Citrus R&T (e-ISSN 2236-3122)
http://citrusrt.centrodecitricultura.br
doi: 10.5935/2236-3122.20130006

Artigo/Article

Genetic variability in *Citrus* sp., related genera and hybrids from germplasm collection evaluated by random amplified polymorphic DNA (RAPD)

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SUMMARY

The tools of molecular biology have enabled the improvement of citrus with significant reduction in identification and selection of new materials, and are usually preferred for genetic diversity assessment, population genetic structure and mapping. Relationships among 24 accessions of *Citrus* species, related genera and hybrids were investigated using random amplified polymorphic DNA (RAPD). A dendrogram based on the unweighted pair-group method for the arithmetic mean method was constructed using a similarity matrix derived from 96 polymorphic RAPD fragments. High polymorphism was detected among the accessions and the number of fragments/primer/accession ranged from 2 to 8. Accession-specific DNA fragments were observed for *Murraya* and *Feroniella oblata*. Within the subtribe Citrinae, the genera *Poncirus*, *Microcitrus, Severinia* and *Atalantia* were more distant from *Citrus*, and *Fortunella* was the most closely related. The *Citrus* species clustered in one group, including possible hybrid species, and the data did not support a separation into the subgenera *Citrus* and *Papeda*. Although RAPD technique sometimes has been criticized, it still can be used with successfully and this study confirmed these markers are useful and efficient for analysis of variability in citrus species. The information generated will be important for breeding programs using citrus germplasm.

Index terms: Aurantioideae, DNA polymorphism, germplasm characterization, molecular markers.

RESUMO

Variabilidade genética em *Citrus* sp, gêneros relacionados e híbridos de uma coleção de germoplasma avaliada por meio de marcadores moleculares RAPD

As ferramentas de biologia molecular têm auxiliado sobremaneira o melhoramento de citros, com uma redução significativa na identificação e no ciclo de seleção de novos materiais, e são preferidos para análise de diversidade genética e mapeamento de populações.

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As relações genéticas entre 24 acessos de diferentes espécies de citros, gêneros afins e híbridos foram investigadas por meio de polimorfismo de DNA amplificado ao acaso (RAPD). O dendrograma com base em agrupamento UPGMA foi construído utilizando uma matriz de similaridade derivada de 96 fragmentos polimórficos de RAPD gerados por 19 iniciadores. Alto polimorfismo foi detectado entre os acessos e o número de fragmentos por iniciadores/acesso variou de 2 a 8. Fragmentos únicos foram observados em dois acessos, *Murraya* e *Feroniella oblata*. Dentro da subtribo Citrinae, os gêneros *Poncirus*, *Microcitrus*, *Severinia* e *Atalantia* foram mais distantes de *Citrus*, enquanto o gênero *Fortunella* foi o mais intimamente relacionado. As espécies cítricas foram agrupadas em um cluster isolado, incluindo possíveis espécies híbridas, e os dados não apóiam a separação nos subgêneros *Citrus* e *Papeda*. Os dados gerados revelaram-se úteis para a análise da variabilidade em *Citrus* e gêneros relacionados e são consistentes com relatos da literatura. Embora a técnica de RAPD, por vezes, tenha sido criticada, ainda pode ser usada com sucesso conforme mostrou este estudo, sendo ferramenta útil e eficiente para a análise da variabilidade de espécies cítricas. As informações geradas têm sido usadas como base aos programas de melhoramento genético de citros, envolvendo a coleção de germoplasma.

Termos de indexação: Aurantioideae, polimorfismo de DNA, marcadores moleculares, caracterização de germoplasma.

INTRODUCTION

The subfamily Aurantioideae of the family Rutaceae is represented by 33 genera, which include *Citrus,* the most widely planted fruit species in the world, and its close relatives. The most usually accepted classifications of the group were established by Swingle and Reece (1967) and Tanaka (1954), but they are very complex and differ considerably in number of species, since Swingle and Reece recognized 16 species and Tanaka, 162 species. This complexity derives from the plants cultivation for thousands of years, their sexual compatibility with most of the species and genera, high level of polyembryony and frequency of somatic mutations and apomictic reproduction (Nicolosi et al., 2000; Moore, 2001).

