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Effects of seed pretreatment with salicylic acid on germination of four fenugreek (*Trigonella foenum-graecum* L.) landraces under salinity stress

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Abstract

Background and purpose: Salinity is an environmental factor that all stages of plant growth and seed germination to fruit production of more or less affected. Germination, is one of the most critical stages of plant growth under salinity stress. Under these conditions, pretreatment is a method of improving the germination and growth in environmental stress conditions. Seed treatment is a method which the seed before putting on bed and exposure to environmental conditions, it can be preparations for germination from aspects physiological and biochemical. This study was carried out to evaluate the effect of pretreatment four landraces of fenugreek with salicylic acid on some germination indices and identify the most tolerant landrace to salinity.

Materials and Methods: In this study was conducted in a factorial arrangement in completely randomized design with three replications in laboratory of 'Unique Plants' in Ferdowsi University of Mashhad in 2013. The treatments included four levels of salinity (0, 60, 120 and 180 mM) of sodium chloride and salicylic acid (at a concentration of 0 and 1 mM). In this experiment was studied the effect of salicylic acid on germination of seed pretreatment fenugreek landraces under salinity stress. Traits measured were germination percentage, germination rate, root length, shoot length, root dry weight, shoot dry weight, seedling dry weight and root dry weight to shoot ratio dry weight.

Finding: The results showed that effect of population, salicylic acid and salinity on measured traits were significant at 1% level ($p < 0.01$). The interaction of landrace \times salicylic acid (exception shoot length) and the interaction of salinity \times landrace and salinity \times salicylic acid (except root/shoot ratio) were significant on the all measured

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parameters. But triple interaction of salinity \times acid salicylic \times landrace was not-significant in all measured parameters. All studied traits were significantly decreased by increasing salinity stress. Application of salicylic acid had no significant effect on increasing of studied traits in the absent of salinity stress (0 mM NaCl), but it had a positive effect on germination under salinity treatments, that it caused germination improvement. In general, among the fenugreek populations, 'Isfahan' and 'Amol' populations were more tolerant to salinity under pretreatment and non pretreatment of salicylic acid conditions with respect to the most studied traits.

Conclusion: According to the results, all treatments had significant effects on the traits while the interaction between landrace and the salicylic acid were not statistically significant on shoot length also, landrace and salinity and salinity and salicylic acid interactions had not significant on root to shoot ratio. Landraces of Isfahan and Amol showed high tolerant in growth stages than Hamadan and Yasooj landraces.

Keywords: Fenugreek seed, Germination, Salinity, Salicylic acid



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Compare nutrition of rose flower (*Rosa hybrida* L. cv. Grian Bdprex) with ammonium fertilizers by Cultan method and nitrate in soil culture

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Abstract

Background and objectives: Nitrogen fertilizers are one of the most important factors in the production of greenhouse rose flower. Nitrate and ammonium are main mineral nitrogen sources, which can be absorbed by higher plants roots. Plants can absorb both nitrate and ammonium ions, but some of species are not able to have an optimum growth with ammonium as a sole nitrogen source. The form and application of fertilizers not only affect nitrogen uptake but also improve the absorption of other nutrients. In this research, the effect of form of nitrogen and application of sulfuric acid on growth and some nutrient elements absorption was investigated by rose plants (*Rosa hybrid* L. cv. Grian Bdprex). Considering that rose is one of the most important flowers in the world, and is ranked as the first in the market of cutting flowers, this experiment is carried out to study the effects of different nitrate and ammonium fertilizers by Cultan method in soil culture.

Materials and methods: The experiment was carried out as completely randomized design with 5 treatments and 4 replications under greenhouse condition. Treatments were organic fertilizer + sulfuric acid + ammonium sulfate, organic fertilizer + sulfuric acid + calcium nitrate, organic fertilizer + calcium nitrate + distilled water, organic fertilizer + ammonium sulfate + distilled water and control (without fertilizer or acid + water). Examined plants were 3-years-old rose cv. Grian Bdprex. Plants irrigated every 4 days and used soil was clay loam.

