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Study of growth and mineral uptake of GF677 (peach and almond hybrid) rootstock in response to matric potential and agar concentration *in vitro*

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Abstract

Background and objectives: Availability and uptake of mineral by explants are recognized as being important in tissue culture. The limiting factor for maximum mineral uptake and optimum growth is that minerals are not enough available to the explants. There are the inter-relationships between plant growth, mineral uptake, medium water potential, and mineral movement through the medium. Plantlets growth with increasing agar concentration in plant tissue culture medium decreased due to various water potentials. Despite importance of mineral uptake *in vitro* is an indispensable part for plantlet growth *in vitro*, to date, less attention has been paid to the control of mineral uptake and aspects of transporting of ions and the role of water potential in mineral diffusion in the culture medium. But, this work can be used to better understand of effects of different agar concentrations on growth and mineral availability of explants.

Materials and Methods: For explant preparation, one year mature dormant shoots were used. After proliferation, three uniform explants (approximately 20 mm in length) were subcultured on same proliferation medium with different concentrations of agar [0 (as liquid medium), 3, 5, 7, 10 and 14 g l^{-1}] for six weeks. The experiments were set up in a completely randomized design (CRD) with four replicates. Data were collected (growth parameters and mineral) at the end of experiment (6^{th} weeks). After data collection statistical analysis was performed using MSTAT-C program and means were separated according to the least significant difference (DMRT) at 0.05 level of probability and charts were drawn by Excel 2007 software.

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Results: Results showed that growth parameters such as fresh weight, dry weight, water content, shoot number, shoot length, leaf number increased significantly (P=0.05) at 5 g l^{-1} agar. Chlorophyll index slightly decreased compared to control at high concentrations of agar. The highest concentrations of macro elements (N, P, K, Mg, Ca) and micro elements (Zn, Fe, Mn, Cu) were obtained at 0.11 MPa water potential and lowest concentrations of mineral at 0.98 and 1.15 MPa water potential. GF677 was a capable rootstock for N uptake. Maximum height of plants was obtained in liquid treatment and best growing conditions and proliferation in 3 and 5 treatments, respectively.

Conclusion: In this experiment, optimum agar concentration for growth and proliferation of GF677 was 3 to 5 g l^{-1}. At concentration of 3 g l^{-1} the highest proliferation and the best growth and proliferation conditions was obtained in 5 g l^{-1} than the higher agar concentrations. of course, at 3 g l^{-1} concentration of agar to prevent the virification explants must be transferred to new media after culture and proliferation and higher than 7 g l^{-1} of agar concentration for this plant is not recommended.

***Keywords*:** Water potential, Mineral uptake, Vitrification, Agar concentration, Chlorophyll

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Effects of ionic toxicity of sodium chloride on some physiological and biochemical characteristics of spearmint (*Mentha spicata* L.)

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Abstract

Background and objectives: Soil salinity is one of the most important and limiting factor of plant productions. Physiological drought resulting from salinity stress is one of the main reasons of limiting water absorption from soil. Moreover, increasing the salt absorption is caused to disorder in physiological and cellular processes. Spearmint (*Mentha spicata* L.) is from Lamiaceae family. This plant grows well in acidic sandy soils and prefers middle light and high soil humidity conditions. Spearmint is rich source of polyphenolic compounds, so it has antioxidant property. This experiment is conducted to study the effect of some physiological and biochemical processes.

Materials and methods: This study was performed in Agricultural Faculty of Ferdowsi University of Mashhad as factorial based on randomized complete design with four replications in 2013. In this experiment the effects of ionic toxicity of Na^+ and Cl^- on some physiological and biochemical characteristics of spearmint was investigated. The treatments of experiment were included five levels of salinity (0, 30, 60, 90 and 120 mM) sodium chloride and three sampling times (90, 100 and 110 days after planting). The measured characteristics were included chlorophyll concentration, leaf relative water contents, electrolyte leakage, proline, soluble sugars, leaf SPAD, antioxidant activity, total phenol and stomatal conductance.

Results: The results showed that salinity stress and sampling time each one individually had significant effect on relative water content, chlorophyll a and b, total chlorophyll, leaf SPAD, antioxidant activity and electrolyte leakage. Salinity

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also had significant effect on stomatal conductance, whereas sampling time had no influence on these characteristics. Interaction effects of salinity stress and sampling time on SPAD, proline and leaf relative water contents were significant.