The study of genetic variability and its distribution in citrus are of great importance for the establishment of genetic relationships, and for the conservation and characterization of germplasm collections (Malik et al., 2013). As a result, this would allow the prevention of genetic erosion of these materials against resource loss. Furthermore, it could help in the design of sampling strategies and the establishment of improvement programs (Federici et al., 1998; Moore, 2001; Novelli et al., 2004; Barkley et al., 2006).

The germplasm collection of the Centro de Citricultura Sylvio Moreira-IAC (CCSM – IAC) Cordeirópolis, SP, Brazil, is one of the most important of citrus in the world, containing approximately 1.700 accessions of *Citrus* and related genera. This collection has been utilized as an excellent source of genotypes in an extensive improvement program for resistance to diseases (Cristofani-Yaly et al., 2005), including studies of genetic inheritance (Siviero et al., 2003; Siviero et al., 2006; Bastianel et al., 2006) and genome mapping (Cristofani-Yaly et al., 1999; Oliveira et al., 2004; Oliveira et al., 2007). Thus, the knowledge of the genetic variability and its pattern of distribution are essential for developing strategies for management, conservation, characterization and germplasm utilization.

In citrus, molecular markers have been applied extensively to fingerprint accessions (Novelli et al., 2004; Novelli et al., 2006), identification of cultivars and hybrids (Oliveira et al., 2002), establishment of phylogenetic relationships (Nicolosi et al., 2000; Froelicher et al., 2011), and to evaluate the level of genetic diversity (Pang et al., 2003; Novelli et al., 2004; Barkley et al., 2006; Ollitraut et al., 2012). Among molecular markers, the main advantages of random amplified polymorphic DNA (RAPD) lies in relatively

inexpensive and applicability to any organism without prior knowledge of nucleotide sequence. Although, RAPD technique sometimes has been criticized, it still has been useful and used with successfully and efficiency for characterization and genetic diversity assessment in citrus (Maya et al., 2012; Malik et al., 2013).

In this study, *Citrus* species, hybrids and related genera, as represented in the CCSM-IAC Germplasm Collection, were evaluated with the aim to assess the level of genetic relationship and diversity to offer support to citrus breeding research programs.

MATERIALS AND METHODS

Plant material

The plants used in this study were obtained from the citrus germplasm collection of the Centro de Citricultura Sylvio Moreira-IAC, located in Cordeirópolis, SP, Brazil, and represent twenty-four accessions for *Citrus* species, correlated genera and hybrids (Table 1).

DNA extraction and RAPD amplification

DNA extractions were performed on two independent events from young leaves (100 mg) using the methodology described by Doyle and Doyle (1990), and concentration was measured using agarose gel electrophoresis according to Sambrook et al. (1989). Sixteen primers from Operon Technologies Inc., USA (OPK01, OPN07, OPR03, OPR04, OPR07, OPR08, OPR10, OPR15, OPR20, OPP2, OPP14, OPP19, OPX07, OPX12, OPX13, OPY09), and three pairs of primers (5.8S and 23S rDNA, and 23S mitochondrial universal primers) were evaluated.

Amplification reactions were prepared in a final volume of 13 µL, containing 7.5 ng of genomic DNA, 1.0 unit of Taq polymerase (Invitrogen), 20 ng of primer, 2.5 mM each of dATP, dTTP, dCTP and dGTP (Invitrogen), 1.3 µL of 10X buffer (Invitrogen), 1.5 mM $MgCl_2$, and 1.04 µL (10 mg/mL) of Bovine Serum Albumin (BSA). Amplification conditions were 36 cycles of 1 min at 92°C, 1 min at 36 ºC, and 2 min at 72ºC, followed by a final extension of 10 min at 72º C. The amplified fragments were visualized on a 1.5% agarose gel in 1X TBE buffer, stained with 0.5 µg/mL ethidium bromide, and amplicons size were estimated using 1Kb DNA ladder (Gibco).

