Application of Morphological Characters and PCR- SCAR Markers for Identification of Dominant Species of Root-knot Nematode (*Meloidogyne* spp.) in Glasshouses of Cold Region of Kohgiluyeh and Boyer-Ahmad Province, Iran

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) are one of the major limiting factors for the successful production of green-house crops, including tomatoes and cucumber. Accurate identification of nematodes is important in the management program. Identification of the dominant species of root-knot nematode in the cold region of Kohgiluyeh and Boyer-Ahmad province (counties of Boyer-Ahmad and Dena), Iran, was done based on morphology of J2s and females as well as PCR-SCAR. For molecular identification, by using DNA extraction kit, DNA extracted from a mixture of eggs and second stage juveniles. The SCAR primers Fjav/Rjav, Finc/Rinc and Far/Rar were used for producing species specific products of 670bp, 1200bp and 420bp. The Fjav/Rjav primer set, used to detect *M. javanica*, amplified a 670bp fragment in all of the studied populations. Based on the morphological and molecular studies, the species of the root-knot nematode was identified as *M. javanica*.

Keywords: Meloidogyne javanica, Morphologic, Morphometric, PCR- SCAR marker

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Effect of Medium, Sugar and Plant Growth Regulators on Micropropagation of Saint Julien A (*Prunus domestica* spp. *Insititia*) Rootstock

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Abstract

Saint Julien A (Prunus domestica spp. Institita) is known as one of the important semi-dwarfing rootstocks with good compatibility with the majority of stone fruits (especially with plum), which no enough research carried out of effective factors on its micropropagation. In this project the effect of 5 types medium (MS, WPM, TK, GNH and mGNH), the interaction effects of PGRs (BAP at concentration of 0.25, 0.5, and 1.0 mg/l and IBA at concentration of 0.0, 0.05, 0.1, and 0.2 mg/l), and also the effect of carbohydrate source (Sucrose and Glucose) each at concentration of 0.0, 10, 20 g/l were studied on mentioned rootstock shoot promotion separately. Besides, for root induction two different methods application of IBA and NAA (gradually pre-application and pulsing) was independently conducted. Results indicated that the highest healthy shootlets N0. (2.125 per each explants) were observed on MS medium supplemented with BAP and IBA at concentrations of 0.5 and 0.1 mg/l, respectively, which had significant effect (α =0.05) in comparison with other treatments. Inspite, the result implicated clearly the significant interaction effects (α =0.01) of sucrose and glucose on both number and length of shootlet, in which the highest shootlet N0. (2.66) with desired growth quality was obtained when MS medium supplemented with both carbohydrates each at 20 g/l concentration, but the longest shoots (8.5 mm) were observed on MS containing 30 g/l sucrose which had significant difference (α =0.05) in comparison with other treatments The obtained results from root induction step significantly supported the reasonable efficiency of pulsing method (with 77.28% rooting and 1.8 root on each shoot) to other conventional method, as well as the superiority of NAA to IBA. The rooted plantlets with 60% success were acclimated in pots containing mixture of perlite and pitmass (3:1) and were transferred into greenhouse.

Keywords: Saint julien rootstock, Plum, Proliferation, Carbohydrate, Pulsing

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Study of Allelic Variation of GLU-B1 Locus in some Iranian Durum Wheat Using STS Marker

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Abstract

Genetic diversity assessment is a feasible approach and useful for germplasm. In this study, 30 genotypes of durum wheat were evaluated for GLU-B1 loci with three specific primer pairs. Nine alleles were present at the GLU-B1 locus. The b (BY8) allele was observed more frequently at the GLU-B1 locus. The amount of genetic variation for each primer that initiates B1.2 (0.85) primer were able to identify polymorphisms more than other primers. Cluster analysis was performed using UPGMA method and the similarity coefficient of 0.62 based on jaccard subdivided the genotypes into eight subgroups. The results revealed that the HMW subunits in durum wheat genotypes are valuable sources and they can be used in breeding program to improve the quality of wheat products.

Keywords: Durum wheat, Genetic variation, Glutenin, Specific primer

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Survey of Genetic Relationships in *Thymus* Accessions, Using Random Amplified Polymorphic DNA (RAPD) Marker

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Abstract

Thymus sp., commonly known as medical plant, is found in the different regions of Iran. Analysis of genetic diversity within species is vital for understanding evolutionary processes at the population and genomic level. Molecular marker technologies are the most advanced and, possibly, the most effective means for understanding the basis of genetic diversity. In the present study, 70 accessions collected from different districts of Iran were analyzed, using 30 RAPD primers. After DNA extracting, PCR performed using different primers. Totally, of amplified 407 bands, 320 bands were polymorphic corresponding to 78.62% polymorphism across the accessions. The average of polymorphism information content (PIC) was 0.34 and D13 and B04 primers had the highest and lowest PIC value, respectively. The UPGMA-based dendrogram constructed among the 70 accessions depends on the similarity matrix results. Results of Principal component analysis confirmed the results of cluster analysis. Assessment of genetically distances using RAPD marker could revealed important information for breeding and improvement of this plant.

