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Application of microsatellite markers for identification and registration of olive cultivars

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Abstract

Background and objectives: Oleaceae family contains approximately 20 species of small trees and ancient world of the Mediterranean, North Africa, Southeast Asia, north to southern China, Scotland and East Australia have a wide dispersion. They are evergreen and have small leaves that are integrated have been met. Olive trees resistant to drought and adapt to poor soils and produce high-value, low-cost and economically very important. In the past, methods for cultivar identification was based on morphological characteristics of leaves, fruits and kernels, but except in specific cases identification using morphological traits alone are not enough. Due to complex identification of young olive cultivars (*Olea europaea* L.) by morphological traits, advance molecular tools have provided a new prospect for DNA fingerprinting.

Materials and Methods: For this study, sampling was done after receiving information from Horticulture Science Research Institute (HSRI) and Seed and Plant Certification and Registration Institute (SPCRI). Sampling was done from young and fresh shoot of trees. Approximately, 30 mg of young leaf were ground in liquid nitrogen and total DNA was isolated using the procedure described Core

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Bio DNA extraction kit and PCR-amplified using 22 pairs of primers flanking SSR sequences. Off-type trees were identified using cluster analysis based on SSR data. The specific molecular keys were verified on 64 olive mother's trees and the results were confirmed at two independent laboratories (SPCRI and Agriculture Biotechnology Research Institute of Iran). Most mother trees of different varieties were classified in separate groups.

Results: Specific molecular keys were identified for 11 Iranian olive cultivars (*Olea europaea* L.) using 10 out of 22 SSR markers. The results showed that 10 SSR markers produced polymorphism or polymorphic bands for studied Iranian olive cultivars. SSR markers MOL 1 in Tokhme Kabki, MOL 7 in Dezfooli, Mavi and Roghani, MOL 8 in Dezfooli and Shangeh, MOL 9 in Dezfooli, Golooleh, Mari and Roghani, MOL 10 in Tokhmekabki, MOL 11 in Dezfooli and Fishemi, MOL 14 in Dehghan, MOL 18 in Golooleh, MOL 20 in Mari produced specific molecular keys. Out of 12 Iranian olive cultivars, four cultivars (Dezfooli, Golooleh, Mavi and Roghani) were identified by SSR marker MOL 9.

Conclusion: Dendrogram of Splits Tree 4 software separated mother trees of studied olive cultivar with 11 SSR markers. The results demonstrated that cluster analysis and structure software, was very appropriate for study of genetic relationships among olive cultivars. The reported specific molecular keys can be efficiently used for identification of olive cultivars for Genetic authentication.

Keywords: Clustering, Fingerprinting, Microsatellite, Model based method, Olive



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"**Technical Report**"

Study on effectiveness of some *Trichoderma* spp. on tomato growth parameters with three inoculation methods

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Abstract

Background and objectives: Different *Trichoderma* species play role as suitable alternative for chemical fertilizers and successful biological tool in sustainable agriculture. These microorganisms show rivalry in food and place sources. They establish in the rhizosphere of most agricultural and non-agricultural plants via producing spores. They also could induce resistance in plants. These properties may lead to pathogen reduction as well as plant growth incitation. Favorite effect of this species on the plant growth depends on type of species, strains and inoculation methods. Therefore this research has been tried to compare growth effects of four different *Trichoderma* species using three methods including seed coating, soil application and foliar spraying on tomato growth factors.

Materials and methods: Four *Trichoderma* spp. including *Trichoderma sp* (T1), *Trichoderma virens* (T2), *Trichoderma harzianum* (T3, T4) and *Trichoderma koningi* (T5) were evaluated on tomato growth under completely randomized design based on factorial method with four replications in greenhouse conditions. Three methods, consisting of seed coating, soil application and foliar spraying were applied under pot experiments and the performance of all three methods was evaluated according to the isolates. Fungal inoculation suspension was prepared for each method separately. For instance in seed coating method, 3×10^6 *Trichoderma* spore on seed surface was estimated. Approximately 10^7 propagule per gram of soil

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was calculated for soil application method and in foliar spraying 10^7 spores per ml was prepared.

Results: Based on results, soil application method showed positive effect on tomato growth traits especially on dry root and foliar weight in comparison to other techniques. Overall, T1, T2 and T5 selected as the best candidates in trial conditions specifically in soil and seed application methods based on their positive effect on tomato growth factors. Isolate T1 increased root and shoot length by 54 and 45.5 percent respectively over controls in soil application treatment. In addition, the means comparisons using LSD method showed no significant difference between T1 and T2 isolates at 5% possibility level especially in soil application method.