Data analysis

The SEQAID II software (Rhoads & Roufa, 1989) was employed to estimate the size of the generated RAPD fragments. Polymorphic bands were scored as present (1) or absent (0). Only those fragments consistently amplified were considered for analysis. Cluster analysis was performed with NTSYS-pc, version.1.7, a numerical taxonomy and multivariate analysis software package (Rohlf, 1992). A similarity matrix was generated using the Jaccard coefficient (Sneath & Sokal, 1973) and a dendrogram constructed using the UPGMA method (Unweighted Pair-Group Method, arithmetic average).

RESULTS AND DISCUSSION

A total of 2,250 fragments were amplified with 19 RAPD primers, in 14 *Citrus* species, 2 intergeneric hybrids and 8 species of related genera, of which 96 bands were polymorphic to study citrus relationships. According to the primer evaluated, the number of polymorphic DNA fragments amplified by primer/accession ranged from 2 to 8, and the size of these fragments ranged from 501 to 3,056 bp (Table 2). Accession-specific DNA fragments were observed in two accessions, *Murraya* (OPN07-977 bp and 707 bp) and *Feroniella oblata* (OPX 13-1440 bp), which helped in drawing a distinction among more close accessions.

UPGMA cluster analysis was perfomed using RAPD markers data and formed an unrooted similarity dendrogram (Figure 1). The germplasm accessions were classified in several clusters and *Citrus* was the most diverse group of species, including *Fortunella* accessions. In this *Citrus* group, *Papeda* and *Citrus* formed distinct clusters. Thus, although *Fortunella* is well-differentiated from *Citrus* on the basis of detailed morphological studies, apparently there is no divergence, at the same level, observed in the molecular studies (Araújo et al., 2003; Barkley et al., 2006; Federici et al., 1998; Nicolosi et al., 2000). Within the subtribe Citrinae, the genera *Poncirus*, *Microcitrus, Severinia, Eremolemon* and *Atalantia* are more distant

Table 1. Citrus species, related genera and hybrids, with *Citrus* subgenus assignments according to the classification of Swingle (1943) and Tanaka (1954)

Accession	Subtribe	Species	Common name	Group[1]	Subgenus (Swingle)	Subgenus (Tanaka)
CV417	Micromelina	*Micromellum tephrocarpa*		--		
CV415	Clauseninae	*Murraya sp*		--		
CV419	Citrinae	*Severinia bruxifolia*		A		
CV413	Citrinae	*Atalantia ceylanica*		B		
CV423	Citrinae	*Fortunella margarita*	Negami	C		
CN835	Citrinae	*Poncirus trifoliata*		C		
CV418	Citrinae	*Microcitrus sp*		C		
CV422	Citrinae	*(Fortunella x mandarin)*	Nippon	--		
CV416	Citrinae	*Eremolemon coachella*		--		
CV378	Citrinae	*Citrus celebica*	Celebis Papeda	C	Papeda	Archicitrus
CN384	Citrinae	*C. hystrix*	Mauritius Papeda	C	Papeda	Archicitrus
MAT	Citrinae	*C. limon*	Eureka limon	C	Eucitrus	Archicitrus
CN700	Citrinae	*C. glaberrima*	Kinukawa	C		Archicitrus
CV458	Citrinae	*C. pseudo-paradisi*		C		Archicitrus
CV460	Citrinae	*C. natsudaidai*		C		Archicitrus
CV379	Citrinae	*C. taiwanica*		C		Archicitrus
MAT	Citrinae	*C. sinensis*	Pêra	C	Eucitrus	Archicitrus
MAT	Citrinae	*C. sinensis*	Hamlin	C	Eucitrus	Archicitrus
CN703	Citrinae	*C. junos*	Yuzu	C		Metacitrus
CV459	Citrinae	*C. yatsushiro*		C		Metacitrus
CV461	Citrinae	*C. keraji*		C		Metacitrus
MAT	Citrinae	*C. reticulata*	Ponkan	C	Eucitrus	Metacitrus
CV421	Citrinae	*×Citrofortunella microcarpa**	Calamondin	C		Metacitrus
CV410	Balsamocitrinae	*Feroniella oblata*		--		

[1] A=primitive citrus group, B= near-citrus group, C=true citrus group

* According GRIN, Database, USDA (2014).

