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Correlative relationships of introduced mandarin and lemon cultivars

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Abstract:
Mandarin (Citrus reticulate Blanco) and lemon (C.limon L.) are among the most cultivated Citrus species in the world.Among different approaches, gamma radiation is widely used to induce mutations in citrus breeding studies. Gamma radiation causes DNA damage, randomly inducing several mutations in the genome. Molecular techniquesarewidely used to detect such induced mutations and genetic diversity in plants.In this study, simple sequence repeats (SSR), inter-simple sequence repeat (ISSR) and single-strand conformation polymorphism (SSCP) markers were used to detectthe induced mutations andgenetic diversityin someirradiated mandarin and lemon genotypes in comparison with standard citrus varieties. The irradiated genotypes used in this study derived from populationsthat had been originally obtained with gamma radiation with different gray doses applied on Yerli Mandarin and Antalya YerliYuvarlak lemon varieties. Out of 82 primers (22 SSCP, 30 ISSR and 30 SSR) tested, 55 primers (9 SSCP, 24 ISSR and 22 SSR primers) successfully amplified a total number of 363 amplicons.A single band with an approximate size of 500 nucleotides was determined using the primer ISSR 868 as differentiating between seedy parent and irradiated seedless mandarin genotypes. The polymorphism information content (PIC) values of SSCP, ISSR, and SSR markers ranged from 0.61–0.99, 0.31–0.96, and 0.33–0.96, respectively. Cluster analysis classified lemons and mandarins into 2 subgroups as mutant and commercial lemon genotypes. ISSR markers were found to be more effective to determine the genetic differences among the varieties in comparison to SSCP and SSR markers. The results clearly showed that SSCP, SSR and ISSR markers are important tools to distinguish mutant genotypes andconfirmed their usefulness for phylogenetic studies.

Key words:
Molecular marker, Morphology, Phenetics, Species identification, Taxonomy, Citrus, genetic dierences, mutation breeding, thornlessness, seedlessness.

Introduction: Citrus is one of the most important fruit crops with an increasing consumptionworldwide (Crowley, 2011). The most important species of citrus are sweet oranges, mandarins, lemons, and grapefruits (Mabberley, 2008). With the rise in consumer population, the demand for citrus fruit production has been increasing, and there has been a great deal of research aiming to develop new citrus varieties (Terolet al., 2008; Crowley, 2011). One of the main goals for the genetic improvement of fresh fruit in the world has been the development of varieties yielding fruits that are seedless, easy-peeling, avorful and aromatic with a long ripening period (Raza et al., 2003).[1] Also, the achievement of thornless trees is desirable because of their ease of handling and laborsaving advantages (Yoshida et al., 1999). Additionally, development of resistant varieties to biotic and abiotic stresses is very important for breeders.Conventional breeding methods could be

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laborious, time-consuming, and expensive. erefore, mutation breeding has been an alternative way of achieving new citrus cultivars. Mutation could be induced articially with the help of various physical and chemical agents called mutagens (Lamo et al., 2017). Techniques such as radiation or chemical mutagens application are advantageous for increasing variability in crop species, since spontaneous mutations occur with an extremely low frequency (Sutarto et al., 2009). Erst experiment on articial mutation was carried out on citrus seeds using x-rays by Haskin and Moore in 1935 (Cameron and Frost, 1968).[2]

