026 and 0.027 mg.l<sup>-1</sup> respectively). The best treatment for producing shoots from callus was MS medium containing 2 KIN mg.l<sup>-1</sup> (40%) and 2 NAA mg.l<sup>-1</sup> (40%). Finally the best treatment for rooting was MS medium containing 1 NAA mg.l<sup>-1</sup> (30%).

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\* Corresponding author; l.fahmideh@uoz.ac.ir



**Conclusion:** In this study, the best plant growth regulators and different type of explants, were optimized for callus, shoot and root production for *in vitro* condition. Based on the results, the flag explant and all of used media were appropriate for the callus induction. The highest rate of shoot production in *In vitro* culture was observed on MS medium supplemented by NAA ( $2 \text{ mg l}^{-1}$ ), KIN ( $2 \text{ mg l}^{-1}$ ) and, for the root production in MS medium containing  $1 \text{ (mg l}^{-1})$  of NAA. Therefore use of these treatments and explants, which showed the best calcification and highest production of shoots and roots, is recommended to reproduce this plant *under in vitro* culture conditions.

**Keywords:** Callus, *Capparis spinosa* L., Explants, Growth regulator, *In vitro* culture



## Study of effects of concentration and time of chemical thinning on improve the quality and quantity characteristics of nectarine "Shabrang"

\*W. Asadi<sup>1</sup>, M. Gholami<sup>2</sup>, N. Kazemi<sup>3</sup> and M. Rasouli<sup>4</sup>

<sup>1</sup>Ph.D. Student, Dept. of Horticultural Sciences, Raisin and Grape Research Institute, University of Malayer, Malayer, Iran, <sup>2</sup>Professor, Dept. of Horticultural Sciences, Bu-Ali Sina University, Hamedan, Iran, <sup>3</sup>Ph.D. Student, Dept. of Horticultural Sciences, University of Tabriz, Tabriz, Iran, <sup>4</sup>Faculty of Member, Dept. of Horticultural Sciences, Malayer University, Malayer, Iran  
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### Abstract

**Background and Objectives:** In most fruit trees, despite the fact that all the flowers are not converted to fruit, the amount of production is more than the tree's capacity, which reduces the quality of the crop and reduces the yield of the tree. While in many fruit trees, the conversion of 5 to 15 percent of the flowers into fruits leads to economic production. The size and quality of the fruit produced should be consistent with market conditions. The goal of horticultural science is to achieve the desirable annual yield and produce throughout the life of the garden. This research was carried out to investigate the effect of a few mineral thinners and a biological regulator on the quantitative and qualitative characteristics of the nectarine "Shabrang" and its comparison with hand-thinning of flowers and fruits.

**Materials and Methods:** The experiment was conducted in a commercial garden in Semirom in 2012-2014 as a randomized complete blocks design with 15 treatments and 5 replications. The treatments consisted of lime-sulfur (6, 8% and twice the application of 6%), ammonium thiosulfate (20, 25 ml per liter and twice the application of 20 ml per liter), urea (4, 8% and twice 4%) and apogee (300, 450 mg / L and twice the use 300 mg / L) at flowering stage (spraying in single stage treatments in 70-80% of flowering and two-stage treatments first in 30-40% and then in 70-80% flowering), flower hand- thinning in the 70-80% flowering stage and then four weeks after the flowering stage were applied to selected trees.

**Results:** The results showed that all treatments had a significant effect on the traits of nectarine "Shabrang" at 1% level. The treatments reduced the fruit set, so that in the hand-thinning treatment of flowers, twice ATS 20 ml per liter and twice lime sulfur 6% reduced the fruit set by 50%. Flower hand-thinning were the best treatment in terms of size (11.33 cm<sup>3</sup>) and weight (151.34 gr) and the highest yield was related to control (90.42 kg per tree) and the least yield was related to fruit hand-thinning treatment (64.04 kg per tree). The yield efficiency in control treatment (3.32 (fruit/trunk cross-section area) was higher than other treatments and its least amount was for the twice lime sulfur 6% (1.99 fruit/trunk cross-section area) and twice apogee 300 mg / lit (2.01 fruit/trunk cross-section area). In terms of qualitative traits, the highest total soluble solids were recorded in flower (17.49%) and fruit (16.53%) hand-thinning respectively, after which the best results were obtained in the twice treatments of thinning compounds. The highest amount of total acid was recorded in lime sulfur 6% (4.88 mg / 100 ml juice) and flower hand-thinning (4.26 mg / 100 ml juice) had the highest index of ripening among all treatments. Also, the highest of leaf area was in twice ATS 20 ml/L (70.42 cm<sup>2</sup>) and twice urea 4% (69.18 cm<sup>2</sup>) and the least of leaf area was

\* Corresponding author; wahab.asadi@gmail.com

recorded in control (55.81 cm<sup>2</sup>). Fruit color as one of the qualitative indices in flower hand-thinning (4.74) was higher than other treatments and the least amount of fruit color was related to lime sulfate 6% treatment (3.38).

