Agric Res (October–December 2012) 1(4):317–324
DOI 10.1007/s40003-012-0045-3

FULL-LENGTH RESEARCH ARTICLE

Assessment of Genetic Diversity in Sweet Orange [*Citrus sinensis* (L.) Osbeck] Cultivars of India Using Morphological and RAPD Markers

S. K. Malik · M. R. Rohini · Susheel Kumar · Ravish Choudhary · Digvender Pal · Rekha Chaudhury

Received: 21 August 2012 / Accepted: 1 November 2012 / Published online: 25 November 2012

Abstract *Citrus sinensis* (L.) Osbeck (sweet orange) is one of the most important commercially cultivated fruit crops of Citrus and occupies the second position after mandarins in India. Genetic diversity and inter-relationship among 22 cultivars of *C. sinensis* were analyzed based on morphological and RAPD markers. A total of 99 bands were generated with 20 RAPD primers, out of which 51 bands were polymorphic (51.83 %). A pair-wise similarity value between cultivars ranged from 0.48 to 1.00 (avg. 0.77). Moderate levels of polymorphism and high genetic similarity within *C. sinensis* suggested that cultivars have a low level of genetic diversity despite having high morphological variability. A dendrogram generated based on UPGMA separated all the cultivars into two main clusters in which two cultivars, Delta Valencia and Sweet Orange, showed distinctiveness from the rest of the cultivars. A two-dimensional plot generated from principle component analysis of RAPD data also supported the clustering pattern of dendrogram. Some primers were able to generate unique fragments, which can be used for identification of the cultivars. This study indicated the presence of low genetic diversity within *C. sinensis*, which could be explained by the fact that much of the phenotypic variation observed may be because of some somatic mutations.

Keywords *Citrus sinensis* · Cultivars · Genetic diversity · RAPD · UPGMA

Introduction

Citrus (L.) is one of the most economically important fruit crops of the world, belonging to the subfamily *Aurantioideae* of the family *Rutaceae*. It is widely distributed throughout the tropical and subtropical regions of the world and believed to have originated in Southeast Asia, particularly northeast India, the Malayan archipelago, China, Japan, and Australia [17, 23]. India has an enormous diversity of Citrus genetic resources, both cultivated and wild. *Citrus* occupies the second position in terms of area (987 ha) and third position in terms of production (9.64 mt) of fruit crops in India [18].

Among the cultivated species, *Citrus sinensis* (L.) Osbeck (sweet orange) is the most important commercial fruit crop of Citrus and occupies the second position after mandarins in India. *C. sinensis* is believed to be a hybrid between pummelo (*Citrus maxima*) and mandarin (*Citrus reticulata*) [7, 20]. It is a highly polyembryonic species. Its fruit pulp is used for preparing fresh juice which is rich in vitamin-C and protein content. The peel of the fruit is used for making perfume and soaps. Cooking oil is extracted from its seeds. Juice extracted from its leaves is used to control several diseases like ulcers, sores, etc. [11].

The genetic diversity of *C. sinensis* is diminishing rapidly because of a number of factors, such as displacement of the natural gene pool due to selection and introduction of genotypes suitable for intensive horticulture forming a limited gene pool [2]. There is urgent need to retain the essential characters of varieties/cultivars and to

S. K. Malik (✉) · M. R. Rohini · S. Kumar · R. Choudhary · D. Pal · R. Chaudhury
Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi 110012, India
e-mail: skm1909@gmail.com; skm@nbpgr.ernet.in

characterize and evaluate the existing genotypes to achieve significant improvement in *C. sinensis* cultivars.

The use of molecular markers has been a valuable and precise strategy to identify *Citrus* species, cultivars and biotypes and to investigate the genetic diversity of *Citrus* species. Molecular marker techniques like RAPD, ISSR, RFLP, SSR, AFLP and other markers have been used for germplasm characterization, studies of genetic diversity, systematics and phylogenetic analysis [24]. Among them, random amplified polymorphic DNA (RAPD) markers have been employed most widely for characterization of plant species [25]. RAPD have gained more attention due to the simplicity of the procedure, the low cost and the very small amount of DNA required for analysis. In Citrus, RAPD markers have been used for cultivar identification, genetic mapping, genetic diversity assessment and other breeding programs [1, 3–6, 9, 14, 16, 19, 21]. In the present study, RAPD and morphological markers have been applied to characterize indigenous as well as exotic cultivars of *C. sinensis* and to establish genetic relationships among these cultivars.

