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Field performance and molecular diversification of lemon selections

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ABSTRACT

Lemon (*Citrus limon* (L.) Burm. f.) is one of the most important Citrus fruit for Turkey because of its great amount of production and export. It has been cultivated for a long time in Turkey, and therefore variations for agronomical traits are likely among cultivated lemons due to bud mutations and, hybridizations. The objectives of this study were to determine variations for some selected agronomical traits and genetic markers among 12 new lemons derived from selections. Tree growth, yield, fruit quality, and molecular diversification of these clones were determined. After four years of evaluation, 'Kutdiken' M-51 indicated the highest canopy volume. For yield per tree, the best clone was 'Kutdiken' M-51. After five years of evaluation, 'Kibris' M-54 had the highest fruit weight and acidity. 'Italian Memeli' M-56 contained the lowest seed number and the highest total soluble solids. Molecular analysis, as assessed with 22 random amplification of polymorphic DNA (RAPD) and 11 inter simple sequence repeats (ISSR) primers, indicated that seven of twelve clones were separated with RAPD markers, whereas four were distinguished with ISSR markers. Combined analysis of RAPD and ISSR data detected that similarity values among the lemons clones were between 0.97 and 1.00. It can be concluded that variations in orchards are abundant and mainly due to mutations.

1. Introduction

Citrus is the most important fruit crop in the world, with an annual production of approximately 110 million tones. Lemon (*Citrus limon*) is the third most important species of citrus after orange (*Citrus sinensis* L. Osbeck) and mandarin (*Citrus reticulata* Blanco), with an 11% of total production. Total Turkish citrus production is about 3 million tones and 23% of this was of lemon (FAO, 2006). Turkey has suitable climatic conditions to grow most citrus cultivars. Especially, east Mediterranean region of Turkey, with an elevation ranging from 0 to 700 m from the sea level and diverse microclimates has important role in citrus production in Turkey (Uzun et al., 2004). Lemon is used for fresh fruit market, pectin, juice and essential oil. Fruit size and peel color are important fruit characteristics for fresh market, while, for processing, soluble solids, juice, pectin and essential oil content are important (Quaggio et al., 2002).

Lemon was reported as a hybrid of citron (*C. medica* L.) and sour orange (*C. aurantium* L.) in recent studies (Nicolosi et al., 2000; Gulsen and Roose, 2001). Most of lemons have highly similar morphological and biochemical characters, and some are reported to have originated by mutation from single parental lemon tree (Gulsen and Roose, 2001). In addition, it was suggested that most of the commercial citrus cultivars originated by spontaneous mutations (Mendel, 1981; Gulsen and Roose, 2001). Lemon-like fruits were difficult to create by hybridization because lemon has high level heterozygosity. Concordantly, chance of selecting a lemon similar to true lemon derived by hybridization is very low.

So far clonal selection has been generally used as one of traditional breeding methods because bud mutations arise frequently in *Citrus* (Iwamasa et al., 1981). This offers advantages to select clones suitable for their aims. Yield and fruit quality can be increased via clonal selection of natural mutants. Clonal selection studies, so far, aimed regular production over years, homogeneous fruit weight, and tolerance to biotic and abiotic stresses. In general, selecting main source trees, testing different ecological conditions, and isolating from pathogens are main steps of clonal selection programmes (Ulubelde et al., 1986).

So far, genetic diversity and phylogeny studies have been carried out in *Citrus* using various marker systems. For instance, RAPD (Federici et al., 1998; Abkenar et al., 2004), RAPD and sequence characterized amplified region (Nicolosi et al., 2000), amplified fragment length polymorphism (Pang et al., 2007), SSR (Barkley et al., 2006), ISSR (Shahsavar et al., 2007), cpDNA restriction fragment length polymorphism (RFLP) (Jena et al., 2009) markers have been used to determine genetic diversity of *Citrus*. The RAPD markers were reported to be more efficient than the RFLPs in *Citrus* cultivar identification (Fang and Roose, 1997). It

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was previously used to determine genetic diversity in lemon (Deng et al., 1995), mandarin (Coletta Filho et al., 1998) mandarin cultivars, and sweet orange hybrids (Oliveira et al., 2003). However, because RAPD analysis does not target those rapidly evolving sequences that may be most likely to differ between mutationally derived cultivars, the detection of polymorphic RAPD markers may require PCR amplifications with many different primers (Fang and Roose, 1997). ISSR markers involve amplification of DNA segment between two identical microsatellite repeat regions. It has been used for characterization, phylogenetic relationships among *Citrus* and related genera (Fang and Roose, 1997; Gulsen and Roose, 2001; Shahsavar et al., 2007). Both RAPD and ISSR markers are among the most utilized markers, since they are produced by techniques that are simple, inexpensive and relatively less labor-intensive (Oliveira et al., 2003). The objectives of this study were to determine variations for some selected agronomical traits and genetic markers among 12 new lemons derived from selections.