**Conclusion:** Flower hand-thinning created a more regular thinning, larger and more colorful fruits and with higher sugar. The results also showed that the flower hand-thinning was better than the fruit hand-thinning. In general, two-step treatments of the compounds produced more suitable effects on the quantitative and qualitative traits of nectarine. However lime sulfur in 8% and twice using 6% caused some leaf burning.

**Keywords:** Ammonium thiosulfate, Apogee, Lime-sulfur, Nectarine, Urea



## Effect of thermo-priming on germination, agronomic characteristics and seed oil of safflower (*Carthamus tinctorius*) genotypes

F. Barazandeh<sup>1</sup>, \*M.R. Sabzalian<sup>2</sup>, M. Rahimmalek<sup>2</sup> and S. Karami<sup>3</sup>

<sup>1</sup>M.Sc. Graduate, Dept. of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran,

<sup>2</sup>Associate Prof., Dept. of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran,

<sup>3</sup>Assistant Prof., Dept. of Agricultural Science, College of Agriculture, Payame Noor University (PNU),  
Tehran, Iran

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### Abstract

**Background and Objective:** Safflower (*Carthamus tinctorius*) is one of the oilseed crops in arid and semi-arid regions. Germination and heading stages are considered as the most sensitive developmental stages of safflower. Therefore, the aim of the present study was to determine the effect of thermo-priming on germination characteristics of the safflower seed in the laboratory and then, to evaluate the effect of this type of priming on some agronomic traits, yield and percentage of seed oil of safflower genotypes in the field.

**Materials and Methods:** In the first stage, a factorial experiment was conducted based on a completely randomized design with three replications in laboratory condition. The experimental factors included thermo-priming (40, 60 and 80 °C) at three times (6, 10 and 20 h) and safflower genotypes (primed and non-primed seeds). Germination percentage of seeds and seed germination rate were measured. Then, based on the results of the first stage, another factorial experiment was conducted based on a randomized complete blocks design with three replications. Safflower genotypes were primed only at 60 °C at two times of 6 and 10 h (non-primed seeds were considered as control) and then days to 50% emergence, days to maturity, number of heads per plant, 1000-seed weight, seed yield per plant and seed oil percentage were measured during the experiment and at the end.

**Results:** The results of the first stage revealed that pre-sowing seed treatment by thermo-priming had a significant effect on the percentage and rate of germination. The highest percentage and rate of germination were obtained at temperatures of 40 and 60 °C in the short period of priming (6 and 10 h). In field, according to the results of the first stage, pre-treatment of seeds at 60 °C resulted in a reduction of the day to 50% seed emergence in primed genotypes compared to the control genotypes. On the other hand, thermo-priming resulted in increasing the day to maturity, seed yield per plant and percentage of seed oil, but the number of heads per plant was not affected by thermo-priming.

**Conclusion:** In general, the results indicated that pre-treatment of seeds with thermo-priming (medium temperatures) resulted in the rapid emergence and establishment of safflower seedlings. Moreover, the results showed that thermo-priming at 60 °C not only had no negative effect on safflower seed yield and percentage of oil, but also significantly increased the traits in primed genotypes compared to non-primed ones. It is worth noting that the spring planting of primed seeds of safflower can lead to improved seed yield and oil yield compared to planting of non-primed seeds.

**Keywords:** Germination rate, Percentage of germination, Phenological traits, Temperature, Yield

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\* Corresponding author; [sabzalian@cc.iut.ac.ir](mailto:sabzalian@cc.iut.ac.ir)



## Investigating the effect of salinity stress and soil microorganisms on the absorption of mineral elements of isabgol (*Plantago ovata* Forsk.)