Results: The results showed that, nutrition with Cultan method that was combination of organic fertilizers, ammonium, and sulfuric acid, in soil with high pH, led to an increase in vegetative growth (leaf fresh and dry weight, flower fresh

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and dry weight, stem fresh and dry weight, root fresh and dry weight and flower stem length) flower diameter, vase life, chlorophyll studied, carotenoids and nitrogen, potassium and iron elements concentrations, and a decrease in zinc concentration in the studied plant. For instance, 85 percent increase was observed in leaf dry weight at nitrate calcium + acid treated plants compared to control. Application of nitrate fertilizer with acid in comparison with nitrate fertilizer increased vegetative growth, flower quality, iron, nitrogen and potassium elements concentration, but decreased Zn element concentration.

Conclusion: Therefore, we concluded that due to positive effect of Cultan method on growth, nutrient concentration, chlorophylla, rose flower yield, and vase life of cut flowers, this method can be used for growing rose flower especially in soils with high pH.

Keywords: Ammonium, Cultan, Nitrate, Nutrition, Sulfuric acid



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Optimization of callus induction and somatic embryogenesis in two genotypes of medicinal chavil plant (*Ferulago angulata* L.)

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Abstract

Background and objectives: In order to produce artificial seed, somatic embryogenesis has been introduced as one of the most effective methods to overcome the problems of cultivation, propagation and conservation of medicinal plants. For this reason, callus induction, embryogenesis and somatic embryos of chavil (*Ferulago angulata* L.) were evaluated on diluted Murashige and Skoog growth medium (1/4 MS) in three separate experiments.

Materials and methods: The first experiment (callus induction) was done in a two factors factorial experiment consists of hormones combination (in 10 concentration combination of naphthalene acetic acid (NAA) and benzyl amino-purine (BAP)) and genotype (Koohegol and Chehel-Cheshmeh genotypes) based on a completely randomized design with three replications in the central laboratory of agricultural college, Yasouj University. Factors of the second experiment (somatic embryogenesis) were consisted of genotype, explant (root and shoot) and light (full light and dark). In the third study (assessment of somatic embryo), after seeing signs of torpedo embryo, number of globular, heart and torpedo embryos formed on the surface of embryogenic calluses in each genotype counted and were compared using T-test.

Results: In callus induction experiment (first experiment), genotype and hormonal composition in combination had a great impact on callus induction, so that the interaction between the two treatment groups was significant at 1% probability level. Explants of root and shoot in both genotypes were produced pre-embryo in the same concentration (1.5 mg/l) of NAA and BAP. Under this condition, the

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lowest callus induction percentage (zero) was observed in the control (hormone-free) and the treatments that high concentrations of auxin or cytokinin concentration was used and or one of the hormones was absent in the growth medium. In the embryogenesis experiment (second experiment), shoot and root explants have formed globular embryo in Koohgol and Chehel-cheshmeh genotypes, respectively. The results of this experiment also showed that under illumination condition, by decreasing auxin concentration the shoot explant in Koohgol and root explant in the Chehel-cheshmeh have produced calluses with white and brittle texture with capability to producing embryo. By transferring calluses with globular embryos to hormone-free medium, development of globular embryo to torpedo was continued. In the third study (assessment of somatic embryo), as embryogenic callus formation conditions were the same, so only two genotypes were compared in terms of the number of somatic embryo and observed that both genotypes answered similarly to somatic embryo formation.

Conclusion: Overall results of this experiment showed that explant type is related to genotype and it is possible to produce somatic embryos and synthetic seed by using callus with white and brittle texture.

Keywords: Callus Induction, Chavil, Growth Medium, Pro-embryo, Somatic Embryogenesis



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Effect of water deficit stress on growth, yield and aloin concentration of *Aloe vera* L. at different harvesting dates

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Abstract

Background and objectives: *Aloe vera* is one of the most economically important medicinal plants in many countries which used in food, cosmetics and pharmaceutical industries; it is widely cultivated throughout the arid and semi-arid regions. Therefore, the current study was aimed to evaluate the effects of different water stress on growth, yield and aloin concentration during plant growth stages.

Materials and methods: This study was conducted in research greenhouse of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. The experimental design was a randomized complete block design with a split plot in time arrangement of treatments in four replicates. Treatments including water deficit stress (irrigation after depleting of 20%, 40%, 60% and 80% field capacity (FC)) and harvesting dates (150, 240 and 330 days after planting). Irrigation treatments and harvesting dates were considered as main- and sub-plots, respectively. Growth changes, yield and aloin concentration were evaluated during growing period.

