Electronic Journal of Plant Breeding,
ISSN 0975-928X Vol 7 No 2 DOI: 10.5958/0975-928X.2016.00041.7

Assessment of molecular diversity in Elite Sweet Orange (*Citrus sinensis L.* Osback) accessions using RAPD markers

C. Madhumathi[1], K.Purushotham[2], R.Chandhrasekhar[3], K.Gopal[4], M.Reddi Sekhar[5] and T.Gowri Shankar[6]

[1]Horticultural Research Station, Anantharajupet, Kodur
[2]Dr.YSR Horticutlrual University, Venkatrammanagudem,
[3]College of Horticulture, Mojerlla,
[4]Horticultural College and Research Institute, Anantharajupet, Kodur
[5]S.V.Agricultural College, Tirupati.
[6]Citrus Research Station, Tirupati.
Email: madhulokanadh@gmail.com

(Received: December 2014, Accepted: June 2015)

Abstract
Random amplified polymorphic DNA (RAPD) markers were used to estimate the genetic diversity among 27 elite sweet orange accessions maintained at the Citrus Research Station (AICRP on citrus), Tirupati. The 52 decamer primers generated 469 randomly amplified DNA fragments, of which 292 were polymorphic (62.26%). The similarity indices measured based on Jaccard similarity coefficient ranged from 0.52 to 0.80, which showed the presence of low to moderate diversity among 27 sweet orange accessions. Based on UPGMA cluster analysis, these accessions grouped into two main clusters. All 26 sweet orange accessions formed a major cluster. HS-3 accession having its clear genetic identity formed another cluster with the rest of the sweet orange accessions. The sweet orange accessions with great economic importance viz., Jaffa, Blood Red Malta, Mediterranean Sweet, Hamlin Sweet, Valencia Late, Pineapple and Kodur Sathgudi showed complete genetic similarity. RAPD analysis showed that most of the accessions of sweet orange were closely clustered, with relatively high genetic similarity (0.75), suggesting that the genetic base of domesticated sweet orange is quite narrow.

Key words: Sweet orange, genetic diversity, germplasm analysis, RAPDs

Introduction

Among the citrus-like trees (Family: Rutaceae, sub family: Aurantiodae), the genus *Citrus* is most important economically, with a high diversity of species, cultivars and clones. The sweet orange *C. sinensis (L.) Osbeck*, is the main evergreen fruit-crop species, accounting for 75% of citrus production used both as fresh fruit and processed juice (Spiegel-Roy and Goldschmidt, 1996). Genetic variability in citrus is related to the high number of taxonomic units (species & hybrid), apomixes, wide sexual compatibility between *Citrus* and related genera, the high frequency of bud mutations and the long history of cultivation and wide dispersion (Scora, 1989). Sweet oranges are vegetative propagated and new cultivars are obtained after careful selection of spontaneous somatic mutations. Genetic improvement of citrus species through conventional breeding methods has been hampered by the long juvenile period, high heterozygosity, large plant size and nucellar embryony (Gmitter *et al.*, 1992). Estimation of genetic diversity is a critical step for germplasm characterization and conservation. RAPD markers are usually preferred for the initiation of this kind of work as the technique is simple, versatile, relatively inexpensive. In case of citrus, RAPD technique has been used for different purposes like the establishment of genetic linkage map for virus resistance gene of citrus (Cristofani *et al.*, 2000), identification of lemon mutants (Deng *et al.*, 1995), genetic diversity study of Japanese and citrus (Abkenar and Isshiki, 2003) and genetic diversity among different taxonomic group of mandarins in Brazil (Coletta Filho *et al.*, 2000). Although citrus is becoming a crop of commercial importance relatively less attention has been paid towards the molecular characterisation of existing elite cultivars of sweet orange available in different parts of the country. Hence, in the present study an attempt has been made to assess the extent of

genetic variation at molecular level in available sweet orange accessions and to utilize the molecular diversity for future crop improvement programmes of this high-value fruit crop.

Materials and Methods

Twenty seven sweet orange accessions collected from different agro-climatic regions of India and maintained at Citrus Research Station (AICRP on citrus), Tirupati were utilized for the present study (Table 1). Genomic DNA was extracted from tender leaves as per Murry and Thompson (1980) protocol using CTAB method with suitable modifications in the procedure. 1g of fresh leaf was sterilized with 70% ethyl alcohol and grounded to a fine powder in liquid nitrogen. The powder was added to 25 ml of extraction buffer (containing 2% (w/v) CTAB, 100 mM Tris-Hcl, pH 8.0, 20 mM EDTA, 1.4M NaCl, 1% Polyvinyl pyrrolidone and 1% β-mercaptoethanol). The contents were then mixed slowly and were incubated in a water bath at 65 ^{0}C for 30 minutes. DNA was extracted with Chloroform:Isoamyl alcohol (24:1). DNA was washed with 70% ethanol and dissolved in TE buffer and stored at -20^{0}C. The quality of isolated DNA was tested by agroase gel electrophoresis and further estimated by Spectropotometry (Nanodrop, USA).