\*A.R. Dehghani Tafti<sup>1</sup>, S. Mahmoodi<sup>2</sup>, H.A. Alikhani<sup>3</sup> and M. Salehi<sup>4</sup>

<sup>1</sup>Ph.D. of Agronomy, University of Birjand, Iran, <sup>2</sup>Associate Prof., Dept. of Agronomy and Plant Breeding, University of Birjand, Iran, <sup>3</sup>Professor, Dept. of Soil Science Engineering, University of Tehran, Iran, <sup>4</sup>National Salinity Research Center, Agricultural Research, Education and Extension Organization (AREEO), Yazd, Iran

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### Abstract

**Background and Objectives:** *Plantago ovata* Forsk has a natural distribution in flora of Iran and its cultivation has economic importance. Salinity is one of the natural features of ecosystems in semi-arid and arid areas. In salinity stress, the high  $K^+/Na^+$  ratio in plant tissues has been considered as one of the important physiological mechanisms in creating salinity tolerance in some plant species. Studies have shown that arbuscular mycorrhizal fungi and inorganic phosphate solubilizing bacteria increase the  $K^+/Na^+$  ratio of the plant and prevent the negative effects of  $Na^+$  ion. These microorganisms play an important role in increasing the availability and absorption of essential elements and ultimately increasing plant production. This research was carried out to Investigating the effect of salinity stress and soil microorganisms on the absorption of mineral elements of isabgol (*Plantago ovata* Forsk.)

**Materials and Methods:** In order to investigate the effect of salinity stress and microorganisms on the accumulation of mineral elements in isabgol (*Plantago ovata* Forsk.) a factorial experiment based on randomized complete blocks with three replications was conducted in 2014 in research greenhouse of the University of Birjand. First factor was three levels of salinity 2.5, 5 and 10  $dS.m^{-1}$ . Second factor was arbuscular mycorrhizal fungi include four levels (None inoculated, *Funneliformis mosseae*, *Rhizophagus intraradices* and *Glomus fasciculatum*) and third factor was phosphate-solubilizing bacteria includes two levels (*Pseudomonas fluorescens* and No bacteria) which was prepared in soil biology lab of university of Tehran. At the stage of physiological maturity, the plants were removed from the pot and dried. The dry weight of each sample was then measured. In order to measure the elements for drying and combining with chloridric acid, the concentrations of mineral elements in the plant were measured.

**Results:** The results showed that salinity increased the absorption of phosphorus, nitrogen and potassium, increased sodium absorption and sodium / potassium ratio, and reduced the dry weight of the stem. Application of soil microorganisms under salinity stress conditions, in addition to increasing the absorption of essential nutrients, reduced the sodium ion absorption and sodium / potassium ratio and increased stem dry weight. Results showed that the highest percentage of phosphorus was 7.21% in the 2.5  $dS.m^{-1}$  + *Rhizophagus intraradices* + *Pseudomonas fluorescens*. The highest nitrogen content was 2.28% in the 2.5  $dS.m^{-1}$  + Arbuscular mycorrhiza (*Rhizophagus intraradices*). The highest potassium content was 48.7 mg/g dry weight in the 2.5  $dS.m^{-1}$  salinity + *Funneliformis mosseae* arbuscular mycorrhizal fungus. The lowest sodium and sodium / potassium ion in the stem were 3.1 mg/g dry weight and 0.06 mg/g dry weight in the treatment composition of 2.5  $dS.m^{-1}$  and the application of

\* Corresponding author; ahmadreza4814@yahoo.com

arbuscular mycorrhiza *Rhizophagus intraradices* were obtained. Also, the highest dry weight of stems was obtained in the treatment composition of 2.5 dS.m<sup>-1</sup> + *Rhizophagus intraradices* + no bacteria intake of 3.9 grams. Although, this difference was not statistically significant with the combination of treatments in which the *Pseudomonas fluorescens* solubilizing bacterium was used.

**Conclusion:** Overall, the results showed that the application of soil microorganisms could compensate for the negative effects of salinity stress by increasing the absorption of essential elements and reducing the absorption of sodium element. Simultaneous use of *Pseudomonas fluorescens* and *Rhizophagus intraradices* can best reflect the absorption of elements under salt stress conditions and increase the production of isabgol.