Materials and Methods

Plant Material and Sample Collection

A total of 22 cultivars of *C. sinensis* were collected from field genebank collection of Regional Research Station, Punjab Agricultural University, Abohar, Punjab and used for morphological and molecular studies (Table 1). A selective sampling strategy was employed, where samples collected from a single plant of a cultivar was given an indigenous collection number (IC number) and treated as an individual accession. Leaf and fruit samples of each accession were collected for confirmation of taxonomic identity, characterization and DNA extraction. Detailed passport information of each accession was recorded in the NBPGR database.

Morphological Characterization

Morphological characterization of 22 cultivars of *C. sinensis* was done using descriptors developed for Citrus by International Plant Genetic Resources Institute (IPGRI), Rome, Italy (now Bioversity International). Characterization data of 43 characters (29 qualitative and 14 quantitative) of leaf, fruit and seed were recorded for the collected germplasm. All the 43 morphological characters were converted into bi- and multi-state code. A pair-wise similarity matrix was generated based on simple matching coefficient method using software NTSYS ver. 2.10e [22]. A cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) based on simple matching coefficient in NTSYS software. Principal component analysis (PCA) was also carried out to study correlations among the variables and establish relationships among cultivars using the same software. The two-way Mantel test [15] for goodness of fit for the UPGMA cluster was also performed using the same software.

Table 1 *Citrus sinensis* cultivars used for the morphological and molecular analyses

Cultivars	Origin	Parentage
Olinda Valencia late	USA	Selection from Valencia
Jaffa	New Zealand	Clone of Palestine beledi tree
Blood red	Mediterranean basin	Unknown
Campbell Valencia	USA	Selection from Valencia
Vanale	Brazil	Unknown
Washington navel	Brazil	Probably selection from Seleta
Moro	Italy	Unknown
Rhode red Valencia	USA	Selection from Valencia
Parent navel	Brazil	Unknown
Declarbe sweet orange	Unknown	Unknown
Satgudi	India	Selection
Malta	Mediterranean basin	Selection
Mosambi	Mozambique, India	Selection
Vaniglia sanguigno	Italy	Unknown
Sweet orange	Italy	Selection
Teneriffe	Spain	Unknown
Temple	Jamaica	Unknown
Mediterranean sweet orange	Mediterranean region	Selection
Seleta	Portugal	Unknown
Valencia late	China	Selection from Valencia
Tardiff	Italy	Unknown
Delta Valencia	South Africa	Selection from Valencia

DNA Extraction

Total genomic DNA was extracted from all the 22 cultivars of *C. sinensis* through the cetyl tri-methyl ammonium bromide (CTAB) method [10]. Quantitation of isolated DNA was done spectrophotometrically and its quality checked by electrophoresis on 0.8 % agarose gel.

RAPD-PCR Amplification

The RAPD primers of Operon Technologies Alameda, CA, USA were used for molecular analysis. A total of 60 primers

were screened in *C. sinensis*, of which 20 primers were selected for final profiling based on banding patterns and reproducibility. The basic protocol reported by Williams et al. [25] for RAPD-PCR amplification was followed, in which a final reaction volume of 20 µl contained 1× Assay buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1U Taq DNA polymerase (Life tech, India), 10 pmol RAPD primer and 20 ng of template DNA. The PCR amplification conditions were as follows: Initial denaturation step at 94 °C for 4 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and extension at 72 °C for 2 min followed by final extension at 72 °C for 7 min. Amplification products were separated by electrophoresis (80 V for 3 h) in 1.5 % agarose gel containing ethidium bromide (10 mg/ml). A photographic record was taken under a UV gel documentation system (Mega Biosystematica, UK).

Data Analysis

Amplified fragments were scored for each accession as presence (1) or absence (0) of homologous bands on the basis of size comparison with standard DNA ladder. Molecular weight of the amplified bands was estimated by using a 1 Kb DNA ladder (Gibco BRL Life Technologies, New York, USA) as standard. A pairwise genetic similarity matrix between cultivars was estimated using Jaccard's coefficient and a dendrogram was constructed based on UPGMA using Software NTSYS ver. 2.01e [22]. The two-way Mantel test [15] for goodness of fit for the UPGMA cluster to the binary data and PCA were also performed using the same software.