Table 2. Primer sequence and features of amplification products

Primer	Sequence	Number of fragments	Size (bp)
OPK01	cattcgagcc	4	774-2177
OPN07	cagcccagag	4	505-2402
OPR03	cccgtagcac	4	1178-2795
OPR04	actggcctga	5	889-3041
OPR07	cccgttgcct	8	501-2171
OPR08	ccattcccca	8	674-2860
OPR10	ggacaacgag	6	669-3056
OPR15	acggcaagga	5	507-2730
OPR20	tcggcacgca	4	511-2167
OPR02	ccagccgaac	5	565-2648
OPP14	ccagccgaac	7	503-2749
OPP19	gggaaggaca	2	770-1780
OPX07	gagcgaggct	3	1020-2040
OPX12	tcgccagcca	3	988-2075
OPX13	acgggagcaa	3	1030-2233
OPY09	agcagcgcac	8	625-2399
5.8s rDNA	-	8	503-2136
23s rDNAn	-	7	507-3048
23s rDNAm	-	2	654-1880

from *Citrus*. *Poncirus trifoliata* is included in the true citrus group, but was distantly related of the others citrus group, as was also observed by Barkley et al. (2006), Herrero et al. (1996) and Pang et al. (2003).

The highest genetic distance (0.2) was observed between *Atalantia ceylanica* with all of the accessions, while the lowest distance (0.75) was observed among *C. pseudo-paradisi* and *C. yatsushiro*. As reported in previous studies with SSR and SNP markers (Novelli et al., 2004; Novelli et al., 2006), *C. sinensis* accessions showed same diversity level among them (100% identity). It has been found through various studies, that it is difficult to differentiate varieties of sweet oranges even using molecular techniques.

Within the genus *Citrus,* one cluster was composed of *C. taiwanica*, ×*Citrofortunella microcarpa*, C. *natsudaidai*, *C. pseudo-paradisi*, *C. yatsushiro*, *C. keraji*, *C. glaberrima* and *C. junos*, which are considered to be possible hybrids (Barret & Rhodes, 1976) and belongs to the true citrus fruit trees (Swingle & Reece, 1967). Some species are pummelo-like fruits and do not have commercial importance, with exception *C. natsudaidai* that has great commercial value in Japan due to low temperature resistant and late maturing, and *C. junos* that is widely cultivated in China and Japan as rootstock due its disease resistance and juvenility (Swingle & Reece, 1967).

Also considering the genus *Citrus*, the present data did not support the subgenus separation as proposed by Swingle (1967). This result is in agreement with RFLP data (Federici et al., 1998), SSR data (Barkley et al., 2006; Pang et al., 2003) and cpDNA data (Araújo et al., 2003; Nicolosi et al., 2000). The *Papeda* subgenus was represented by only two accessions (*C. hystrix* and *C. celebica*), where it would be interesting to study other species of this genus and to expand the number and type of molecular markers in order to clarify their position among *Citrus* relationships.

Microcitrus is closest to *Citrus*, such as *Fortunella*, which implies a relationship between these genera. In the fact, several studies demonstrated that they originated from a common ancestor (Araújo et al., 2003; Pang et al., 2003). The genetic proximity of the *Citrus* explains an easy hybridization and could be very useful in citrus breeding programs given its wide range of ecological adaptations (Herrero et al., 1996).

This study with RAPD analysis is in partial agreement with the known taxonomy of subfamily Aurantioideae (Table 1), since all the genera forming the true citrus group (C) were distinct from the primitive citrus group (A), near-citrus group (B), subtribe Balsamocitrinae (*Feroniella*), and subtribe Clauseninae (*Murraya*). Furthermore, the dendrogram indicated that accessions in the true citrus group are close but located a large distance from the others of citrus group. Although *Micromellum tephrocarpa* is considered a remote citroide group, in this study, it was found to be closely related to the true citrus group. However it has been observed that the morphological leaf traits of this accession, in the germplasm collection of the Centro de Citricultura Sylvio Moreira-IAC, do not correspond with descriptions in previous literature (data no shown). Further studies, using additional markers and more *Micromellum* accessions are needed to clarify its correct classification.