Results: The results demonstrated that the growth and yield values decreased with increasing water stress severity. Generally, the highest number, width and thickness of leaf were observed when the plants were irrigated at 20% FC, which was increased by 19, 22 and 16%, compared with irrigation after depleting 80% of the field capacity, respectively. Irrigation at 40% FC increased plant height and leaf length by 16% and 21%, compared with the irrigation at 80% FC respectively. The highest leaf and gel fresh weight were observed in 330 days after planting at 40%

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FC which increased by 44 and 49% compared with irrigation at 80% FC. Results also showed that the pup number decreased with increasing water stress. The maximum pup number was observed when plants were irrigated at 40% FC at 150 days after planting. The aloin concentration and Total Soluble Solids (TSS) increased in *Aloe vera* plants under severe water stress. The highest aloin and TSS content was observed when the plants were irrigated at 80% FC at 150 and 330 days after planting, respectively.

Conclusion: In general, severe water stress decreased leaf yield and plant growth while caused a significant increase in aloin concentration and TSS. Finally, irrigation after depleting of 40% of soil water content was the best treatment for growth and yield, also depleting of 80% of soil water content was the best treatment for aloin and TSS during all growth period.

Keywords: *Aloe vera*, Growth, Field capacity, Yield, Harvest date



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Study of genetic diversity of some Iranian acid lime (*Citrus aurantifolia* Swingle) genotypes using AFLP marker

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Abstract

Background and objectives: Citrus is one of the most important crops of semi-tropical regions in the world and acid lime (*Citrus aurantifolia*) constitute a great proportion of this group. Cultivation of limes are common in southern regions of Iran from many years ago and has an important role in the economy of these regions. A large number of limes in these regions were affected and destroyed by many reasons and knowledge about genetic of this plants for desining of breeding programs to find suitable plant is helpful. Therefore, in this survey, the genetic diversity of some acid lime genotypes were identified in major cultivation regions of this crop in south of Iran with comparison by six commercial cultivars.

Materials and methods: After selecting young and well expanded leaves, their genomic DNA were extracted. AFLP method was done by using four primer combinations of *EcoRI* and *MseI* including ECGC/MAGA, ECCA/MAGA, ECCA/MAGT and ECGC/MAAG. Genetic relationship between 30 local genotypes from three regions Darab (Fars), Manoojan (Kerman), Minaab (Hormozgan) and six foreign cultivars were considered. Collected data was analysed by Jacard similarity coefficient and UPGMA algorithm.

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Results: The four combinations produced 126 scorable bands that had %70.63 polymorphism. Number of amplified bands for each primer combination were 26 to 37 (with average of 22 bands for each primer combination). The maximum number of polymorphic bands was observed 27 in ECCA-MAGT combination. Polymorphic information content (PIC) was measured 0.4 to 0.5 for all combinations with an average of 0.48. Similarity range obtained by Jacard similarity coefficient was observed 0.24 to 0.96. Minimum similarity was found between sweet lime (*Citrus limetta*) and D8 genotype from Darab and maximum similarity was between two genotypes of Minaab region (M4-2 and M4-2). Cluster analysis arranged samples in four groups that this grouping was not consistent with studied regions. In other words, samples were not separated on basis of collection regions but samples of Minaab region had been shown high level genetic similarity with each other in comparison with samples of Manoojan and Darab regions. This is due to imitated cultivated citrus varieties in the region.

Conclusion: Significant polymorphic percent and polymorphic information content (PIC) obtained from primer combinations used in this study showed ability of these markers to identify different genotypes of acid lime. By using these markers remarkable genetic diversity between genotypes of acid lime were found. But this diversity was not such that could make the difference between genotypes of various locations. It seems that this separation could be achieved by increasing the number of primer combinations and using other markers such as SSR and SNP. Also, results of this study showed that Iranian acid lime genotypes have high level of genetic diversity because they were propagated sexually.

Keywords: Citrus, Primer combination, Polymorphic Information Content (PIC), Cluster Analysis, Genetic diversity



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The effect of micro- and nanoparticles of silicon dioxide (SiO₂) on some qualitative characteristics and nutrient elements of strawberry fruit (*Fragaria ananassa* Duch.)