Materials and methods: 1) Nine stable mutant thornlessness lemons, oneparent [nonmutant Antalya Yerli Yuvarlak Limonlemon (L. Parent)], 34 stable mutantseedless mandarins,1 parent [nonmutant Yerli Mandarinmandarin (M. Parent)] obtained from a mutation breeding project (Turgutoğlu et al., 2014) were used. 3commercial lemon, 5 mandarin, 1 citron, 1tangelo and 1 tangor varieties were obtained from the Batı Akdeniz Agricultural Research Institute (BATEM) Citrus genetic gene pool, and were also included in the study as references.Stable mutant genotypes were chosen at the M1V3 stage and from dierent applications of 60Co doses as shown in Table 1. 2.2. DNA isolationTotal genomic DNA was isolated from young leaves using the CTAB method as described by Doyle and Doyle (1990). e quality and concentration of the extracted DNAs were checked with a spectrophotometer (ermo Scientic™ NanoDrop, V, Finland) and by 1% high resolution agarosegel electrophoresis.2.3. SSCP analysisExpressed sequence tags (ESTs) primers (Table 2) were developed by NCBI (National Center for Biotechnology Information) screening. Primer design was carried out using Primer 3 soware (Rozen and Skaletsky, 2000), allowing the prevention of hairpin loops and dimers. PCR reactions for SSCP based molecular markers in citrus were performed according to the published protocol Simsek it) all. [3]

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Table 1. Plant materials, their cultivar or code name

Table 2. List of SSCP primersand their bases sequences 5'-3'

DNA	Variety or	^{60}Co doses	DNA	Variety or	^{60}Co doses

No	Forward	Reverse
1	GACCACTTTGATGGCTGTGA	GCAGAAGTCAGTAAACC
2	CATCACCAGCATATGGGACA	CAGAAGCGGCAAGAATGT
3	ATCGTGTTATCGCCATGGTT	CGTAACGTGCCAAAGTTTTT
4	CGTGTTGCCTGATTCATTTG	GCTTCAGGAATAACCCCAGA
5	AGTGAAATGCATTATCTGTTGCAG	TTATCAATATCTCTTGATTGCACA
6	CTGACCCCACTGAGATGGAT	CCCTTATTGCCATGATGCTT
7	CGTCGACTATGCCTGCTACA	GCAGGTCTCTTCACCGAGTC
8	CAAAATTCACGATCGCCTTT	AGGGCCAAACCTTTTCTCAT
9	TGGAATTGAAGGCAAAAAGG	TGCTCTTCTGGCAGTTCTCA
10	TGCTAGCTCCAAGGACAGGT	CGACCACAAGCTGATAGCAA
11	AGCAACTGGCTGGTCTTACAA	CCTCTTCGAATGGCTGAAAC
12	TTCTCTCTCAGGTTCCTTGTACTTT	GCTTTCTTTTCTATTTGTTTTCTGA
13	TGTTTCCCCTCTGCTTTCAC	ATTTGGCAAGGCCTCTCAG
14	AAGAAGAAATGGGGGAAACG	CTTGAGGCCATGGAGGATT
15	AATTCTAATTGGGGCCAACC	AATGCAAAATTCCGTGAACC
16	ACCGTTTGATCGAGTATGC	GCTGTCACTCCACCCGTAGT
17	AGCGTGCTCTCTCGTTAGAT	GAAGGATCATTGTCGAAACC
18	TCTGCAATTCACACCAAGTA	GGAAATCTAACGAGAGAGCA
19	CTGCAATTCACACCAAGTAT	CCAAGGAAATCTAACGAGAG
20	TTGCGTTCAAAGACTCGATG	CTGCGCCAAGGAAATCTAAC
21	TCAAAAATTCAAGGGTTCAC	GCCAAGGAAATATAACGAGA
22	AGCTCGAGAGGCTTTTGTTT	CGTGAACCCTTGAATTTTTG

Citrus is a genus of horticultural plants cultivated for its fruit either as table fruit or food additives Citrus also known as aromatic plants from which the essential oils is extracted from the peel or leaves for pharmaceutical and industrial needs due to the distinctive refreshing aroma [4,5]. Several Citrus species are cultivated to other purposes, such as rough lemon (Citrusjambhiri lush), which is used as rootstock for commercial Citrus species due to its adaptability [6], disease

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resistance [7], and positive effects on scion's performances and yield [8].Molecular analysis of the origin of Citrus species confirmed that rough lemon was a hybrid between citron and mandarin [9]. Phylogenetic analysis revealed rough lemon's origin from C. Medica and C. Reticulata [10]. Evidence suggests Citrus's Southeast Asian origin [11]. It gained species diversity from admixture among progenitor species through interspecies hybridization followed by clonal propagation and cultivation [12]. Accurate species identification is very important for the exploration of biodiversity [13].