2. Materials and methods

2.1. Plant materials

Twelve clones of lemons collected from the citrus growing regions of Turkey between 1979 and 1983 were used in this study. They were iniatially selected for agronomical traits by observing for 2–3 years. They consisted of eight'Kutdiken' clones, (44-M, 45-M, 46-M, 47-M, 48-M, 49-M, 50-M, 51-M), 'Lamas' 52-M, 'Yediveren' 55-M, 'Italian Memeli' 56-M and 'Kibris' 54-M clones. 'Kutdiken' is the most widely produced lemon cultivar in Turkey with high yield, good fruit quality and long-term storage without significant loss of quality. 'Lamas' and 'Yediveren' are local Turkish lemon cultivars believed as selections from 'Kutdiken'. The former has good adaptation and the latter bears fruits year around, therefore being suitable for 'Verdelli' type lemon production. 'Italian Memeli' and 'Kibris' are also local lemon cultivars in Turkey.

2.2. Orchard management

Budwoods were taken from selected main source trees and grafted on sour orange (*Citrus aurantium* L.) rootstock. The grafted trees were planted to field in randomized complete block design with five replicates in 1991 in Alata Horticultural Research Institute by the Mediterranean coast in Mersin Province, Turkey. Tree spacing was 7 m × 7 m. The soil was loamy sand (70% sand, 20% silt, and 10% clay) having 5.64% $CaCO_3$ and a pH of 8.1. The trees were managed according to standard local commercial practices, pruned annually, and watered as needed using a drip irrigation system. Fertilization was managed based on soil and leaf analysis. In December, each tree was fertilized with 1.0 kg of potassium sulphate (50% K_2O) and 1.0 kg of triple super phospate (42% P_2O_5). Three kilograms of ammonium sulphate containing 21% of nitrogen was also applied two times (1/2 in February, 1/2 in May). In May, foliar sprays of manganese and zinc were also applied. Herbicides were used for weed control. Pest populations were kept under control a recommended pest management program.

2.3. Characterization for tree performance and fruit quality

Selected clones were evaluated as follows.

2.3.1. Tree growth

Tree height, canopy diameter, and scion trunk circumference were recorded annually for 4 years (2003–2006). Tree height and canopy diameter were converted into canopy volume. Canopy volume (CV, m^3) was estimated as follows: $4/3\pi ab^2$ where a = canopy diameter; b = tree height (Westwood, 1988). Scion trunk circumference was measured 10 cm above the bud union and was converted into trunk cross-sectional area (TCSA, cm^2).

2.3.2. Yield effciency

Yield by tree (kg/tree) were recorded annually during 4 years of production. Tree yield by canopy volume (kg/m^3) and trunk cross-sectional area (kg/cm^2) were also estimated.

2.3.3. Fruit quality

Twenty-five fruits per tree in December, commercial harvesting season of lemons in Turkey, were selected. All fruit samples were assessed for fruit weight (g), rind thickness (mm), juice percentage (%), total soluble solids (%), acidity(%) and seed number per fruit for 5 years (2002–2006) according to Ozsan and Bahcecioglu (1970).

2.4. Molecular analysis

The total genomic DNA was extracted from young leaves by the CTAB method as described by Doyle and Doyle (1990). DNA concentration was measured with a NanoDrop, ND 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA), and 10 ng/μL DNA templates were made using TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0).

A total of 11 ISSR primers evaluated previously (Fang and Roose, 1997) and 22 RAPD primers (Operon Technologies, Huntsville, Alabama, USA) were used for all clones (Table 1). For ISSR and RAPD each 15 μL reaction consisted of 1.33 mM of primer, 200 μM of each of dNTPs, 1.5 μL of 10× PCR Buffer (Bioron, Nantes, France), 2 mM of $MgCl_2$ as a final concentration, 0.8 μg/μL Bovine serum albumin, (Biological Industries, Beit Haemek, Israel) 7.2 μL ddH_2O, 1 unit of Taq polymerase (Bioron, Nantes, France) and 20 ng of template. DNA Thermal Cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK) was used for PCR amplifications. For ISSR analysis, cycling parameters included as follows: 2 min at 94 °C for initial denaturation, following 38 cycles of 1 min at 94 °C, 1 min at 50 °C, and 1.15 min at 72 °C, and then one cycle of 7 min at 72 °C for extension. For RAPD analysis, cycling parameters were as follows: 2 min for initial denaturation at 94 °C, following 38 cycles of 30 s at 94 °C, 1 min at 38 °C and 1.15 min at 72 °C, and then one cycle 7 min at 72 °C. PCR products were separated on 2% agarose gel in 1× TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 3 h. The fragment patterns were photographed under UV light for further analysis. A 1 kb and 100 bp standard DNA ladder as the molecular standard in order to confirm the appropriate markers were used for RAPD and ISSR analysis, respectively.

2.5. Data analysis

For tree and fruit characters, data were analyzed using SPSS 11.0 (SSPS, Chicago, IL, USA) and means were separated and grouped (a, ab, abc, abcd, b, ..., etc.) using Tukey's test ($P < 0.05$). Molecular analysis was performed as follows: each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 1993). A similarity matrix was constructed using RAPD and ISSR data based on simple matching coefficient. Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the clones studied. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA-based dentdrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test (Mantel, 1967). The result of