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Abstract

Background and objectives: Silicon (Si) is one of the beneficial nutrient elements for the most of the plants. Recent studies have shown that Si increases the yield and crop quality and plays an important role in plant resistance against the environmental stresses. Strawberries are the good resource of bioactive compounds being an important and valuable fruit in the world from the economical and commercial point of view. For the agricultural purposes, feeding strawberry plants by different nutrient elements such as silicon can affect nutritional value and fruit quality. With science development and nanoparticle production, their application in different industry such as agriculture introduced. The aim of this study was to evaluate the effect of different concentrations of micro- and nano-SiO₂ with two methods of usage including foliar spray and root feeding applied through the growth stage of strawberry plants on some qualitative characteristics and nutrient elements of fruit.

Materials and methods: This study was conducted in the research laboratory and greenhouse of Bu-Ali Sina University in a factorial experiment based on a completely randomized design with 3 replications and 4 plants in each replicate. Foliar spray and root feeding were carried out using 20, 40, 60 and 80 mg L⁻¹ micro- and nano-SiO₂ at two separate growth stages. After fruiting, the ripened fruit were harvested, and some nutrient elements as well as qualitative characteristics of fruit including phosphorus, potassium, magnesium, iron, nitrate, total soluble sugar, vitamin C, TSS and juice acidity measured, and finally the data were statistically analyzed.

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Results: Application of silicon increased the amounts of potassium, magnesium, iron, total soluble carbohydrate, vitamin C, TSS and TA significantly, but reduced phosphorus amount, and nitrate remained unchanged. Among the treatments, 60 mg L⁻¹ nano-SiO₂ in root feeding method showed the better effects on studied traits than the other treatments as well as the control. Overall, the application of nano-SiO₂ had a better result than micro-SiO₂.

Conclusion: Application of silicon had a significant influence on nutrient elements and quality of strawberry fruits. The effect of silicon on nutrient elements and quality of fruits depended on the form, concentration and application method of silicon. Based on the results, utilization of SiO₂ particularly in nano-scale during the growth stage increased the majority of nutrient elements and quality of strawberry fruit. In general, in soilless culture and greenhouse production of strawberries, the application of 60 mg L⁻¹ nano-SiO₂ through the root feeding method is recommended.

Keywords: Foliar spray, Micro silicon, Nano silicon, Root feeding



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Evaluation of genetic diversity among clones of different seedless and seeded grapes cultivars in arak township by morphological characteristics

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Abstract

Background and objectives: Grapevine is one of the most important fruit crops in Iran which is used as table grape and raisin consumption. Among producing countries of grape, Iran is ranked ninth globally and the grape production is estimated to be two million tons. Ampelography was utilized for evaluation of genetic structure in grapevine. It is the science of phenotypically identifying of grapevines to distinguish thousands of grape cultivars, without the aid of genetic markers. This study has been conducted to investigate of some seeded and seedless clones of grape cultivars in Arak township in order to identify desirable clones for the development and cultivation of them. In addition, the identification of clones and desirable cultivars based on investigation of different characteristics from botany and horticultural perspective can be used for germplasm preservation and grapevine breeding programs.

Materials and methods: In this study, genetic variation among forty-nine clones of seeded and seedless grapevine belonging to *Vitis vinifera* species were evaluated based on fifty morphological characters of leaf, shoot, fruit and seed. These accessions were collected from different geographical sites in Arak township. Leaf and fruits samples were collected randomly from various parts of trees at normal fully mature of each accession. In order to evaluate of traits related to leaf, thirty leaves were selected randomly from each clone with three replications and the characters of them were recorded. To investigate the traits related to fruit, three bunches and thirty berries from bunch were chosen randomly from each clone with three replications and the characters of them were recorded.

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Results: Preliminary results illustrated that some traits such as bunch weight, the number of berries in bunch, berry weight, total soluble solids percentage, titratable acidity percentage, seed fresh weight, seed dry weight and TSS/TA index have shown high variability among grapevine clones. The genotypes have been divided in two main groups according to the results derived from cluster analysis so that the seed cultivars were separated from seedless cultivars. Simple correlation analysis among eighteen main traits showed that positive correlations were detected among traits. The principle component analysis results revealed that the first thirteen components explained 85.38% of the total variation for studied accessions. The first five components which related to fruit and leaf explained about 59.43% of the total achieved variability. In PCA, fruit weight, fruit length, fruit diameter, fruit size simultaneously character related to seed such as seed fresh weight, seed dry weight, seed moisture percentage, seed dry matter percentage were predominant in the first components, indicating that they were useful for the assessment of grapevine clones characterization.