Results

Morphological Characterization

The tree is medium sized, profusely branched with elliptic to ovate leaf lamina and narrowly winged petiole. Fruit shape varies from spheroid to ellipsoid with rounded or truncated apex. Most of the fruits have pitted surface texture with either conspicuous or strongly conspicuous oil glands. *C. sinensis* shows mostly seeded cultivars and few cultivars like Jaffa and Delta Valencia are seedless. Seeded cultivars have 2–18 seeds with an average of five seeds per fruit. Seeds were clavate in shape with wrinkled seed surface. Mature seeds were cream to brown in colour with cream or light yellow-cream cotyledon and brown chalazal spots. Most of the *C. sinensis* cultivars are polyembryonic with two to four embryos. Among the studied cultivars, Vaniglia sanguigno reported the highest percentage of polyembryony (70 %) with four embryos per seed. The only cultivar with 100 % monoembryonic seeds was Temple.

Pair-wise similarity values among the cultivars of *C. sinensis* ranged from 0.18 to 0.64 with an average of 0.39 based on morpho-metric data. A dendrogram generated based on morpho-metric data grouped all the 22 cultivars into five major clusters (Fig. 1). The first cluster comprised of the cultivar Washington navel which was the most distinct from all other clusters and separated with similarity value of 0.30. A second cluster was comprised of two cultivars, namely Jaffa and Delta Valencia, which were closely related with similarity value of 0.52. The third cluster comprised of only one cultivar, Malta, which was separated from the fourth cluster with similarity value of 0.32. The fourth cluster was the biggest one comprising 16 cultivars, viz. Mosambi, Declarbe sweet orange, Valencia late, Parent navel, Mediterranean sweet orange, Teneriffe, Satgudi, Blood red, Temple, Sweet orange, Rhode red Valencia, Morro, Vaniglia sanguigno, Tardiff, Vanale, and Campbell Valencia. Within this cluster the cultivars Campbell Valencia and Vanale were most similar morphologically showing a similarity value of 0.64. A fifth cluster comprised of two cultivars, viz. Olinda Valencia late and Seleta, which were closely related by a similarity value of 0.44.

Based on Mantel *Z* statistics [15] the correlation coefficient (*r*) was estimated as 0.84. A value of 0.84 is considered a good fit of the UPGMA cluster pattern to the data. A two-dimensional plot generated from PCA showed five

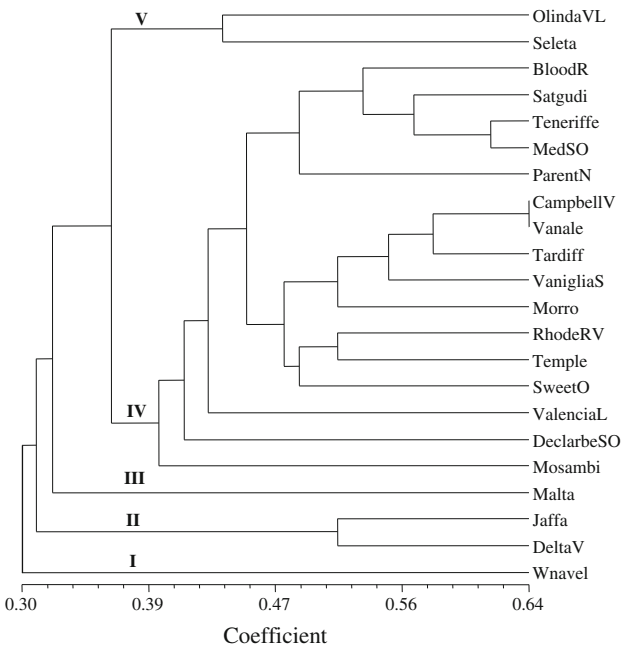

Fig. 1 Dendrogram generated based on morphological traits of 22 *C. sinensis* cultivars using the unweighted pair group method with arithmetic average (UPGMA)

groups which was found to be less similar to the clustering pattern of the UPGMA dendrogram. In a 2D plot, cultivar Delta Valencia alone constituted one group whereas in UPGMA clustering, Delta Valencia and Jaffa were grouped together in one cluster. Jaffa also formed a separate group in 2D plot but in the dendrogram it was grouped along with Delta Valencia. Malta and Washington navel together constitute one group in the 2D plot, whereas in the dendrogram they were present in two different clusters. The analysis gave first ten principal components, which contributed 91.36 % of the total variability of the collected accessions. The first five principal components accounted for 70 % of the total variability and the first three accounted for 53.77 % of the variance, in which maximum variability was contributed by the first component (26.85 %) followed by the second component (14.84 %), and the third component (12.07 %). The first PC was most highly influenced by characteristics of the fruit morphology, viz. fruit weight, fruit length, fruit diameter, fruit rind thickness and TSS (Table 2). In the second PC, the traits contributing to the total variability were fruit adherence of albedo to pulp, leaf apex, leaf lamina shape and petiole wing width. The third PC was mostly influenced by fruit shape, leaf length, leaf width, and oil gland size.