Conclusion: The soil application method was suggested as preferred technique in current study. In addition, it seems that application of T1 and T2 isolates in the form of individual or combined formulation which can be used in the field level and could improve tomato growth and yields significantly.

Keywords: Growth factors, Tomato, *Trichoderma* spp



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Interaction effects of weeds and seed priming on growth Indices of different watermelon (*Citrullus lanatus*) seed lots

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Abstract

Background and objectives: Seedy watermelon (*Citrullus lanatus*) is a crop that is planted as a second crop after winter crops in some parts of country. This crop is more attended by farmers due to the short time between planting and maturity, low production costs and greater profitability rather than other spring crops. In order to evaluate the interaction effects of weeds and seed priming on growth indices of different seed lots of seedy watermelon (*Citrullus lanatus*) an experiment was conducted in the Research Farm of Faculty of Agriculture, Ferdowsi University of Mashhad in 2013.

Materials and methods: This experiment was conducted in factorial design based on completely randomized block, design with three replications. First factor was three seed lots of seedy watermelon (Kalaleh, Neishabour and Bojnord), second factor was seed hydropriming in two levels (primed and non-primed seeds) and third factor was weedy and weed free conditions. In this experiment, plant height, leaf area index, fruit setting, fruit number per plant, seed number per fruit, 1000 seed weights and seed yield were measured.

Results: Results showed that weeds had effect on growth and yield components of seeds, which caused reduction in seed yield in all seedy watermelon lots by 79%. Seed priming reduced the effects of weeds on leaf area index and thousand seed weight, but didn't have any significant effect on seed yield. In general, the most seed yield and thousand seed weight were observed in Neishabour seed lot with 1581 Kg ha⁻¹ and 144 g, respectively.

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Conclusion: Based on the results, on weed interference treatment all traits, especially seed yield decreased significantly, and then yield reduction could compensate to a certain extent with removal of weeds or in the weedy condition can achieve optimum yield with sowing appropriate varieties (Neishabour seed lots) and seed priming.

Keywords: Hydro-priming, Neishabour, Seedy watermelon, Seed yield, Weed



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An investigation of genetic diversity of some hawthorn genotypes using morphological characterization of leaf and fruit

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Abstract

Background and objectives: Hawthorn is a forest fruit tree which is grown in the temperate regions around the world. This plant is known as the most traditional herbal remedy for heart failure and cardiovascular disease in worldwide. This study has been conducted to investigate the genetic variability and classify hawthorn genotypes based on quantitative and qualitative characteristics of leaf and fruit. In addition, the identification of desirable genotypes based on investigation of different characteristics from botany and horticultural perspective can be used for germplasm preservation and breeding programs of this crop.

Materials and methods: In this study, genetic variation among thirty hawthorn genotypes belonging to four species was evaluated based on seventeen morphological characters of leaf and fruit. These accessions were collected from different geographical sites in north and west of Iran. 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 genotype with three replications and the characters of them were recorded. To investigate the traits related to fruit, ten fruits were chosen randomly from each genotype with three replications and the characters of them were recorded.

Results: Preliminary results illustrated that some traits such as fruit weight, leaf area, and leaf length, depth of leaf sinus, seed length and petiole length have shown high variability among accessions. Results derived from cluster analysis, divided the genotypes into two main groups which *pontica* and *microphylla* species were

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separated from others species including *monogyna* and *pentagyna*. Simple correlation analysis among traits showed that positive correlations were detected among them. Factor analysis showed that 85% of total variance was indicated by five main factors. Two first factors were related to leaf and fruit characteristics, constructed 55% of the total variance. In PCA, leaf area, petiole length and number of leaf in the node simultaneously characters related to seed and fruit such as seed weight, seed length, fruit dry matter and fruit moisture content were predominant in the first components, indicating that they were useful for the assessment of hawthorn genotypes characterization.

Conclusion: Finally, the results of this study showed that there is high variability among hawthorn genotypes in terms of quantity and quality characteristics of fruit. Also, the fruit weight significantly varies among hawthorn accessions, and some genotypes which belong to *pontica* species had quite high weight. Therefore, these species can be used to be cultivated commercially or to be utilized in breeding program to achieve desirable progeny.