Conclusion: Finally, the results of this study showed that there are high variability among clones of grapevine cultivars in terms of quantity and quality characteristics of fruit. Also, some clones belonging to seeded and seedless grape cultivars had desirable traits. Therefore, these clones can be used for commercial cultivation or utilization in grapevine breeding program to achieve desirable progeny. Among seedless cultivars, the maximum of berry weight (3.34 gr) has been estimated in sample 23 (Askary clone) which was collected from Hazaveh village of Arak. Also, the maximum of berry weight (5.24 gr) is obtained in sample 40 (Shahani clone) among seeded cultivars. In general, the production of seedless grape with largest berry is important to table grape breeding and these results illustrated that with identification of grape cultivars and desirable parent selection for breeding programs, new seedless cultivars with large berry from crosses of seeded and seedless grapes can be produced.

Keywords: Grapevine, Clone, Traits, Correlation, Variability



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The effect of potassium permanganate on biochemical characteristics of onion (*Allium cepa*)

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Abstract

Background and Aim: Plant growth regulators play important roles in plant growth and development. Ethylene is the only gaseous plant hormone that its impact on plants was known about a century ago. Nowadays, ethylene absorbents like powdered potassium permanganate in waterproof packages are widely used to increase horticultural products shelf life, but there is no report on its application as spraying and soaking and its effects on plants. This study aimed to investigate effect of spraying and soaking on onion bulbs with a solution of potassium permanganate.

Materials and Methods: In order to evaluate the effect of potassium permanganate on biochemical characteristics of onion (*Allium cepa*), an experiment based on a randomized complete block design with four replications was performed at the farm of Gorgan University of Agricultural Sciences and Natural Resources in 2013-2014. For this purpose, onion bulbs were treated by soaking for 48h before planting or spraying directly on the foliage after germination (without soaking), with different concentrations of potassium permanganate (0, 5, 10, 20, 40, 60, 80 and 100 ppm). For measurement of samples characteristics, they were taken to the laboratory of horticultural sciences and characteristics such as anthocyanins and chlorophyll of leaves, potassium, phenols and flavonoids of onions and ethylene, fresh and dry weight of onions were measured during the experiment.

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Results: The results demonstrated that the use of potassium permanganate as spray resulted in absorption of the produced ethylene by plant and then it changed the physiological characteristics of the plant. The maximum amount of fresh weight (146.94 g) and potassium ($66.497 \text{ mg.g}^{-1} \text{ DW}$) were recorded at 100 ppm spray treatment. The highest phenols ($0.627 \text{ mg.g}^{-1} \text{ DW}$) and flavonoids ($1.017 \text{ mg.g}^{-1} \text{ DW}$) content were obtained at 5 ppm spray treatment and soaking control, respectively. In addition, the maximum anthocyanin content ($0.00022 \text{ } \mu\text{mol.g}^{-1}$) and ethylene ($1.607 \text{ nmol.h}^{-1}$) were related to 20 ppm soaking and 5 ppm spray treatments, respectively. The result of group comparisons showed that there was a significant difference between two treatment application methods for flavonoids, chlorophyll a, fresh and dry weight.

Conclusion: Although there is no report about use of potassium permanganate for spraying and soaking. Based on the results of this research, we can conclude that using of potassium permanganate as soaking or direct spray on foliage can have different physiological and biochemical effects on plants.

Keywords: Biochemical characteristics, Ethylene, Onion, Soaking, Spraying



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“Technical Report”

Effect of salicylic acid and jasmonic on growth traits and ionic interaction of lavender in salt stress

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Abstract

Background and objectives: Lavender (*Lavendula officinalis* L.) is the most important medicinal plant, belongs to lamiaceae family in which had a role in ancient medicine. Growth and yield of plants are being limited by the abiotic environmental stresses including salinity in many places throughout the world, which have caused a widespread harm to the plants, also it is considered as an important functional limitations for yield around the world. This study was done to evaluate effect of salicylic acid and jasmonic acid on some growing traits and sodium and potassium content of the root and shoot in lavender in salinity stress conditions. Also evaluation of the foliar application of salicylic acid and jasmonic effect to reduce the harmful effects of salinity stress and reach to the effective introduction of new inputs was examined.