Table 2 Eigenvectors of morphological variables explained by the first three principal components

Traits	PC1	PC2	PC3	Mean	SD
Fruit shape	1.2556	0.9653	2.188	–	–
Fruit base	0.8527	0.6747	0.8541	–	–
Fruit apex	1.0050	−2.5711	−1.9157	–	–
Fruit colour	0.3905	−0.7748	−2.6671	–	–
Adherence of albedo to pulp	0.1089	2.1354	2.0969	–	–
Nature of oil glands	1.2926	−2.0343	2.7404	–	–
Oil gland density	−0.1170	0.1792	−0.3351	–	–
Oil gland size	1.3175	−0.7800	1.9100	–	–
Albedo colour	0.1043	−0.0002	−0.5795	–	–
Pulp colour	0.2142	0.3790	−1.2273	–	–
Pulp firmness	−1.1916	0.7850	−0.2665	–	–
Juice content	1.6040	−0.0903	−1.6302	–	–
Seed shape	−3.3331	0.0783	−0.6235	–	–
Seed surface	−4.0378	−0.7243	0.0108	–	–
Seed colour	−3.5283	−1.6338	−0.4604	–	–
Cotyledon colour	−4.0246	−0.4604	−0.3159	–	–
Chalazal spot colour	−3.8574	−0.8183	0.3892	–	–
Seed embryony	−3.7246	0.9268	0.3361	–	–
Intensity of green colour	−0.1170	0.1792	−0.3351	–	–
Leaf lamina attachment	0.5696	2.3253	−3.1472	–	–
Leaf lamina shape	0.2590	3.1294	−0.6731	–	–
Leaf lamina margin	−0.7697	−3.2917	1.4873	–	–
Leaf apex	0.8941	2.6532	−2.7717	–	–
Petiole wing width	0.5696	2.3253	−3.1472	–	–
Petiole wing shape	1.7383	−2.5972	−1.2645	–	–
Fruit weight	3.2275	−1.4676	−0.046	231.45	36.14
Fruit diameter	3.8789	−1.3272	0.1426	73.94	3.78
Fruit length	3.5309	−1.6249	0.8997	75.93	4.32
Epicarp width	1.0082	−0.3820	0.3959	2.34	0.27
Fruit rind thickness	2.1625	−0.5059	1.7416	5.08	0.62
No. of segments	1.8554	−1.7922	0.8276	11	0.56
Total soluble sugar (TSS)	2.4093	1.9652	−0.5539	7.90	0.59
No. of seeds	−4.1594	−0.4949	0.1761	5.00	1.27
Ten seeds weight	−4.1594	−0.5014	0.1743	1.76	0.06
Seed moisture (%)	−4.1630	−0.4887	0.1633	37.32	0.80
Leaf lamina length	0.2068	2.8000	2.0826	61.9	0.52
Leaf lamina width	−0.3258	3.5558	2.4584	28.1	0.30
Leaf lamina ratio	0.9224	−2.6776	−1.6189	2.25	0.18
Leaf lamina thickness	−1.3183	1.9818	2.5039	0.21	0.02

Genetic Polymorphism among Cultivars

Twenty primers were selected for the RAPD analysis based on the reproducibility and banding patterns. PCR amplification of the genomic DNA isolated from 22 cultivars of *C. sinensis* yielded a total of 99 bands, of which 51 were polymorphic and 48 were monomorphic (Table 3). Representative gel profiles generated using primers OPC-08 and OPG-17 are shown in Fig. 2. The total number of amplified DNA bands ranged from three to eight, with an average of five bands per primer. The maximum number of polymorphic bands (5) was obtained with two primers, i.e. OPG-08 and OPA-01 and the minimum number (1) was generated with primer OPT-01. The polymorphism percentage ranged from 12.5 (primer OPT-01) to 83.33 % (OPA-01). Average polymorphism across all the 22 cultivars was 51.83 %. Overall size of the PCR amplified fragments ranged from 300 to 3,000 bp.

Five RAPD primers gave seven unique bands in specific sweet orange cultivars. These primers produced a specific DNA fragment which distinguished one cultivar from the rest. Primer OPB-18 generated two unique bands, one in Satgudi and another in Tardiff, while primer OPC-08 also generated two unique bands each for Seleta and Temple. Each OPO-04, OPG-17 and OPA-12 primers also generated single fragments in Satgudi (500 bp), Valencia late (900 bp) and sweet orange (600 bp), respectively.