**Keywords:** Arbuscular mycorrhizal fungi, Haloculture, Macro elements, Phosphate-solubilizing bacteria



## Investigation of the effects of ABA on alleviating drought stress in *Lilium ledebourii* Bioss under *in vitro* condition

\*E. Chamani<sup>1</sup>, S. Hosseini Niari<sup>2</sup>, H. Maleki Lajayer<sup>3</sup>,  
Y. Poorbeyrami-Hir<sup>4</sup> and M. Torabi Gayglou<sup>4</sup>

<sup>1</sup>Professor, Dept. of Horticultural Science, Faculty of Agricultural Science and Natural Resource, University of Mohaghegh Ardabili, <sup>2</sup>M.Sc. Graduate, Dept. of Horticultural Science, Faculty of Agricultural Science and Natural Resource, University of Mohaghegh Ardabili, <sup>3</sup>Assistant Prof., Dept. of Plant Sciences and Medicinal Plants, Faculty of Meshkin-Shahr Agricultural Science, University of Mohaghegh Ardabili, <sup>4</sup>Assistant Prof., Dept. of Horticultural Science, Faculty of Agricultural Science and Natural Resource, University of Mohaghegh Ardabili  
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### Abstract

**Background and Objectives:** The *Lilium ledebourii* Bioss is an endangered and wild species native to native to forest and moist area of Iran, having a high potential for unique beauty in Iran and world flower market. Water deficit produces a cascade of physiological responses that ends-up in decreased production and reduced crop quality. Some factors involved in plant responses to water stress. Several mechanisms that plants utilize to cope with water stress are mediated by abscisic acid. So, the aims of this study were to assess the effects of ABA in alleviating the adverse effect of polyethylene glycol- induced drought stress in lilium plants under *in vitro* condition.

**Materials and Methods:** This study was carried out as factorial based on completely randomised design with 5 replications in tissue culture laboratory of Department of Horticultural Science, Faculty of Agricultural Science and Natural Resources, University of Mohaghegh Ardabili. *In vitro* bulblets derived from hormone- free MS medium were cultured on MS medium containing different concentrations of PEG (0, 15, 30, 60 gr/l) and ABA (0, 1, 10, 100  $\mu$ m). Vegetative traits such as bulblet number, bulblet fresh weight, plant fresh weight, root length and root number, plantlet fresh weight, leaf and scale number, and also some physiological traits in response to drought stress such as proline, chlorophyll, anthocyanins, phenols and flavonoids content were measured after 70 days from the beginning of the culture.

**Results:** Results showed that application of ABA and PEG into the cultural medium affected all of the studied traits, significantly ( $P \leq 0.01$ ). Vegetative parameters of plantlets such as, root number and root length, bulblet fresh weight, bulblet number, bulblet scale, leaf number and plant fresh weight were declined by increasing the severity of drought stress. Application of lower concentrations of ABA (1 and 10  $\mu$ m) enhanced plant growth rate, but a higher level (100  $\mu$ m) of this plant hormone reduced growth rate under drought stress. Accumulation of phenolic compounds, anthocyanins, flavonoids and proline in tissues of plantlets subjected to severe drought stress were respectively 5.5, 4.5, 3.15 and 2.15 times more than control. While, there was a considerable drop in leaf chlorophyll content of stressed plantlets compared to the control. Moreover, application of ABA into cultural medium under drought stress condition, increased proline content, anthocyanins, flavonoids and phenolic compounds in the leaves. This

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\* Corresponding author; echamani@yahoo.com

increased concentrations of these secondary metabolites and osmolytes might resulted in an increased plantlet growth under drought stress.

**Conclusion:** According to the results it can be deduced that growth rate and bulblet formation in liliium plantlet were reduced as a result of drought stress. However, using ABA at lower concentrations in cultural medium enhanced plantlet growth rate by increasing secondary metabolites and osmolytes. While, this compound at higher concentrations showed an inhibitory effect on *in vitro* plantlets.

**Keywords:** Bulblet, Chlorophyll, Proline, Secondary metabolites





## Effect of sodium nitroprusside on physiological and biochemical response of *Solanum tuberosum* cv. Agria under salinity stress and *in vitro* condition

Zh. Mohammadi<sup>1</sup>, A.R. Motallebi Azar<sup>2</sup>, F. Zaree Nahandi<sup>2</sup>,  
A.R. Tarinejad<sup>3</sup> and \*Gh.R. Gohari<sup>4</sup>

<sup>1</sup>M.Sc. Graduate, Dept. of Horticulture Science and Engineering, Faculty Agriculture, University of Tabriz, Tabriz, Iran, <sup>2</sup>Associate Prof., Dept. of Horticulture Science and Engineering, Faculty Agriculture, University of Tabriz, Tabriz, Iran, <sup>3</sup>Associate Prof., Dept. of Biotechnology, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran, <sup>4</sup>Assistant Prof., Dept. of Horticulture Science and Engineering, Faculty Agriculture, University of Maragheh, Maragheh, Iran