Keywords: Polymorphism information content, Cluster analysis, Similarity matrix, Principal component analysis

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Clustering of the Dominant Broad-Bean Root Nodulating Bacteria Based on 16S rDNA and Their Phenotypic Pattern

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Abstract

Broad bean is one the most important legume plants in the world. Characterization and clustering of the root nodulating bacteria on this plant is a noteworthy issue. In spring 2011 root samples were collected randomly from broad bean fields in Lorestan province. A total of 65 bacterial strains were isolated which among them 58 strains could nodulated broad bean roots under green house condition. Phenotypic characteristics of these strains were determined based on the standard bacteriological methods. Results showed that they were belongs to two species including *Rhizobium leguminosarum* and *R. etli*. Total DNA from some representatives strains were isolated and they were subjected to PCR using specific primers for amplification of 16S rRNA encoding gene. Sequence analysis of the amplified PCR band from a broad-bean root nodulating representative strain by NCBI blast software revealed that the tested strains has high sequence similarity to *R. leguminosarum* bv. *viciae* strain BIHB. Clustering of the isolated strains showed a single separated cluster for representative strain with high similarity among the diverse *R. leguminosarum* bv. *viciae*.

Keywords: Rhizobium leguminosarum, Rhizobium etli, R. leguminosarum bv. viciae, Fabaceae

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Evaluation of Chlorophyll Content and Genes Expression (Catalase and DREB1) in Soybean Cultivars Under Drought Stress Condition

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Abstract

Drought stress is one of the important factors that restrict crop production in tropical and semi-tropical areas. This experiment has taken place in Golestan province Agriculture research station in split plot format with RCBD design with four replicates. Drought stress as a main factor included three levels (irrigation after 50, 100, and 150 millimeters evaporation from the A class evaporation pot) of which 50 millimeters evaporation is considered as control. The sub factor included factorial combination of 3 varieties (DPX, Sari and WE6) and three sampling stages (flowering, packing pod and grain filling). Evaluated traits included A and B chlorophyll amount and alternation in gene expression. The amount of chlorophyll was increased on first step of the stress condition (100 ml evaporation) and decreased again, on the second step (150 ml evaporation). The TBARM level was increased under the stress condition as well as growth stages. The gene expression analysis was carried out by using QRT-PCR technique. The results showed at first level of drought stress condition catalase gene expression had the most amounts. It was quite similar to DREB1 gene expression. DPX variety showed the most DREB1 gene expression first level of drought stress condition in packing pod stage, and least reduction in second level of the drought stress condition. Sari variety showed the most reduction in DREB1 gene expression in second level of the drought stress condition. Chlorophyll amount also showed a positive correlation with the catalase and DREB1 genes expression.

Keywords: Gene expression, Drought stress, Soybean, Catalase, Chlorophyll amount, DREB1

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Assessment and Genetic Characterization of Iranian Two-Humped Camel Using New World Camelidae Microsatellite Primers

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Abstract

In Iran camels are providers of milk, meat, fibers, sports and capital. This work was carried out to document the structure and assess the genetic diversity within Iranian two-humped camels (Camelus *bactrianus*). Using of 85 reproductive individuals belonging to four sampling locations of bactrian camels in Ardabil province, Nine microsatellite markers (CVRL07, CVRL01, CVRL05, CMS9, CMS15, VOLP10, LCA66, YWLL38 and YWLL59) were analyzed to assess polymorphism in this population. DNA extraction was conducted with optimized and modified salting-out method. The polymerase chain reactions (PCR) for 85 individuals were successfully done with all primers and then amplification products were resolved on 8% SDS PAGE and stained with silver nitrate. Eight of these markers were polymorphic, producing a total of 31 alleles. Significant deviations from Hardy-Weinberg equilibrium (HWE) occurred for all loci (P<0.005). Polymorphism ratio (P) for nine microsatellite primers in this study calculated 88.89%. Average allelic and polymorphism information content (PIC) for all loci estimated 3.4444 and 0.4726, respectively. The average expected heterozygosity excluding monomorph locus calculated 0.5242 and ranged 0.3869 to 0.7665. A phylogenetic analysis showed that Iranian bactrian camels can be divided into two main groups including many subgroups with some remote individuals. Hence the Iranian two-humped camels population have acceptable genetic diversity yet and can preserve of this valuable genetic resources from extinct with a proper management and breeding programs in Iran.