Genetic Diversity and Relationships

The pair wise similarity values obtained between 22 cultivars of *C. sinensis* ranged from 0.48 to 1.0. A maximum

Table 3 Details of amplified bands generated in 22 cultivars of *C. sinensis* based on 20 RAPD primers

Primer	Sequences (5′–3′)	Total no. of amplified bands	No. of polymorphic bands	Percentage of polymorphism (%)
OPA-08	GTGACGTAGG	5	3	60.00
OPA-12	TCGGCGATAG	4	3	75.00
OPB-18	CCACAGCAGT	6	4	66.66
OPC-08	TGGACCGGTG	8	4	50.00
OPC-18	TGAGTGGGTG	4	2	50.00
OPG-05	CTGAGACGGA	5	3	60.00
OPG-08	TCACGTCCAC	8	5	62.50
OPG-16	AGCGTCCTCC	3	2	66.66
OPG-17	ACGACCGACA	6	3	50.00
OPM-06	CTGGGCAACT	4	3	75.00
OPM-13	GGTGGTCAAG	4	2	50.00
OPO-06	CCACGGGAAG	4	3	75.00
OPO-12	CAGTGCTGTG	4	2	50.00
OPA-04	AATCGGGCTG	4	0	0.00
OPO-04	AAGTCCGCTC	4	3	75.00
OPA-19	CAAACGTCGG	4	3	75.00
OPT-08	AACGGCGACA	4	0	0.00
OPA-01	GAGGCCCTTC	6	5	83.33
OPQ-18	AGGCTGGGTG	4	0	0.00
OPT-01	GGGCCACTCA	8	1	12.5
	Total	99	51	51.51

similarity value of 1.00 was observed between cultivars Declarbe sweet orange and Rhode Red Valencia, indicating that they are most genetically similar, whereas Washington naval and sweet orange, Delta Valencia and Mosambi, and Satgudi and Delta Valencia showed the lowest similarity coefficient value of 0.48. Average similarity across all the cultivars was 0.77. A dendrogram generated based on the UPGMA method grouped all the 22 cultivars into two major clusters (Fig. 3). The first cluster comprised of the cultivars Delta Valencia and sweet orange. The biggest formed cluster was the second cluster consisting of the remaining 20 cultivars. Within this cluster, the cultivars Declarbe sweet orange and Rhode red Valencia were genetically most similar, showing 100 % genetic similarity; while Seleta and Vanale cultivars were individually separated into distinct clades from the rest of the cultivars with similarity value of 0.79.

Based on Mantel Z statistics [15], the correlation coefficient (r) was estimated as 0.93. A value of $r > 0.90$ is considered a very good fit of the UPGMA cluster pattern to the data. A 2D plot generated from principle component analysis (PCA) of RAPD data also supported the clustering pattern of the UPGMA dendrogram, except Seleta, which was distinctly separated in the 2D plot, while in the dendrogram, it was grouped in cluster II. First and second principal components accounted for 23.18 and 14.35 %, respectively, of the total variation.

Discussion

Experiments with *C. sinensis* cultivars have demonstrated the potential of RAPD markers as a rapid, reproducible and useful method for distinguishing different cultivars of *C. sinensis* and clustering genotypes into different groups. The moderate level of genetic polymorphism (51.83 %) was observed among the 22 cultivars of *C. sinensis* based on 20 primers. This could be explained by the fact that somatic mutations are the main source of variability in cultivars of this species. However, a high level of polymorphism (70.13 %) was reported within the naval sweet orange cultivar based on RAPD markers [8]. In contrast to these results, Natividade Targon et al. [19] and Novelli et al. [21] did not observe polymorphisms among the cultivars of *C. sinensis* based on RAPD and microsatellites markers.

Pair-wise similarity analysis of 43 morphological characters in the 22 cultivars of *C. sinensis* revealed that maximum similarity (0.64) occurred between the cultivars Campbell Valencia and Vanale, both of which are exotic in origin and are most similar in terms of qualitative fruit, leaf and seed characters. Minimum similarity (0.18) was observed between the cultivars Washington navel and Mosambi, which may be attributed to their different centres of origin where they have developed their distinct characters. Both cultivars are different in fruit characters, as