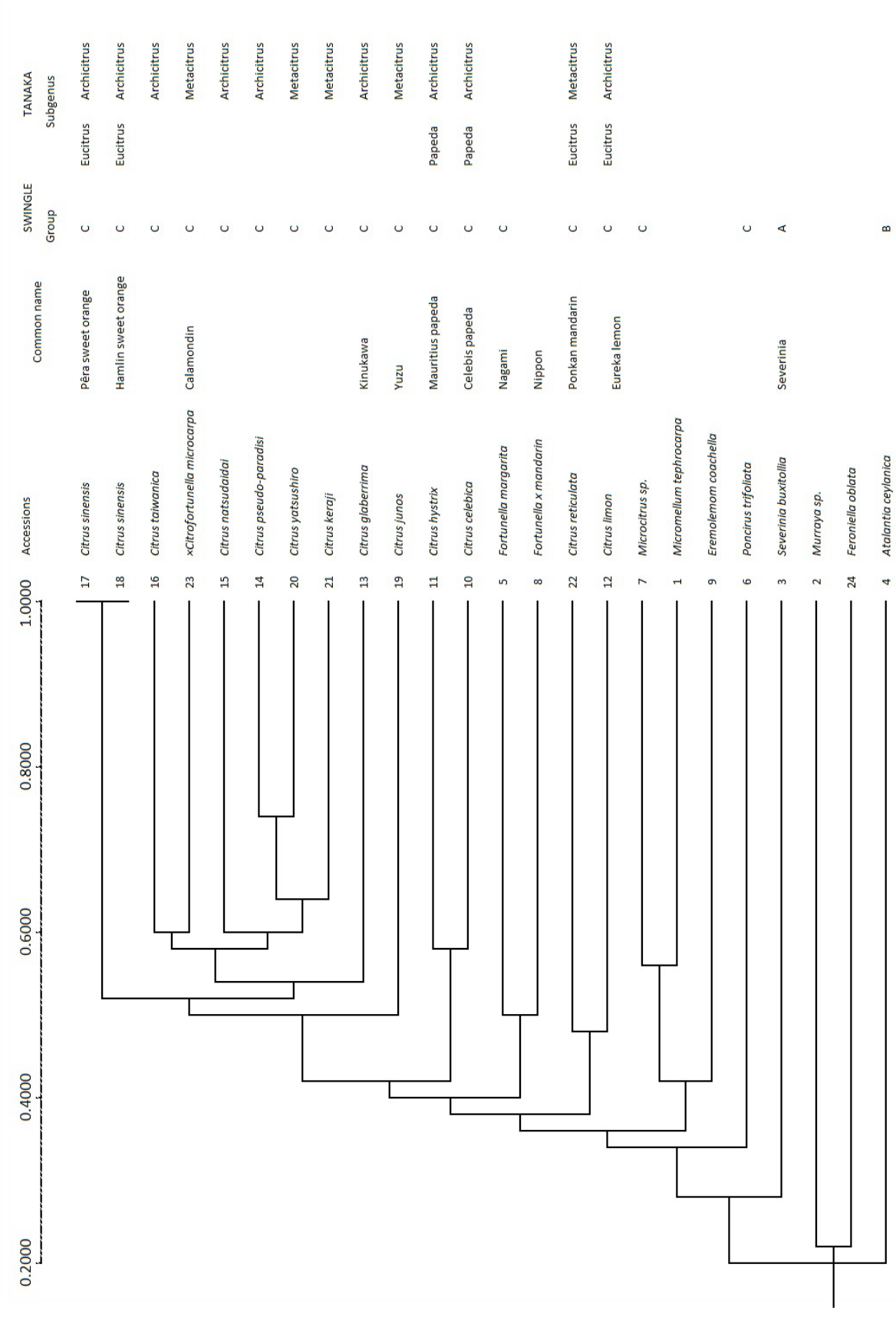

Figure 1. UPGMA dendrogram of Citrus species and related genera based on RAPD data.