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### Abstract

**Background and Objectives:** Salinity stress is the crucial factor that seriously limits agricultural production in various regions especially in arid and semi-arid areas. Nitric oxide is a bioactive molecule that synthesizes via enzymatic and non-enzymatic pathways under stress conditions in different organs of plant, regulates and adjustments defense reactions of plant. Sodium nitroprusside (SNP) is used as a releasing compound of nitric oxide. Many studies have shown that this compound can protect plant under oxidative stresses and maintain chlorophyll. SNP could improve the effects of salinity and increased chlorophyll.

**Materials and Methods:** This experiment was conducted at the plant tissue culture laboratory in Department of Horticultural Sciences, University of Tabriz, Iran, during spring–summer of 2017. This study was investigated the effect of sodium nitroprusside as a nitric oxide donor, on growth characters (leave number, plantlet height, fresh and dry weight), physiological (chlorophyll a, b and carotenoids) and biochemical (antioxidant activity, total phenol, protein and glycine betaine) characters of *Solanum tuberosum* cv. Agria under salinity stress and *in vitro* condition. For treatment the single node of *S. tuberosum* cv. Agria stem were cultured in MS medium with ½ MS medium, four levels of sodium nitroprusside (0, 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> mM) and two levels of salinity (0 and 70 mM). Treatments were factorial combinations of salinity as completely randomized design with 3 replications.

**Results:** Obtained results showed that under salinity stress leaves number, height and fresh and dry weight of plantlets decreased, also chlorophylls, carotenoids, protein and total phenol of plantlets showed significant reduction. In contrast, antioxidant activity and glycine betaine content of *in vitro* plantlets significantly increased. Application of sodium nitroprusside in media culture caused to improvement of growth, physiological and biochemical characters under salinity stress.

**Conclusion:** Antioxidant activity, total phenol and protein were decreased salinity stress. In addition, antioxidant activity and glycine betaine content during salt stress period was decreased application of nitric oxide. The glycine betaine content of plantlets in general condition with application of sodium nitroprusside increased but under salinity stress, sodium nitroprusside had negative effect on glycine betaine content. The results of this research showed that with increasing antioxidant activity, total phenol, protein can tolerate salt solution and also applying the SNP enhance plant tolerance to salinity. Further studies are necessary to determine optimum concentration and duration of NO application in order to achieve maximum benefit of this chemical in *Solanum tuberosum* tissue culture.

**Keywords:** Antioxidant activity, Glycine betaine, Nitric oxide, Salinity stress

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\* Corresponding author; gholamreza.gohari@gmail.com



Gorgan University of Agricultural  
Sciences and Natural Resources

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## **Effect of 24- Epibrassinolide foliar application on the “Camarosa” strawberry plant growth and fruit yield under salinity stress condition in soilless culture**

**\*S.M. Zahedi<sup>1</sup>, Z.S. Asgarian<sup>2</sup>, R. Gholami<sup>3</sup> and F. Karami<sup>4</sup>**

<sup>1</sup>Assistant Prof., Dept. of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh, Iran, <sup>2</sup>M.Sc. Graduate, Dept. of Horticultural Sciences, Bu-Ali Sina University, Hamedan, Iran, <sup>3</sup>Assistant Prof., Horticultural Crops Research Department, Kermanshah Agricultural and Natural Resources Research and Education Center, AREEO, Kermanshah, Iran, <sup>4</sup>Assistant Prof., Horticultural Crops Research Department, Kurdistan Agricultural and Natural Resources Research and Education Center, AREEO, Sanandaj, Iran

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### **Abstract**

**Background and Objectives:** Salinity stress is one of the most important environmental stresses that significantly reduce growth and yield of most herbaceous species. It is considered as the most important threatening factor in production of crops in several parts of the world. Brassinosteroids are the first steroid hormones found in plants that have growth stimulator activity. They are also effective in reducing the adverse effects of environmental stresses. 24-Brassinolide is known as a reducing agent against various biological and non-biological stresses. Therefore, the present study was carried out with respect to the effect of brassinosteroids on salinity. This study was designed and implemented to investigate the effect of different concentrations of 24-epibrassinolide on the quantitative and qualitative characteristics of strawberry fruit under salinity stress.