The highest (106.72%) and lowest (65.38%) relative water contents were observed at control and first sampling time, and 90 mM sodium chloride and third sampling time, respectively. The highest (50.49%) and lowest (19.42%) electrolyte leakage were obtained in 120 mM sodium chloride and control, respectively. Sampling time had significant effect on soluble sugars, total phenol and proline. Relative water content, membrane stability, chlorophyll a, b and total chlorophyll decreased over the time.

Conclusion: According to the obtained results, rising the salinity levels cause to decrease relative water contents, chlorophyll and stomatal conductance, increase electrolyte leakage and antioxidant activity, and had no effect on proline, soluble sugars and total phenol. It appears that spearmint is sensitive to salinity stress and it can not tolerate salinity levels more than 30 mM sodium chloride.

Keywords**:** Salinity, Antioxidant activity, Leaf relative water content, Spearmint.

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Optimization of callus induction and micropropagation of four varieties of grape (*Vitis vinifera* L.)

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Abstract

Background and objectives: Grape has high nutritional value and it is an economically important horticultural plant in the world. Iran has seventh producer grape rank in the world. Today tissue culture is one of technique which can be used in grape propagation and breeding.

Material and Method: In order to optimization of callus induction and micropropagation of grape *in vitro* condition, this investigation was conducted in two separate experiments. In the first experiment the effects of two explants including middle meristem and leaf segments and two plant regulators inducing Benzylaminopurine (BAP) and Naphthalene acetic acid (NAA) at five levels (0, 0.5, 1, 1.5, 2 mg L^{-1}) on callus induction of four grape varieties (Askari, Siyah, Gavi, Rish-baba) were investigated. Two months after explants cultured and treatments application, length, height, volume, fresh and dry weight of callus, and their relative humidity were measured. In the second experiment the effect of BAP at two levels (2 and 1.5 mg L^{-1}) and NAA at four levels (2, 1.5, 1, 0.5 mg L^{-1}) on shooting and rooting percentage of four above mentioned grape varieties were tested. Shooting percentage, leaf number, shoot fresh weight and shoot length in shooting experiment, and rooting percentage in the rooting experiment were recorded. Both experiments were conducted using factorial experiment in a completely randomized design with three replications. Data were subjected to analysis of variance and the best treatments were introduced based on treatment means comparison.

Results: Results of analysis of variances showed that plant growth regulators and their interactions were significant for all measured traits in the both experiments.

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The best treatment for callus induction and callus characters was 1.5 mg L^{-1} BAP and 2 mg L^{-1} NAA in middle meristem of Rish-baba cultivar. Results of second experiment showed that 2 mg L^{-1} BAP and 2 mg L^{-1} NAA were the best plant growth regulator combination for shooting of Askari and Gavi cultivars. This treatment was resulted 100 and 67 shooting percentage in Askari and Gavi cultivars respectively. Whereas combination of 1.5 mg L^{-1} BAP and 0.5 mg L^{-1} NAA in Siyah cultivar with 33 shooting percentage and combination of 1.5 mg/L^{-1} BAP and NAA in Rish-baba cultivar with 67 shooting percentage had the best responses for shooting experiment. The all treatments showed very good responses to root generation because there were no significant differences between control and other plant growth regulators for rooting percentage and they produced 100% root tissue organ.

Conclusion: In general, based on this results callus induction and regeneration conditions were optimized for four grape cultivars which can be used in grape production.

Keywords: Grape, *In Vitro* culture, Explants, Callus, Micropropagation

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Effect of integrated application of manure and urea and foliar application of micro elements on growth and physiological indices of medicinal pumpkin (*Cucurbita pepo* var styriaca)

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Abstract

Background and objectives: Pumpkin (*Cucurbita pepo* var. styriaca) is one the most important cultivated medicinal plants, because of oil quality and its uses as edible and herbal medicine. High oil production of pumpkin by lower amounts of chemical fertilizers usage is one of the important aims of this plant cultivation. Because of plant nutrition importance and growth and physiological indices on photosynthetic condition and production, this experiment designed and performed.

Materials and methods: In order to evaluate the effects of integrated application of manure and urea and microelements foliar application on growth and physiological indices of medicinal pumpkin, a field experiment was performed as split plot basis on randomized complete block design with three replications at University of Tehran, college of Abouraihan. The animal manure and urea as pellet with four levels (150 Kg urea, 50 Kg urea + 3.5 ton animal manure, 100 Kg urea + 1.5 ton animal manure and 150 Kg urea + 1.5 ton animal manure) as main factor and microelements foliar with three levels (1000, 2000 and 3000 (mg kg^{-1}) including of iron, zinc, manganese in chelate form and boron in boric acid forms as minor factor were considered.