Keywords: Cluster analysis, Fruit weight, Hawthorn, Species



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Effects of biofertilizer with organic and chemical fertilizers on quantities yield and physiological parameters in Indian senna (*Cassia angustifolia* Vahl)

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Abstract

Introduction and aim: Sennai (*Cassia angustifolia* Vahl) is one of the valuable plants which used to constipation treatment. Production of organic Plants is one of the goals of medicinal plant production. This is possible with the application of bio-fertilizers. In the past decades due to the use of chemical fertilizers numerous environmental impacts, including soil and water pollution problems in the health of humans and other living things came into being. Bio-fertilizers are useful microorganism in the area around the plant roots. They can coloniz in plant roots and increase growth by improving the absorption of minerals nutrients, especially phosphorus. Microorganisms in biofertilizers can convert mineral elements in the soil into the vitamins and other minerals in the soil and reach them to the roots. The use of bio-fertilizers is cheaper and do not cause pollution in the ecosystem. This emperiment was conducted. In order to study the effects of different types of biological fertilizers with organic and chemical fertilizers on quantities yield, photosynthesis pigments and nutrient elements concentration in Indian senna.

Materials and Methods: A field experiment was conducted as factorial design with three replications at University of Zabol in 2012. The experiment consisted of three different types of biological fertilizer: B₁= Nitroxin, B₂= Biosulfure, B₃= Barvar phosphate biofertilizer 2 and B₄= combined of B₁+B₂+B₃ as a first factor and F₁=manure, F₂=compost and F₃=chemical fertilizer was as the second factor.

Results: Results showed that, biofertilizer had significant effect on grain yield, number of pod per plant and the amount of phosphorus in shoot and seed of Indian senna. The highest grain yield and phosphorus in this study was obtained from

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Barvar phosphate biofertilizer and Nitroxin and the number of pods per plant was obtained from Nitroxin. The results showed that, organic and chemical fertilizer and interaction between biological fertilizer and organic fertilizers had significant effects on the grain yield, biological yield, number of branches per plant and number of pods per plant. The highest values of these traits were obtained at B₁F₁ treatment. The use of biological fertilizers although had no significant effects on photosynthesis pigments and soluble carbohydrate in leaves but organic and chemical fertilizer have a significant effect on the chlorophyll a and soluble carbohydrate in leaves of Indian senna.

Conclusion: The results of this experiment showed that, the best treatment in this study was the use of Nitroxin biological fertilizer with organic manure. This treatment improved quality and physiological parameters in Indian senna.

Keywords: Biofertilizer, Grain yield, Indian senna, Nutrient elements, Photosynthesis pigments



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Freezing tolerance of grass species under controlled conditions

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Abstract

Background and objectives: Cold stress is one of the most important limiting factors for growth and development of grasses in temperate regions. Freezing tolerance is highly different between plant species and between regions. The importance of grasses is due to widespread application in lawn beautifying, animal feeding, and erosion control. There are many differences between grass species in regards to freezing tolerance as well. Researchers have used several traits such as Lethal Temperature 50 percent (LT₅₀), electrolyte leakage, plant height, and leaf area to identify more freezing tolerant plant species. Due to little information exists about freezing tolerance of grasses, this experiment was conducted in order to identify more tolerant species of grasses using some physiological and morphological traits.

Materials and methods: In order to evaluate the freezing tolerance of some grass species under controlled conditions, an experiment was conducted at Faculty of Agriculture, Ferdowsi University of Mashhad, as a completely randomized design using factorial arrangement with three replications in 2009. Treatments were included 14 freezing temperatures (from 0 up to -19.5 with 1.5 °C intervals) and six grass from *Lolium*, *Festuca*, *Bromudagrass* species. Grass seeds were sown in plastic pots with 10 cm diameter in late September. In order to do cold acclimation, plantlets were grown to 4-6 leaves stage in natural conditions. Pots were irrigated 24 h prior to be transferred to thermogradient freezer. Such traits as crown and leaf membrane stability, survival percentage, dry matter per plant, LT₅₀, RDMT₅₀, and tiller number were evaluated.