A total of 52 decamer oligonucleotides (Operon technologies) listed in Table2 were utilized for PCR amplification by following the protocol of Williams *et al.* (1990) with minor modifications . Polymerase chain reactions of genomic DNA were carried out in 25 µl reaction volume containing 100 ng of template DNA, 2U of Taq polymerase (Bangalore Genei Pvt.Ltd.), 2.5mM $MgCl_2$, 0.2 mM dNTPs (Fermentas),1 pmole of primer and 10X Buffer (Genei). The PCR amplification was performed in a thermal cycler (Corbett Research Inc.) for an initial denaturation cycle of 2 min at 94 ^{0}C followed by 45 cycles comprising 1 min each at 94 ^{0}C, 1 min at 37 ^{0}C and 2 min at 72 ^{0}C for final extension. Amplification products were separated by electrophoresis (100V for 3 hours) in 1.5 % agarose gels and stained in Ethidium Bromide. one kb DNA marker (Fermentas) was used to measure the size of bands formed in the electrophoretic gel. The gel was visualized and photographed under UV light using the gel doc system (Alpha Innotech Inc.). Reproducibility of the pattern was tested by running parts of the reaction in duplicate.

Polymorphism was detected by scoring the presence (+) or absence (-) of the reproducible bands. . The data from 52 primers were used to estimate the similarity on the basis of the number of shared bands using the NTSYS-pc version 2.0 software. A genetic similarity matrix was constructed using Jaccard's Coefficient method and was subjected to cluster analysis using UPGMA and dendrogram was generated.

Results and Discussion

A total of 469 unambiguous amplified DNA fragments were produced from a total of 52 RAPD primers. The number of amplified fragments varied from 2 to 19, with an average of 9.02 fragments per primer (Table 2). The size of the fragments ranged from 190 bp to 6000 bp. This was comparable with results generated by polymorphic bands ranging from 71 bp to 1.5 kb in citrus cultivars and rootstocks (Das *et al.*, 2004), 430 bp to 2.3 kb in sour orange accessions (Siragusa *et al.*, 2006), 150 bp to 2100 bp in Navel sweet orange cultivars (Dehesdtani *et al.*, 2007) and 400 to 3200 bp in citrus cultivars and clones (Hvarleva *et al.*, 2008).

According to Guerra (1984) the citrus genome size is reported to be 563 mbp. In the present study, a total number of 292 polymorphic bands were produced with the use of 52 RAPD markers which appears to be adequate. Out of 469 bands that were obtained in the present study, 292 were polymorphic (62.26%) with the average number of 5.62 polymorphic markers per primer. This value seems to be higher than those reported by Corazza Nunes *et al.* (2002) where in it was revealed 4.6 per primer among grapefruit accessions and less than 7.06 in mandarin oranges (Das *et al.*, 2004) and 7.25 per primer in Navel sweet orange cultivars (Dehesdtani *et al.*, 2007).

Among the 52 primers used, OPM 08, OPM 17 and OPE 08 generated the highest level of polymorphism (100%). The total number of amplified fragments generated per primer had no correlation with proportion of polymorphic bands. Similar pattern was observed by Siragusa *et al.* (2006) in sour orange. RAPD profiles illustrated typical level of polymorphism present in accessions of sweet orange. Based on the estimated genetic similarity matrix (Table 3), HS-1 to HS-2 and Mediterranean Sweet to Valencia were found to be most genetically similar (0.98) and HS-3 to Nadempalli selection

were found to be least similar genetically (0.52) with an average similarity coefficient of 0.75 among the group of accessions studied. Among 27 accessions, 26 sweet orange accessions grouped in cluster I and only HS-3 formed cluster II which diverged with rest of the accessions at similarity index of 0.55 (Fig. 1).. The cluster I was again divided into three sub-clusters IA, IB (Ananthapur selection) and IC (HS-4) at a similarity coefficient of 0.79. The sub cluster IA was the largest cluster comprising of 24 accessions which was again sub divided into IA-a (22 accessions) and IA-b (CIP Sathgudi and Nandeli selection) at a similarity index of 0.84.