Severinia and *Atalantia,* when compared with genus *Citrus*, formed a cluster that is consistent with the established taxonomic classification (Swingle & Reece, 1967), and were distant from other accessions. In this study, *Atalantia ceylanica* was the most distant accession (0.2). This supports previous suggestions that *Atalantia* is a variable genus (Herrero et al., 1996). In fact, even *Severinia* and *Atalantia* have been considered distant from the true citrus group, and these accessions could be important genetic resources for *Citrus* rootstock breeding. Additionally, *Severinia buxifolia* has graft compatibility with *Citrus* and shows important traits, such as resistance to boron and high salinity (Swingle & Reece, 1967), and resistance to citrus tristeza virus (Herrero et al., 1996).

Although *C. sinensis* has heterozygous nature, high genetic similarity is expected and the RAPD data have been agreement with previous surveys using others molecular markers. According Herrero et al. (1996), the low intraspecific polymorphism in cultivated species is directly correlated with the narrow genetic base of these cultivars and to the use of vegetative propagation.

The RAPD markers showed genetic diversity and proved to be useful for analysis of variability in *Citrus* and related genera, and the results are consistent with what has been reported in the literature. This technique still is very fast and simple, and it can be used as a cheaper alternative to characterization and management germplasm, and development of a citrus core collection (Maya et al., 2012; Malik et al., 2013).

Finally, the data here obtained were important to determine the level and distribution of diversity within a germplasm collection and to assess relationships among citrus species, and provided basic information for citrus breeding program.

ACKNOWLEDGEMENTS

To Capes, CNPq and Embrapa for supporting this work. V.M.N., M.C.Y., M.B., C.R.L. and M.A.M. are recipients of research fellowships from CNPq.

REFERENCES

Araújo EF, Queiróz LP, Machado MA 2003. What is *Citrus*? Taxonomic implications from a study of cp-DNA evolution in the tribe Citreae (Rutaceae subfamily Aurantioideae). Organism Diversity & Evolution 3:55-62.

Barkley NA, Roose ML, Krueger RR, Federici CT 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theoretical and Applied Genetics 112(8):1519-1531.

Barret HC, Rhodes AM 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Systematic Botany 1:105-136.

Bastianel M, Oliveira AC, Cristofani-Yaly M, Guerreiro Filho O, Freitas-Astua J, Rodrigues V, Astua-Monge G, Machado MA 2006. Inheritance and heritability of resistance to citrus leprosis. Phytopathology 96: 1092-1096.

Cristofani-Yaly M, Machado MA, Grattapaglia D 1999. Genetic linkage maps of *Citrus sunki* Hort. ex. Tan. and *Poncirus trifoliata* (L.) Raf. and mapping of citrus tristeza virus resistance gene. Euphytica 109(1): 25-32.

Cristofani-Yaly M, Novelli VM, Perin MS, Oliveira AC, Oliveira RP, Bastianel M, Machado MA 2005. Programa de Melhoramento de Citros por hibridação controlada no Centro APTA Citros Sylvio Moreira em 1997-2005. Laranja 26(1): 121-134.

Doyle JJ, Doyle JL 1990. Isolation DNA from fresh tissue. Focus 12:13-15.

Federici CT, Fang GDQ, Scora RW, Roose ML 1998. Phylogenetic relationships within the genus *Citrus* (Rutaceae) and related genera as revealed by RFLP and RAPD analysis. Theoretical and Applied Genetics 96(6-7):821-822.

Froelicher Y, Mouhaya W, Bassene J-B, Costantino G, Kamiri M, Luro F, Morillon R, Ollitrault P 2011. New universal mitochondrial PCR markers reveal new information on maternal citrus phylogeny. Tree Genetics & Genomes 7:49-61.

Herrero R, Asins MJ, Pina JA, Carbonell AE, Navarro L 1996. Genetic diversity in the orange subfamily

Aurantioideae. II. Genetic relationships among genera and species. Theoretical and Applied Genetics 93(8):1327-1334.