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Results: The results showed that freezing temperature significantly ($P < 0.01$) reduced survival percentage of grasses. All of the grasses were survived at -15°C , but with decreasing temperature survival percent was reduced, while at -16.5°C all grasses species died. Nevertheless, survival percentage between genotypes were different, as California and Starlet plants showed 50 percent survival at -12°C , while, survival percentage of Masterpiece plants were less than 15 percent in this temperature. In general, cell membrane stability of crown was higher than leaves and at -13.5°C cell membrane stability was higher than 60 percent, but at -15°C this index for both of them was zero. With reducing the temperature, no. of tillers and plant dry weight after recovery significantly ($P < 0.01$) decreased. The correlation between Lethal Temperature 50 (LT_{50}) and survival was negatively significant ($r = -0.98^{**}$). Based on LT_{50} , *Bermudagrass* and *Poa pratensis* had the highest and the lowest survival, respectively. *Festuca arundinacea* (var. Starlet) showed the highest recovery in regrowth period based on temperature for 50% reduced of plant dry matter ($RDMT_{50}$). Starlet cultivar also showed the highest membrane stability under freezing temperatures.

Conclusion: In overall, it seems that LT_{50} , $RDMT_{50}$, survival percentage and membrane stability index are suitable indices to determine freezing tolerant of grasses. Based on the results, *Bermudagrass* and *Festuca arundinacea* (Starlet cv.) had known as freezing tolerance grass species.

Keywords: Cell membrane stability, Cold stress, LT_{50} , $RDMT_{50}$, Survival



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Effect of organic fertilizers and salicylic acid on the yield and some secondary metabolites of lemon balm (*Melissa officinalis* L.)

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Abstract

Background and objectives: Lemon balm is a medicinal herb that its essential oil has wide range of applications in the pharmaceutical, health and food industries. It also contains rosmarinic acid (RA) which is presently utilized against HIV1 viruses and treatment of AIDS disease. In this research, the effects of organic fertilizers (cattle and sheep manure, leaf mould and vermicompost) combined with foliar application of salicylic acid (SA) on yield and some secondary metabolites of lemon balm were investigated.

Materials and methods: The experiment was conducted as completely randomized block design with factorial arrangement in pots with three replications. The parameters such as shoot fresh & dry weights, oil percentage, essential oil components, the amount of rosmarinic acid (RA) and the antioxidant activity were measured. Essential oil was extracted by Clevenger, the essential oil components were identified by GC/MS, RA was estimated by HPLC and antioxidant activity was measured by spectrophotometer.

Results: Based on the analysis of variance, the effects of organic fertilizers and SA on oil percentage, the amount of RA and antioxidant activity were found to be significantly different. Maximum oil percentage (%0.536) was recorded in the treatment of cattle manure combined with 10^{-2} M SA and the lowest level (%0.203) was observed in cattle and sheep manures without SA. The essential oil analysis revealed that Citral (E & Z) at the rate of 61.88 % was the highest essential oil component of lemon balm. The highest and the lowest amounts of RA were found

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in plants treated with sheep manure 10^{-4} M SA and control plants respectively (29.47 and 14.33 mg g⁻¹ dry weight, respectively).

Conclusion: In conclusion, the results of this experiment showed that the application of organic fertilizers and salicylic acid may be effective in improving the performance and production of secondary metabolites in lemon balm.

Keywords: Essential oil, GC/MS, HPLC, Lemon balm, Rosmarinic acid



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Growth analysis and qualitative traits of pumpkin (*Cucurbita pepo* L.) affected by application of chemical and organic fertilizers

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Abstract

Background and objectives: Production and application of fertilizers in conventional agriculture has many environmental problems. While the application of organic fertilizers instead of chemical fertilizers have an important role on soil fertility, increase product quality and safety of agricultural ecosystems. Pumpkin is an important annual herbaceous plant belongs to the Cucurbitaceae family due to a high percentage of protein accumulation, especially oil in seeds have high nutritional value. Therefore, the aim of this study was to investigate the effect of different sources of fertilizer and their combination on qualitative and quantitative characteristics of pumpkin.

Materials and methods: In order to study growth analysis and qualitative traits pumpkin, affected by application of chemical and organic fertilizers, a field experiment was conducted based on RCBD with three replications during the 2010-2011 growing seasons in Gorgan University of Agricultural Sciences and Natural Resources, Iran. The treatments included N0: (control), N1: 66-66-99 Kg ha⁻¹ NPK, N2: 132-132-198 Kg ha⁻¹ NPK, N3: 300-200-200 Kg ha⁻¹ NPK, H: 30 t ha⁻¹ manure, HN1: (1/2 H + N₁), HN2: (N₂ + 1/2 H), HN3: (N₃ + 1/2 H), C: 30 t ha⁻¹ municipal compost, CN1:(1/2 C + N₁), CN2: (1/2 C + N₂), CN3: (1/2 C + N₃) and HN: (1/2 C + 1/2 H). In this study, crop growth rate, relative growth rate, seed yield, biological yield, harvest index, oil content, linoleic and oleic acid were measured.