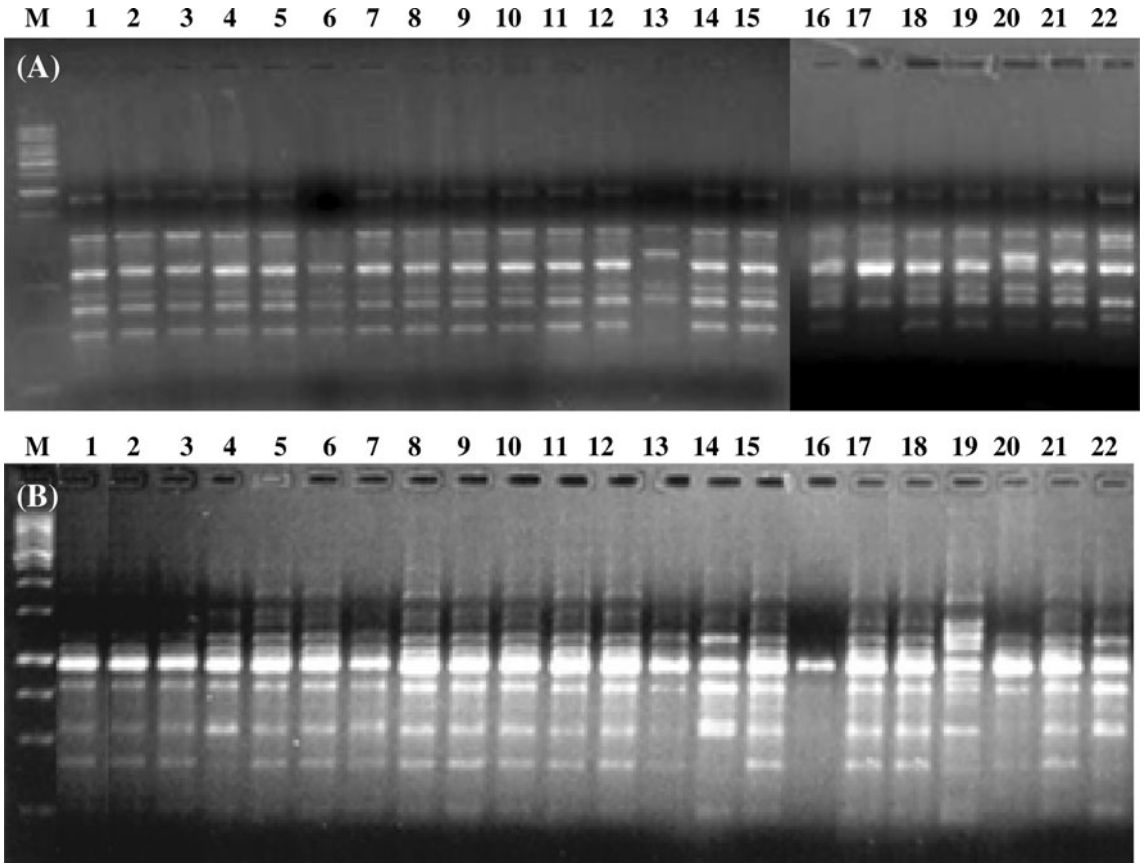

Fig. 2 Representative gel profiles of 22 cultivars of *C. sinensis* based on random amplified polymorphic DNA (RAPD) primers. *M* represents 1 kb DNA ladder. **a** OPC-08. **b** OPG-17

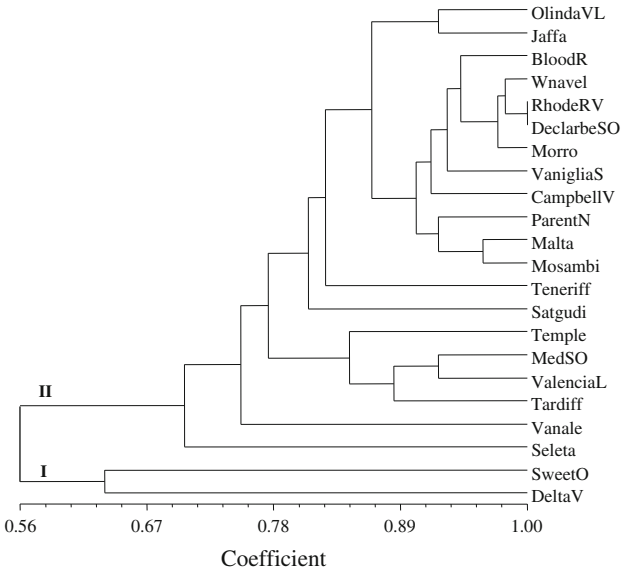

Fig. 3 Dendrogram generated based on random amplified polymorphic DNA (RAPD) data of 22 cultivars of *C. sinensis*