Materials and methods: This experiment was done at Azad Islamic University, Yadegar-e-Imam Khomeini (RAH) Shahre-Rey branch and greenhouse located in the Region 4 in Jun 2015. The experiment was done as factorial based on completely randomized blocks design with four replications. The experimental factors were included salt stress (NaCl) in four levels (0, 25, 50, 75 mM), salicylic acid in two levels (0 and 0.7 mM), and jasmunic acid in two levels (0 and 100 μ M).

Results: The average comparison simple effects of salinity showed that this factor significantly reduced the morphological traits of root and shoot dry weight, shoot length, leaves surface, also made decreased in root and shoot potassium, and increased the root and shoot sodium. The results indicated that only triple

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interaction effect of experimental factors were significant on dry weight of root. At this condition, the optimum root dry weight equivalent 2.21 gr was gained in non-applications of salinity stress, salicylic acid and jasmonic acid. According to findings, the highest root potassium contents with 1.20 and 1.96 element weight percent per dry matter conducted in non-salt stress treatment and 0.7 mM salicylic acid or 100 μ m jasmonic acid application conditions alternatively.

Conclusion: Based on the results, it seems that potassium adsorption in root and shoot has a role in enhancing salt resistance as an effective mechanism. However, in most of experimental traits, the role of salicylic acid in enhancing growth traits potassium content of root and shoot and at the other side, sodium decrease in root and shoot of lavender was more effective than jasmonic acid.

Keywords: Jasmonic acid, Lavender, Salicylic acid, Salt stress, Morphological traits



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“Technical Report”

Evaluate the effects of winter stress (freezing and snow) on some morphological and physiological reactions of commercial citrus in Tonekabon region

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Abstract

Background and objectives: Citrus is a subtropical crops, therefore susceptible to freeze stress. This fruit tree has a different response to freeze stress, according to the genotype and environment climate. Therefore, the objective of this project was to evaluate the damage of freeze and snow stress (February, 2014) on commercial citrus of Tonekabon region.

Materials and methods: In this project, the physiological characters linked to the freeze and snow stress such as electrolyte leakage, water content, proline and total chlorophyll of leaf, beside broken branches in 15-year-old trees of Thomson Navel orange (*Citrus sinensis*), Satsuma (*C. unshiu*) and Page [(*Citrus reticulata* × *C. paradise*) × (*C. clementina*)] on sour orange (*C. aurantium*) rootstock were evaluated based on randomized complete block design (average was evaluated by LSD).

Results: Analysis of variance showed that the effect of cultivar had a significant effect on electrolyte leakage, proline, total chlorophyll, water content of leaves and broken branches. However, the block factor had significant on water content of leaves ($p \leq 0.05$). Therefore the highest electrolyte leakage (53.06%) was recorded in Thomson Navel orange and the lowest mean 12.29 and 17.92 were record in Satsuma and Page mandarin respectively. The highest leaf proline content (30.3 and 29.21 mg/g fresh weight) were observed in Satsuma and Paige mandarin

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respectively and the lowest value of this indicator (16.35 mg/g fresh weight) was record in Thomson Navel orange leaf. Breaking branches caused by the accumulation of snow on the tree crown. Thus evaluation samples showed that the highest of this damage (with an average of 28.66%) has been in Page mandarin. In this research the highest (2.32 mg/g fresh weight) and lowest (1.24 mg/g fresh weight) total chlorophyll were recorded respectively in Satsuma mandarin and Thomson Navel orange. Leaf water content of Satsuma (63.11%) and Page (60.47%) mandarins' cultivars were significant on a same statistical level.

Conclusion: Among the three commercial cultivars of studied citrus in the Tonekabon region, electrolyte leakage, destruction of chlorophyll and proline content were increased in winter of 2014 due to low temperature stress -4.5 °C. In conclusion, it can be said that in winter stress with snow and freezing, in citrus breaking branches and trunks to burn leaves and twigs (due to freezing), more destructive effects. Therefore on this research data, the greatest damage was observed in Page mandarin, due to breaking branches.

Keywords: Citrus, Freeze, Genotype, Snow, Tonekabon