Most studies on rough lemon are related to its role as rootstock for commercial Citrus. These have focused on morphology [14], growth and physiology under salt stress [15], and rooting and budding performance as rootstock [16]. Research on rough lemon's potential for other functions, such as antibacterial [18] and insecticide [17], have been linked to its secondary metabolite compounds. The potential of rough lemon as nutritious food product in India showed that fruit juice and peel of rough lemon was processed into several value-added products such as squash, pickle, candy, and jelly [19]. Meanwhile, [20,21] reported the essential oils content of rough lemon. The economic importance of rough lemon is particularly its role as vigorous, highly tolerant, and highly adaptable rootstock for commercial plantations of other Citrus species [22]. The essential oils extracted from fruit rind of rough lemon was reported as having antimicrobial activity against Gram positive bacteria and yeast [23]. In this 2case, the importance of Citrus species as source of ecologically friendly botanicals has been mentioned by [24]. Meanwhile, the medicinal importance of rough lemon has been recognized as having therapeutic properties in Ayuverda formulations as antidiarrhea and to improve digestion . The medical benefit of rough species is high in crude fiber and minerals such as magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn) relative to another Citrus [25]. It has been processed into value-added food products , its essential oils are reported to have antimicrobial effects , and it is used in Ayurvedic medicine . Rough lemon is particularly popular as a food product in Indonesia's South Kalimantan and Central Kalimantan provinces, while in North Sumatra, it is sold as food flavoring. Rough lemon has high intraspecific variation, especially in fruit morphology [26]. This causes misrecognition with morphologically similar species such as C. Hystrix and C. Amblycarpa, which can be overcome through studies on characterization, intraspecific variation, and taxonomic relationships of rough lemon with other species.

Studies reveal variation within a species plays an important role as an initial step in mapping the wealth of biological resources and serves as basis for formulating strategies for developing its potential. Such studies start with comprehensive characterization on species of interest. Characterization is needed to identify accessions with similar genotypes, taxonomic relationships, and effective germplasm management of germplasm. Phenotypic characterization is generally based on morphology while genotypic characterization uses molecular markers. There have been no publications regarding rough lemon's taxonomic relationship with morphologically similar species, and the potential of C.jambhiri for food and other purposes is underexplored. This research was performed with the aim of producing comprehensive characterization of C.jambhiri based on morphological characters and Inter Simple Sequence Repeats (ISSR) markers, as well as revealing taxonomic relationships with C. Amblycarpa and C. Hystrix.[27]

C. jambiri samples were collected from South Kalimantan and Central Kalimantan. Nasnaran mandarin (C. Amblycarpa) and kaffir lime (C. Hystrix) samples were obtained from Central Java and Yogyakarta: varied collection sites ensured control for environmental factors. 18

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total samples were used (Table 1). Leaf samples for DNA isolation were preserved in silica gel. Morphological characterization was based on observation of 20 leaves and 5 mature fruits. Morphological characters examined referred to [28]. Morphological data used for characterization and analysis of taxonomic relationships consisted of 49 characters obtained from observations of plant habit, leaves, and fruit (Table 2). For each character, binary or multiple character states were determined according to sample conditions. For purposes of cluster analysis, each character state was converted into numerical data, referring to research on morphological characterization of C. Maxima [29].

Summary

Tangerines and oranges are citrus fruits that look similar, but are not alike. They belong to the same species, but they have slight differences such as taste, size, shape, origin, etc. We have a lot to learn about their differences.

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