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Results: The results showed that chemical fertilizer application with FYM increased physiological indices. Application integrated treatment of 132-132-198 Kg NPK + 15 tons of manure per hectare, produced the highest seed yield with an average of 1044 and 1148 Kg per hectare respectively in first and second year of the experiment. The highest oil content and harvest index also were obtained of integrated treatment of 132-132-198 Kg NPK + 15 tons of manure per hectare in two years, respectively. Application of FYM (H) produced the highest fruit weight, biological yield, linoleic and oleic fatty acids. Application of chemical fertilizers in high level (300-200-200 kg NPK per hectare) produced the least oil content among different level of chemical fertilizers.

Conclusion: In general, the results showed that application of extensive chemical fertilizers had negative effect on all traits except harvest index. Instead of chemical fertilizer, application of 15 tons ha⁻¹ manure + 132-132-198 Kg ha⁻¹ NPK could improve the qualitative and quantitative yield of pumpkin seeds and it considered a step towards achieving sustainable agriculture.

Keywords: Compost, Medicinal plants, Nitrogen, Sustainable agriculture



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Evaluation of genetic diversity in some loquat genotypes based on pomological characteristics in Golestan province

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Abstract

Background and objectives: Loquats have formed different ecological types during their cultivation and adaptation in different parts of the world. The first step in the breeding operation is the identifying of the distribution center accompanied the studying of the variation between genotypes. In this research, 20 loquat genotypes were evaluated using fruit morphological markers to identify and select the superior genotypes in Golestan province.

Materials and methods: The fruits of the genotypes were harvested in the ripening stage and quantitative and qualitative characteristics of 25 fruit were assessed in the laboratory. The maximum, minimum, mean values, diversity coefficient index, correlation between traits and the cluster and principal component analysis were performed.

Results: The results showed that there were some significant differences among genotypes based on physicochemical characteristics. Among the genotypes, fruit weight and length varied from 12.87 to 23.80 g and 25.67 to 33.70 mm, respectively. Fruit was roughly round in majority of genotypes. Fruits contained 1.14 - 4.33 seeds depending on the genotypes. Fruit flesh ranged from approximately 2.5 to 6 times of seeds weight in genotypes 2 and 20, respectively. The highest (87.96%) and lowest (77.61%) amount of flesh humidity were recorded in genotypes 2 and 20, respectively. The percentage of seed dry matter differed from 41.13 to 51.27%. The highest total soluble solids (17.73%) were

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observed in genotype 13, whereas genotype 3 had the highest titratable acidity (1.16%) and vitamin C (38.10 mg/100g fresh weight). In addition, to determine the main discriminator traits among the genotypes, the principal components analysis of 19 quantitative traits was conducted and the evaluated traits were categorized within 5 factors, accounted for 89.95% of total variance. The first factor was related to fruit physical characteristics while the second factor was related to seed characteristics. These two factors accounted for 55.5% of total variance. Factor analysis showed that characteristics such as: fruit length, diameter, weight and volume, seed length, fresh and dry weight, seed dry matter percent, seed length to diameter ratio and seed number had the highest effects on genotypes diversity. Cluster analysis of 19 quantitative traits divided the genotypes into five main groups. Fresh flesh weight showed positive correlation with fruit length and diameter, weight and volume at $P < 0.01$, with fruit weight and volume at $P < 0.05$, and with fruit flesh thickness at $P < 0.05$.

Conclusion: Finally, genotypes with superior fruit characteristics such as: higher fruit weight, lower seeds within fruit or including higher qualitative traits may be introduced as promising ones for future evaluation, breeding or propagation programs and commercial orchards establishment. In this research genotype number 20 with the highest flesh to seed weight, genotype number 16 with the lowest seed number and higher flesh to seed ratio and genotype number 13 with the highest total soluble solids and flesh to seed ratio are introduced promising genotypes.

Keywords: Cluster analysis, Factor analysis, Loquat, Pomology

Contacts

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