Keywords: Iranian two humped camel, Microsatellite markers, Genetic structure and diversity

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Genetic Characterization of Native *Bacillus thuringiensis* Strains Isolated from Forst Soil Samples of Mazandaran Province

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Abstract

Native *Bacillus thuringiensis* (Bt) strains were isolated from forest soils samples of different regions in Mazandaran province and some encoding genes (*Cry1*) for insecticidal crystal proteins were detected in the isolated Bt strains. A total of one hundred forty four *Bacillus* strains were isolated from 32 soil samples using selective inhibitory property of sodium acetate. Insecticidal crystal proteins were detected in 32.63% of the isolates following colonies culturing, specific staining and microscopic identification. *Cry1* gene was recognized in seven isolates as consequence of molecular analysis of the strains. Gene content investigation was carried out to detecting four *Cry1A* (*Aa*, *Ab*, *Ac* and *Ad*), *Cry1B*, *Cry1C*, *Cry1D*, *Cry1E*, *Cry1F*, *Cry1G*, *Cry1H*, *Cry1I*, *Cry1J* and *Cry1K* genes by using 14 specific primers. *Cry1Ac* and *Cry1I* genes were detected in all isolates, but *Cry1Aa*, *Cry1E* and *Cry1G* were not found in all isolates. Based on the results, the described molecular procedure can be exploited for identification of the insecticide-proteins encoding genes.

Keywords: Crystal protein, Cry1 gene, Molecular characterization of Bt

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The Effect of Ethyl Methane Sulfonate on Some of Growth and Developmental Parameters in Plants Regenerated Petunia (*Petunia hybrida* Vilm.)

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Abstract

In order to evaluate the effect of Ethyl Methane Sulfonate (EMS) on some of growth and developmental parameter in regenerated plant of *Petunia hybrida* Vilm., seeds of Petunia were cultured on MS medium. Then the large number of plant was reproduced from each single plant. Leaf and stem segments were treated with EMS 1% and 2% for 15, 30 and 60 minutes and were transferred to medium containing cytokinin BAP (benzyl amino purine). Finally photosynthetic pigments, diameter vascular system and root formation in regenerated plant were recorded. Protein from leaf segment was extracted and the total protein, and the protein patterns was evaluated using SDS-PAGE. The Results showed that treated plant with higher concentration of EMS for longer duration time, showed the significant differences in all parameters compared with control and other plants. These differences were similar with differences in protein assay and SDS-PAGE pattern of protein. However, the concentration and duration of EMS treatment resulted in change the plant is different for each plant and it is depending on the plant used for the study.

Keywords: Petunia hybrida, Ethyl methane sulfonate, Chlorophyll, Protein

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Molecular Studys of *Fusarium graminearum* Species group isolated of Wheat in Ardabil Province Iran

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Abstract

One of the most important diseases on wheat is Fusarium head blight (FHB) which causes high losses on wheat crop quantitatively and qualitatively. The main species caused FHB are Fusarium graminearum, F. culmorum and F. avenaceum. Fusarium graminearum isolates are concerned as the major agents to produce deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, and zeralenone in wheat. Regarding to high DON contamination of 2011 wheat crop production at Ardabil province, population analysis of the Fusarium graminearum species complex isolated from wheat crop of this area was considered as the purpose of this study. During Jun till July of 2011 the FHB wheat contaminated samples were collected from wheat fields and harvested wheat crop through Ardabil area and Fusarium isolates obtained from wheat samples using suitable laboratory methods. 321 F. graminearum isolates were identified based on morphological characters. The identification of all 321 isolates was confirmed molecularly using Nicholson's specific F. graminearum primers. To resolve the different members of the F. graminearum clade, the species specific primers which designed for partial sequences of the ammonia ligase 2 (CTPS2) gene were used. F. asiaticum, F. meridionale, and other members of the F. graminearum clade can be distinguished based on differences in the length of the CTPS2-derived amplicons by these primers. The primers for the ammonia ligase 2 gene produce fragments of 162 and 311bp in isolates of F. asiaticum, while the 311bp fragments are observed in all other members of the F. graminearum clade To find the phylogenic relation four isolates were selected as the representative isolates and their ammonia ligase 2 gene amplicons were sequenced. The results of PCR products showed that 89.4% of F. graminearum isolates (287 out of 321isolates) were F. asiaticum due to producing both 162 bp and 311 bp bands while in 10.6 % of isolates only one band (311 bp) was produced which indicated this minority of isolates were not F. asiaticum but they were in the clade of F. graminearum. The sequences of the ammonia ligase 2 gene amplicons of four representative isolates confirmed the identification of F. asiaticum as the dominant species of F. graminearum clade on FHB contaminated wheat in Ardebil province. The results showed that F. asiaticum was the predominant species causing Fusarium head blight (FHB) of wheat suggested resulting in DON production throughout all regions of Ardabil province.

Keywords: Wheat, Ardabil, Fusarium asiaticum, Fusarium graminearum complex species

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