Mosambi has an ellipsoid shape with rounded apex while the Washington naval has spheroid shape with truncated apex. The average similarity value of 0.39 indicated that cultivars show moderate to significant variability among these cultivars with respect to morphological traits. Based on RAPD markers, a high similarity value of 1.00 was found between two cultivars, Declarbe sweet orange and Rhode Red Valencia, showing very close genetic relationships between them which may be due to their common origin by mutation. Low genetic similarity (0.48) between cultivars Washington naval and sweet orange; Delta Valencia and Mosambi; and Satgudi and Delta Valencia may be due to different sources of origin of these cultivars. High genetic similarity (avg. 0.77) was recorded within this group, which showed a narrow level of genetic diversity existed within *C. sinensis*. This was also congruent with a moderate level of polymorphism that occurred within this group. Similarly, Fang and Roose [12] also reported low genetic variation among the cultivars of *C. sinensis* based on ISSR markers. This further supports the view that a majority of *C. sinensis* cultivars derived from a single ancestor through somatic mutation [13].

A search for unique bands was made for all the cultivars tested, in which a total of seven unique bands were generated in six cultivars by five RAPD primers. In Satgudi, a maximum of two unique bands were given by primer

OPB-18 (600 bp) and OPO-04 (500 bp). Similarly, unique bands were generated in Sweet orange, Teneriffe, Seleta, Valencia late and Temple by RAPD primers. These unique fragments can be used as a marker for identification of these cultivars, which will be useful for future conservation, maintenance and breeding programmes. These accessions can also be used for developing the core collection of *C. sinensis* germplasm.

The UPGMA dendrogram divided all the cultivars into five main clusters based on morpho-metric data. The cultivar Washington naval was the most distinct from rest of the clusters mainly with respect to its fruit and leaf morphology. Jaffa and Delta Valencia were grouped together due to their similarity in fruit morphology and seedlessness. Indigenous cultivars of *C. sinensis* were clustered together in one group because of their similarity in most of the observed traits. A 2D plot showed five groups which was found less similar to the clustering pattern of UPGMA dendrogram. In the 2D plot, cultivars such as Delta Valencia, Jaffa, Malta, Washington navel and Seleta were found very distinct from rest of the cultivars as all are exotic and might have originated from different sources. The UPGMA clustering pattern based on RAPD data also indicated the genetic relatedness of *C. sinensis* cultivars by grouping 20 cultivars out of 22 into a single cluster. This shows that most of the cultivars of *C. sinensis* originated through somatic mutation (bud sports). However, two cultivars, Delta Valencia and sweet orange, were clearly separated from the rest of the cultivars and grouped in the same cluster. This indicated that both cultivars may have originated from the same genotype.

Comparison of morphological and molecular characterization data is of immense importance to conclude the extent of genetic diversity present in the set of cultivars. Although the correlation between the morphological and RAPD data was low in the analyzed cultivars of *Citrus sinensis*, as correlation values were found to be much less than 0.5, both methods allowed fare groupings of cultivars based on the analyzed traits. This is clear from the clustering pattern of the cultivars, where UPGMA dendrogram based on morphological data divided 22 cultivars into five major clusters whereas the dendrogram based on the RAPD marker divided them only into two major clusters. This shows that in spite of the wide phenotypic variations present within the cultivars they had a very narrow genetic base. The cultivar Washington Navel, which was morphologically most distinct from other cultivars, showed maximum genetic similarity with the rest of the cultivars. In the same way the Delta Valencia which was genetically most distinct showed some extent of morphological similarity with the cultivar Jaffa.

Based on the PCA of morphological characters, the first three principal components accounted for 53.77 % of the variance, in which maximum variation was given by the first component. Morphological characters, viz. fruit shape, fruit weight, fruit length, fruit diameter, fruit rind thickness, TSS, adherence of albedo to pulp, leaf apex, leaf lamina shape, leaf length, leaf width, petiole wing width, and oil gland size, represent maximum variability revealed by the first three components which were identified for developing a minimal descriptor for describing the species.

The moderate level of polymorphisms, in spite of the high morphological variability, could be explained by the fact that somatic mutations may be one of the sources of variability in *C. sinensis*. These results can be further used to manipulate genetic determinants of horticulturally important traits and to characterize the basis of productivity of *C. sinensis* cultivars in India. In the present study, RAPD markers proved to be useful for germplasm characterization and diversity analysis in *C. sinensis* cultivars.

Acknowledgments The authors are grateful to Prof. KC Bansal, Director, NBPGR, New Delhi for providing laboratory facilities and for providing financial support. A grant from NAIP-World Bank is greatly acknowledged for the germplasm collection for this study. Thanks are also due to Director, Regional Station, PAU, Abohar, Punjab for a generous supply of germplasm.