Results: The results showed that the usage of pelleted manure and urea after 15 days had no significant effect on total dry matter production variation. Main plot had significant effect at the next growth stage ($P<0.01$). Microelements had no significant effect on total dry matter until 45 days after planting. Sixty days after planting, microelements had significant effect on total dry matter at 5% levels; in the next growth stage had significant effect at 1% level. Maximum LAI (3.62 and

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3.51) and CGR (5.15 and 4.35 g d^{-1}) were achieved at 150 Kg urea + 1.5 ton animal manure as pellet and 2000 (mg kg^{-1}) of microelements application. Because of the importance of leaf area index in photosynthesis, maximum dry matter was 2397 and 2094 g m^{-2} in these two treatments. Minimum LAI and dry matters (0.12 and 4.48 g d^{-1}) were achieved at 50 Kg urea + 3.5 ton animal manure as pellet. Among different microelements levels, minimum LAI (0.13) was achieved at 2000 mg Kg^{-1} application and minimum dry matter (4.5 g day^{-1}) was achieved at 1000 mg Kg^{-1} usage.

Conclusion: Result showed that 150 Kg urea + 1.5 ton animal manure as pellet and 2000 (mg Kg^{-1}) of microelements can be suggested as the best option for achievement of the maximum yield characteristics of pumpkin medicinal plant.

Keywords: Dry matter production, CGR, LAI, Yield, *Cucurbita pepo* var styriaca

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Callogenesis and plantlet regeneration from two Iranian accessions of bitter melon medicinal plant (*Citrulus colosynthis* L.) *in vitro* condition

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Abstract

Background and objectives: Bitter melon is one of the anti-cancer medicinal plants that it's wide level with in vitro propagation and production of anti-cancer material was necessary. Different factors such as genotype, selected explants' type and it`s preparation conditions influenced regeneration content. Plant growth regulator`s effect on callogenesis and indirect regeneration from Parangipetai Indian accession of bitter melon assessed and regeneration method by using stem explants was presented. Their result showed that maximum amount of callus induction was obtained in MS medium supplemented with 0.5 mg L^{-1} of IAA and 2,4-D hormones and 1 mg L^{-1} 6-BA. By attention that we have not any report about indirect plantlet regeneration from Iranian accessions of this plant. For this, in this research we assessed callogenesis and plantlet regeneration in two Iranian accessions of bitter melon.

Materials and methods: After surface sterilization and deleted external hard bark, kernel of this accessions were planted on MS medium and after one month, from obtained plantlets stems and leaves explants were transferred to the MS medium supplemented with different hormonal combinations of BAP, NAA, 2,4-D and Kinetin. For indirect regeneration different hormonal combinations of BAP, NAA in MS, ½MS and ¼MS mediums was examined. Regenerated plantlets were transferred in small pots containing sterile soil (soil and sand in 3:1 ratio). This plantlets were changed in complete plants and be able to well established and grow.

Results: Callogenesis in both accessions in different hormonal combinations of 2,4-D and Kinetin was more little than mediums supplemented with different hormonal combinations of BAP and NAA. In Dehdasht accession in different hormonal combinations of BAP and NAA and in Gachsaran accession in different

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hormonal combinations of Kinetin and 2,4-D were obtained approximately 100 and 66.6% Callogenesis, respectively. Shooting in both accessions was started at five weeks after callus segments culture. Leaf explants, in hormonal combination of 3 mg L^{-1} BAP in ½MS medium in Dehdasht accession and stem explants, in hormonal combination of 2 and 3 mg L^{-1} BAP in MS medium in both accession showed that the best indirect plantlet regeneration. The best rooting medium was hormonal combination of 0.5 mg L^{-1} BAP with 0.5 mg/liter NAA. Rooting in both accessions was done with this hormonal combination with this difference that in Gachsaran accession rooting percent more little than Dehdasht accession.

Conclusion: The results of this research showed that Dehdasht accession with regard to Gachsaran accession and stem explants with regard to leaf explants have better response to indirect regeneration. All together, both Iranian accessions of this plant have suitable response to callogenesis and indirect regeneration.

Keywords: Regeneration, Callogenesis, Genotype, Bitter melon

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Effects of plant density on flower, essential oil yield and some important agronomic indices of Borage (*Borago officinalis* L.)