**Materials and Methods:** The present study investigated the effect of extrinsic application of 24-epibrassinolide as an active 24-brassinosteroid (0, 2 and 4  $\mu\text{mol}$ ) on fruit yield, number of fruits, flowers and leaves, leaf area, fresh and dry weight of root, relative water content of leaves, fruit size, leaf necrosis, total soluble solid, firmness, soluble carbohydrates, proline, total chlorophyll, electrolyte leakage, superoxide dismutase and catalase under salinity stress with different concentrations ( $S_1=0$ ,  $S_2=15$ ,  $S_3=30$ ,  $S_4=45$  Mm). It was carried out as a factorial experiment in the form of random complete blocks design with 3 replications in non-greenhouse conditions of University of Maragheh.

**Results:** The results of this study showed that salinity of 45 mM increased necrotic leaves (15.8%), ion leakage (52%), and proline (137  $\mu\text{g/g}$ ) in stressed plants, i.e. the plants increase their proline content, which is part of the antioxidant system, to resist stress. Using 24-brassinolide at the concentration of 4  $\mu\text{mol}$  showed the greatest effect on yield (59.25 g/plant), leaf area (96.25  $\text{cm}^2$ ), number of flowers (8.96), fruits (5.78) and leaves (17.41), total chlorophyll levels (1.63  $\mu\text{g/mg}$ ), fresh (7.01 g) and dry (1.26 g) weight of root, relative water content of leaves (60.50%), firmness (1.52  $\text{kg/cm}^2$ ), total soluble solid (10.58%), fruit size (3.61  $\text{cm}^3$ ), and reduction of ion leakage (43%) as well. Increasing the activity of catalase and superoxide dismutase enzymes in plants treated with 24-epibrassinolide and salinity stress versus the ones that were only exposed to salinity stress showed activation of the antioxidant system and plant protection by 24-Brosinosteroids and reduction of oxidative damage in these plants.

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\* Corresponding author; s.m.zahedi@maragheh.ac.ir

**Conclusion:** The present study showed that using plant biochemical regulator such as brassinosteroids have successfully reduced the adverse effects of salinity stress on growth parameters and fruit yield of strawberry cv. Camarosa. Therefore, the results of this research can be used to progress cultivation of strawberry cultivars in saline areas.

**Keywords:** 24-epibrassinostroide, Proline, Salinity stress, Strawberry, Superoxide dismutase



## Effect of nitrogen source on growth parameters, chlorophyll content and some elemental concentration of basil (*Ocimum basilicum* L.) in soilless culture conditions

A. Ansari<sup>1</sup>, \*A. Hassani<sup>2</sup>, B. Dovlati<sup>3</sup> and F. Sefidkon<sup>4</sup>

<sup>1</sup>M.Sc. Graduate, Dept. of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran,

<sup>2</sup>Professor, Dept. of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran,

<sup>3</sup>Assistant Prof., Dept. of Soil Science, Faculty of Agriculture, Urmia University, Urmia, Iran,

<sup>4</sup>Professor, Research Institute of Forests and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

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### Abstract

**Background and Objectives:** Basil (*Ocimum basilicum* L.) is an annual, aromatic and herbaceous plant belonging to the Lamiaceae family which is used as fresh vegetable and traditionally used to treatment many diseases. Plant nutrition is one of the important factors that affect quantitative and qualitative characteristics of plants. Nitrogen is one of the most important nutrients for plant growth and development. Plants can absorb nitrogen either as nitrate (NO<sub>3</sub><sup>-</sup>) or ammonium (NH<sub>4</sub><sup>+</sup>), and for optimal uptake and growth each plant species requires a different nitrate to ammonium ratio. This experiment was conducted to investigate the effect of different nitrate to ammonium ratios on morphophysiological characteristics and element concentration of basil.

**Materials and Methods:** To study the effect of different nitrate to ammonium ratios (100:0, 75:25, 50:50, 25:75 and 0:100) on hydroponically grown basil, a pot experiment was conducted in a completely randomized design with four replications. In order to evaluate the growth parameters (plant height, stem diameter, number of leaves, leaf area, number and length of axillary shoots, fresh and dry weight of leaves, stems and roots), chlorophyll content and nutrients concentration (nitrogen, phosphorus, potassium, iron, copper, manganese and zinc in the leaves and roots), plant sampling was carried out at flowering stage.