References

1. Abkenar A, Isshiki S (2003) Molecular characterization and genetic diversity among Japanese acid *Citrus* (*Citrus* spp.) based on RAPD markers. J Hort Sci Biotech 78:553–556
2. Arora RK (2000) In situ conservation of biological diversity in *Citrus*. In: Proceedings of the Global Citrus Germplasm Network, app. 7, GCGN Meeting, 7–8 Dec
3. Bretó MP, Ruiz C, Pina JA, Asíns MJ (2001) The diversification of *Citrus clementina* Hort. ex Tan., a vegetatively propagated crop species. Mol Phylogenet Evol 21:285–293
4. Cai Q, Guy CL, Moore GA (1994) Extension of the linkage map in *Citrus* using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor Appl Genet 89:604–614
5. Coletta Filho HD, Machado MA, Targon MLPN, Moreira MCPQDG, Pompeu J Jr (1998) Analysis of phylogenetic diversity among mandarins (*Citrus* spp.) using RAPD markers. Euphytica 102:133–139
6. Das A, Sarkar J, Mondal B, Chaudhury S (2004) Genetic diversity analysis of *Citrus* cultivars and rootstocks of Northeastern India by RAPD markers. Indian J Genet 64:281–285
7. Davies F, Albrigo LG (1992) Taxonomy, cultivars and breeding. In: Gmiter FG Jr, Grosser JW, Moore GA (eds) *Citrus*. CAB International, Wallingford, pp 12–51
8. Dehesdtani A, Kazemitabar SK, Rahimian H (2007) Assessment of genetic diversity of navel sweet orange cultivars grown in Mazandaran province using RAPD markers. Asian J Plant Sci 6:1119–1124
9. Deng ZN, Gentide A, Nicolosi E, Domina F, Vardi A, Tribulato E (1995) Identification of in vivo and in vitro lemon mutants by RAPD markers. J Hort Sci 70(1):117–125
10. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

11. Dugo G, Giacomo Di (2002) A *Citrus*: The genus *Citrus*, medicinal and aromatic plants—industrial profiles. Taylor & Francis, London
12. Fang DQ, Roose ML (1997) Identification of closely related *Citrus* cultivars with inter-simple sequence repeat marker. Theor Appl Genet 95:408–417
13. Hodgson RW (1967) Horticultural varieties of *Citrus*. In: Reuther W, Webber HJ, Batchelor LD (eds) The citrus industry, vol I. University of California Press, Berkeley, pp 431–591
14. Luro F, Laigret F, Bove JM, Ollitrault P (1992) Application of random amplified polymorphic DNA (RAPD) to *Citrus* genetics taxonomy. Proc Int Citriculture 1:225–228
15. Mantel NA (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220
16. Maya MA, Rabbani MG, Mahboob MG, Matsubara Y (2012) Assessment of genetic relationship among 15 *Citrus* fruits using RAPD. Asian J Biotech 4:30–37
17. Moore GA (2001) Oranges and lemons: clues to the taxonomy of *Citrus* from molecular markers. Trends Genet 17:536–540
18. National Horticulture Board (2010) National Horticulture Database. Ministry of Agriculture, Gurgaon
19. Natividade Targon MLP, Machado MA, Coletta Filho HD, Cristofani M (2000) Genetic polymorphism of sweet orange (*Citrus sinensis* [L.] Osbeck) varieties evaluated by random amplified polymorphic DNA. Acta Hort 535:51–53
20. Nicolosi E, Deng ZN, Gentile A, La Malfa S (2000) *Citrus* phylogeny and genetic origin of important species as investigated by molecular markers. Theor Appl Genet 100:1155–1166
21. Novelli VM, Cristofani M, Machado MA (2000) Evaluation of microsatellite markers in cultivars of sweet orange (*Citrus sinensis* (L.) Osbeck. Acta Hort 535:47–49
22. Rohlf FJ (2000) NTSYS-pc: numerical taxonomy and multivariate analysis system, ver. 2.10e. Exeter Ltd., Setauket
23. Swingle WT, Reece PC (1967) The botany of *Citrus* and its wild relatives. In: Reuther W, Batchelor LD, Webber HJ (eds) The citrus industry, vol I. University of California Press, Berkeley, pp 190–340
24. Weising K, Nybom H, Wolff K, Kahl G (2005) 'DNA fingerprinting in plants' principles, methods and applications, 2nd edn. Taylor & Francis, Boca Raton
25. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18:6531–6535