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Abstract

Background and objectives: *Borago officinalis* belongs to Boraginaceae family that has been used widely as traditional medicine since long times. The increasing tendency toward herbal medicine for curing diseases has made necessity for cultivation of various medicinal plants in worldwide level and Iran. The production of medicinal plants like to other types of plants is affected by agronomic factors such as plant density per unit area. Hence, this experiment was carried out to evaluate the effects of plant density on flower and essential oil yield and some important agronomic indices in Borage (*Borago officinalis* L.) under climatic condition of Guilan province.

Material and methods: This experiment was performed based on randomized complete block design with three replications, at Shalman research station of agricultural and natural resource research center of Guilan province, Iran, in 2013. Plant densities including of 20, 40, 60, 80 and 100 plant per m^2 comprised the experimental factors.

Results: The results showed that effect of plant densities were significant for flower yield, essential oil, seed and oil yield. The greatest flower yield (based on dry weight) and essential oil content of *Borago officinalis*, were obtained from 40 plants per m^2 density. But, the lowest essential oil content was observed at 100 plants per m^2 density. In addition, the results showed that the greatest seed oil content and oil yield of *Borago officinalis* obtained from 40 plants per m^2 density.

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Conclusion: In general, measured traits of *Borago officinalis* decreased due to enhancement of plant density per unit area. Based on results of this experiment, the determination of plant density per unit area is necessity to achieve the highest production of *Borago officinalis*. Thus, the density of 40 plants per m^2 could be recommendable in order to increase borage productions under Guilan province and similar climatic conditions.

***Keywords*:** Borage, Plant density, Dry matter and Essential oil

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The effect of opportune harvesting time on the fruit quality of pomegranate Shekar, Shirin and Sangak cv.

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Abstract

Background and objectives: Pomegranate, belonging to Punicaceae family, is native to subtropical regions of Iran and its neighborhood countries. Iran has the richest germplasm resource of pomegranate and the first grade in cultivation area and production worldwide. Physical and chemical properties of pomegranate cultivars vary in different developmental stages and have a large impact on the nutritional values of the fruit. These traits have not been evaluated yet in details in Iranian pomegranate cultivars during maturation stages. This study aimed to investigate the influence of harvesting time on fruit quality in three pomegranate cultivars.

Materials and methods: In this experiment, three native pomegranate cultivars of Kolbad region, located between Golestan and Mazandaran province, including Shekar, Shirin and Sangak, were studied at two stages of the fruit maturation, including the early harvest (early days of traditional harvesting season on 10 Sep. 2012) and opportune harvest (last days of traditional harvesting season on 10 Nov. 2012). The experiment was conducted in a factorial design in three replications. In each replication, four fruits were used to measure some physiochemical traits. Data were analyzed by SAS version 9.1 and the means were compared by LSD method.

Results: The results showed that there were some significant differences in physicochemical attributes between these two harvesting times and among the three cultivars. In all cultivars, most of the physical properties were the highest in the opportune harvest, except for the thickness and the percent of the fruit peel. Among the cultivars, Shekar had the heaviest and largest fruits. In contrast, Shirin had the heaviest and largest arils. Shekar had the highest percent of fruit juice,

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while there was no difference between Shirin and Sangak. The results showed that there was not any significant difference between the harvesting times and in fruit crown length and fruit density among the cultivars. In all cultivars, pH, total soluble solids, total phenol, total antocyanin, and total sugar were highest in the opportune harvest, while EC (electrical conductivity), titratable acidity, and vitamin C were lowest. Shekar had the highest fructose and vitamin C; while, Shirin had the highest glucose and antioxidant activity.

Conclusion: In general, the results showed that most of the physical and chemical characteristics changed from early harvesting time to opportune harvesting time in fruits of the studied cultivars. Opportune harvest may highly improve the nutritional values of pomegranate fruits; therefore, in this part of the country and other parts the pomegranate fruits must harvested in the appropriate time.

***Keywords*:** Harvesting time, Maturity, Physicochemical characteristics, *Punica granatum*

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Effect of medium, explants and BA on somatic embryogenesis induction in two Iranian native orchids

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Abstract

Background and objectives: Temperate terrestrial orchids are an important group in Orchidaceae family that in the recent years attract attention in horticulture. *Epipactis veraterifolia* is the common orchids and *Dactylorhizba umbrosa* is the shadow like orchids are members of Iranian native temperate terrestrial orchids. Unfortunately, the low propagation, high price and high demand in the market interest, is due to collect them from the wild habitat. As a result, their number has decreased and most of temperate terrestrial orchids are endangered species. On the other hand, despite progress for micropropagation and in vitro cultivation of some orchids, there are a few reports in temperate terrestrial orchids. Somatic embryogenesis has high potential for large-scale micropropagation especially for plants with specific reproduction cycle (as orchids). To establish an efficient method, a study was conducted using somatic embryogenesis as a rapid vegetative propagation technique for conservation. The aim of this report was to finding better condition for somatic embryogenesis induction of this native species, for mass propagation in large scale.