**Results:** The results showed that plant growth parameters (such as number of leaves, number and length of axillary shoots, fresh and dry weight of leaves, roots and stems) and chlorophyll index (SPAD) were significantly affected by different nitrate to ammonium ratios. The different nitrate to ammonium ratios had no significant effect on plant height, stem diameter and leaf area. The growth parameters and chlorophyll content decreased with increasing of ammonium in nutrient solution and the 75:25 ratio of nitrate to ammonium was the most suitable treatment for most growth parameters and chlorophyll content. Also nitrogen (N), phosphorus (P) and copper (Cu) concentration in leaves and potassium (K), iron (Fe), manganese (Mn) and zinc (Zn) content in leaves and roots were significantly affected by nitrate to ammonium ratios. As nitrate to ammonium ratio decreased, N, P, Fe and Zn content of leaves increased, while K, Cu and Mn content of leaves and K, Fe and Mn content of roots decreased.

**Conclusion:** The findings of this study showed that basil plant requires lower concentrations of ammonium in nutrient solution for proper growth. Therefore, a solution containing 75:25 of nitrate to ammonium ratio is recommended for basil plant production under hydroponic culture conditions.

**Keywords:** Ammonium, Basil, Hydroponic, Micronutrients, Nitrate

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\* Corresponding author; a.hassani@urmia.ac.ir



## The effect of cropping pattern and manure rates on competitive indices and yield of spinach (*Spinacia oleracea* L.) and garlic (*Allium sativum* L.)

\*E. Azizi<sup>1</sup>, Gh.A. Asadi<sup>2</sup>, R. Ghorbani<sup>3</sup> and S. Khorramdel<sup>2</sup>

<sup>1</sup>Associate Prof., Dept. of Agronomy, Payame Noor University, Iran,

<sup>2</sup>Associate Prof., Dept. of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran,

<sup>3</sup>Professor, Dept. of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

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### Abstract

**Background and Objectives:** Intercropping is cultivation of two or more crops in a land in a growing season that the important goal of this is optimal use of growth resources and labors. The most important advantages of intercropping is higher yield in a certain land by using the various traits such as canopy structure, the ability of root development, height, nutritional needs and efficient use of growth resources. The studies show that the responses of plant species were different in intercropping and monoculture cropping systems. The present research aimed to investigate the planting pattern and different levels of manure on competitive power and agronomic traits of garlic and spinach.

**Materials and Methods:** This experiment was conducted as split plot based on completely randomized blocks design with three replications at the Agricultural Research Station, Ferdowsi University of Mashhad, during growing season of 2011-2012. The treatments were manure rates at three levels (0, 10 and 20 ton.ha<sup>-1</sup>) in main plots and 6 cropping systems (garlic and spinach monocultures and garlic-spinach intercropping with 1:1, 2:2, 3:3 and 4:4 ratio) in sub plots. Studied traits were included in height, fresh and dry weight of spinach and garlic, Land Equivalent Ratio, Aggressivity Coefficient and Competition Ratio.

**Results:** The results indicated that with increasing manure level to 20 ton per hectare, spinach fresh weight increased, significantly but this trend wasn't observed in spinach dry weight. Also with changing cropping pattern, dry and fresh weight of spinach was affected, significantly. The highest dry and fresh weight of spinach was observed in garlic and spinach intercropping with 4:4 ratio. The investigation of interaction effect of planting pattern and manure showed that the highest fresh and dry weight of spinach were obtained in garlic and spinach intercropping with 1:1 and 4:4 ratio under 20 ton per hectare manure, respectively. The lowest spinach dry weight was in garlic and spinach intercropping with 3:3 ratio under 20 ton per hectare manure. Garlic dry and fresh weights weren't affected by manure rates, but cropping pattern affected dry and fresh weights of garlic, significantly. The highest fresh and dry weight of garlic was obtained in garlic monoculture without manure. In studied intercropping systems, land equivalent ratio (LER) was more of one. Also, the highest LER was obtained in intercropping with 3:3 ratio without manure.

**Conclusion:** In general, the best treatment for Aggressivity Coefficient, Competition Ratio and Land Equivalent Ratio was obtained in garlic and spinach intercropping with 1:1 ratio and 20 ton per hectare manure.

**Keywords:** Dry weight, Intercropping, Land Equivalent Ratio (LER), Monoculture

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\* Corresponding author; [azizi40760@gmail.com](mailto:azizi40760@gmail.com)