Malik SK, Uchoi A, Kumar S, Choudhary R, Pal D, Kole PR, Chaudhury R, BHAT KV 2013. Molecular characterization of Citrus macroptera Montr. (Satkara): An endangered wild species from northeast India, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana, DOI:10.1080/11263504.2012.751063.

Maya MA, Rabbani MG, Mahboob MG, Matsubara Y 2012. Assessment of genetic relationship among 15 Citrus fruits using RAPD. Asian J Biotechnol 4: 30–37.

Moore GA 2001. Oranges and lemons: clues to the taxonomy of *Citrus* from molecular markers. Trends in Genetics 17(9):536-540.

Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G, Tribulato E 2000. *Citrus* phylogeny and genetic origen of important species as investigated by molecular markers. Theoretical and Applied Genetics 100(8):1155-1166.

Novelli VM, Takita MA, Machado MA 2004. Identification and analysis of single nucleotide polymorphisms (SNPs) in citrus. Euphytica 138(3):227-237.

Novelli VM, Cristofani M, Souza AA, Machado MA 2006. Development and characterization of polymorphic simple sequence repeats (SSRs) in sweet orange (*Citrus sinensis* L. Osbeck). Genetics and Molecular Biology 29(1):90-96.

Oliveira AC, Garcia A, Cristofani-Yaly M, Machado MA 2002. Identification of citrus hybrids through the combination of leaf apex morphology and SSR markers. Euphytica 128: 397-403.

Oliveira RP, Cristofani-Yaly M, Machado MA 2004. Genetic linkage maps of Pêra sweet orange and Cravo mandarin with RAPD markers. Pesquisa Agropecuária Brasileira 39 (2): 159-165.

Oliveira AC, Bastianel M, Cristofani-Yaly M, Amaral AM, Machado MA 2007. Development of genetic map of citrus varieties Murcott tangor and pera sweet orange by using fluorescent AFLP markers. Journal of Applied Genetics 48: 219-231.

Ollitrault P, Terol J, Garcia-Lor A, Bérard A, Chauveau A, Froelicher Y, Belzile C, Morillon R, Navarro L, Brunel D, Talon M 2012. SNP mining in *C. clementina* BAC end sequences; transferability in the *Citrus* genus (Rutaceae), phylogenetic inferences and perspectives for genetic mapping. BMC Genomics 13:13, doi:10.1186/1471-2164-13-13.

Pang XM, Hu CG, Deng XX 2003. Phylogenetic relationships among *Citrus* and its relatives as revealed by SSR markers. Acta Genetica Sinica 30(1):81-87.

Rhoads DD, Roufa DJ 1989. SEQAID II Version 3.8. Molecular Genetics Laboratory, Kansas State University, Manhattan.

Rohlf FS 1992. NTSYS-pc: numerical taxonomy and multivariate analysis system. New York: State University of New York.

Sambrook J, Fritsch EF, Maniatis T 1989. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY.

Siviero A, Cristofani-Yaly M, Machado MA 2003. QTL mapping associated with rooting stem cuttings from *Citrus sunki* vs. *Poncirus trifoliata* hybrids. Crop Breeding and Applied Biotechnology 3(1): 83-88.

Siviero A, Cristofani-Yaly M, Furtado E, Garcia AAF, Coelho AS, Machado MA 2006. Identification of QTLs associated with citrus resistance to Phytophthora gummosis. Journal of Applied Genetics 47:23-28.

Sneath PHA, Sokal RR 1973. Numerical taxonomy: the principles and practice of numerical classification. W H Freeman, San Francisco, CA, 573p.

Swingle WT, Reece PC 1967. The botany of citrus and its wild relativies. In: Reuther W, Batchelor LD,

Webber HJ, editors. The Citrus Industry. VI Berkeley: University of California Press, p.190-430.

Tanaka T 1954. Species problem in citrus. A critical study of wild and cultivated units of *Citrus*, based upon field studies in their native homes. Japonese Society for the Promotion of Science, Ueno, Tokio, 152 p.

USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN Database)*. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?313479 (02 April 2014).

Recebido: 24/07/2013 – Aceito: 21/05/2014
(CRT 062-13)