Materials and methods: In this study, two levels of BA (0 and 3 mg l^{-1}) and four different culture media (1/2 MS, 1/4 MS, 1/8MS, FAST) were investigated for two Iranian native orchids regeneration via direct somatic embryogenesis. Hence, four different explant types of (leaf, stem, crown, protocorms) *Epipactis veratrifolia* and two explants of (leaf, crown) *Dactylorihza umbrosa* were used.

Results: Results showed that there were no significant differences in somatic embryogenesis of *E. veratrifolia* among medium treatments, but BA and different

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explants interaction was significant and protocorm explants by produced 13 embryos in each explant had the highest embryogenesis frequency in 1/4 MS medium contain BA (3 mg l^{-1}). There was significant difference among medium treatments in *D.umbrosa* and FAST medium had the best response. Crown explants had the highest embryogenesis frequency (2.66 embryos per explants). Also 3 mg l^{-1} BA had positive effect on embryogenesis.

Conclusion: The observation showed the positive effect of 3 mg l^{-1} BA in both species. Therefore, the usage of BA and study on the effect of different dosage of this hormone were suggested. In *Epipactis veratrifolia* there was no significant difference between the media, while FAST medium has significant difference with other media in *Dactylorihza umbrosa* species. Protocorm explants in *Epipactis. veratrifolia* and crown explants in *Dactylorihza. umbrosa* showed the most efficient explants. In both species leaf explants has no response.

Keywords: *In vitro culture media, Protocorm, Epipactis veratrifolia, Dactylorihza umbros*

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Effects of animal manure levels and bulb weights on yield, yield components and essential oil contents of black zira (*Bunium persicum* Bioss.)

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Abstract

Introduction: Black zira (*Bunium persicum* Bioss.) is an important aromatic and medicinal plant that belongs to the Apiaceae family with persistent tuberous roots. It is native to the Middle East particularly, Iran. Black zira is found in the mountains of the eastern and central parts of Iran, west of Afghanistan and Pakistan and northwest of India and Turkmenistan. It can be propagated sexually by seed or by transplanting the tuberous roots. After 2 or 3 yr of vegetative growth it enters the generative phase and then can set seed. Black zira seeds are harvested for medicinal purposes with additional uses as a spice. Soil is the natural habitat for microorganisms that they play an important role in soil processes and determine plant productivity. Soil fertility can be maintained and improved by using either organic manure or inorganic fertilizers. Positive effects of cow manure on plant yield and soil physico-chemical properties have been reported in many studies. It is a porous material with high surface area thus it can significantly affect soil moisture and nutrient dynamics. Cow manure has a higher capacity for cation adsorption due to its greater surface area, and greater charge density.

Materials and Methods: In order to study the effects of different levels of animal manure and bulb weights on growth criteria, yield components, seed yield and essential oil yield of black zira, an experiment was conducted as factorial based on a randomized complete block design with three replications at the Agricultural

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Research Station, Ferdowsi University of Mashhad, during two growing seasons of 2012-2014. Five animal manure levels (0, 10, 20, 30 and 40 t ha^{-1}) and three bulb weights (<2, 2-4 and >4 g) were considered as treatments. Studied traites were plant height, canopy diameter, yield components such as umbel number per branch, umbellet number and 1000-seed weight, biological yield, seed yield, essential oil content and essential oil yield of black zira. For statistical analysis, analysis of variance (ANOVA) and least significant difference test (LSD) were performed using SAS version 9.1.

Results and discussion: The results showed that the simple effect of animal manure levels and bulb weights were significant on yield components, biological yield, seed yield and essential content of black zira. Interaction effect between animal manure level and bulb weight was significant on plant height, canopy diameter and essential oil yield. The highest seed yield was belonged to the maximum animal manure level with 164.20 g m^{-2}. Seed yield was improved up to 27% by increasing bulb weight from <2 g to >4 g. The maximum essential oil yield (14.25 g m^{-2}) was observed for 40 t ha^{-1} animal manure + bulbs with>4 g.

Conclusion: Generally, the results revealed that the cow manure consumption and planting of higher bulbs improved growth and yield of black zira.

Keywords: Essential oil, Seed yield, Canopy diameter, Cow manure, Medicinal plant