The group IA-a had 22 accessions which included all the exotic cultivars (Jaffa, Blood Red Malta, Hamlin Sweet, Valencia Late, Mediterranean Sweet, Pineapple, Valencia and Mosambi), all Tirupati Sweet orange selections (TS-1, TS-2, TS-3, TS-4, TS-6, TS-8, TS-9, TS-10 and TS-11), locally grown commercial cultivar (Kodur Sathgudi) and local selections (HS-1, HS-2, Nadempalli selection and Ankalamma Gudur) at 0.87 similarity index. CIP Sathgudi and Nandeli selection were grouped together (IA-b) with similarity index of 0.89. Local selection Ananthapur selection (IB) and HS-4 (IC) formed separate groups at a similarity index of 0.78 in dendrogram.

Genetic similarity, was observed among the accessions originating from widely divergent locations as evident from the present study where in exotic cultivars were grouped together with other local selections in cluster IA-a and also HS-1 and HS-2 belonging to cluster IA-a shared genetic similarity with the exotic collection (Pineapple). Though they differed morphologically, they were similar at the DNA level. This would suggest a distinct genetic identity and rather large genetic divergence from most plant selections of the geographically closer ones (Das *et al.,* 2004). The other possible reason might be sharing a similar gene pool before their geographical separation (Jaiswal and Amin, 1992). This could be a reason for less genetic distance observed among the exotic cultivars in this study. Intra-location genetic divergence among the clones was quite evident from the grouping of plants collected from Himakuntla in sub cluster IA (HS-1 and HS-2) and sub cluster IC (HS-4) and Cluster II (HS-3). Similar kind of findings was reported in mandarins by Das *et al.*(2004).

The low level of genetic variability among the TS selections which yielded very similar pattern with RAPD, would have caused the accessions to be grouped together suggesting either that they originated from a common cultivar or that the technique was not able to detect cultivar variation, such as point mutations which can not be detected by RAPD (Dettori and Palambi, 2000). These results were in agreement with the reports of Coletta Filho *et al.* (2000) and Shaaban *et al.* (2006). Motohashi *et al.* (1992) also reported high mutation rate and variability among citrus species. They reported that the role of hybridization in genetic diversification is low, while the frequent mutations followed by subsequent selections are the major factors of diversification in citrus species. The molecular differences observed between commercial and exotic cultivars and other local selections in the present study might be attributed to different mutations of their subsistence in evolutionary process coupled with subsequent propagation from mutant part of mother rootstock over a prolonged period. These Results were in agreement with the findings of Domingues *et al.* (2004) and Dehesdtani *et al.* (2007) and corroborate the findings of the present study.

The varieties with great economic importance and distinct morphological characteristics (pulp colour, rind thickness, number of seeds, TSS, acidity) such as Jaffa, Blood Red Malta, Mediterranean Sweet, Hamlin Sweet, Valencia Late, Pineapple and Kodur Sathgudi showed complete genetic similarity indicating that these accessions were clonally derived from a single ancestor or they are derived from somatic mutations that were not detected by the molecular markers used in the present study. Gulsen and Roose (2001) also observed great similarity between popular cultivars of lemon Eureka and Lisbon cultivars which did not form discrete clusters. Low polymorphism among commercial cultivars of grapefruit with RAPD has also been documented by Corazza-Nunes *et al.* (2002).

Low to moderate level of genetic diversity among sweet orange accessions coming from different locations and low intra-specific variability were also observed by Gulsen and Roose (2001) in lemons and Siragusa *et al.* (2006) in sour orange which corroborated the results of the present study. On contrary, Das *et al.* (2004) observed high level of genetic diversity among elite clones of mandarins with

RAPD analysis, which might be attributed to the heterogeneity of the species with cultivars ranging from facultative apomicts to completely sexual types (Barret & Rhodes, 1976).

It is to conclude that RAPD analysis of 27 elite clones of sweet orange indicated the existence of a high degree of genetic diversity among sweet orange accessions. Further, a finer molecular analysis of sweet orange accessions using other molecular marker techniques like Amplification Fragment Length Polymorphism (AFLPs) and Inter-Simple Sequence Repeat Markers (ISSRs) could also be helpful to explored to the genetic diversity present between exotic and local accessions.

References

Abkenar, A.A. and Isshiki, S. 2003. Molecular characterization and genetic diversity among Japanese acid citrus (*Citrus spp*.) based on RAPD markers. J. of Horti. Science and Biotechnology 78(1): 108-112.

Barrett, H.C. and Rhodes, A.M. 1976. A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Systematic Botany 1:105-136.

Coletta Filho, H. D., Machado, M.A., Targon, M. L. P. N. and Pompeu-Junior, J. 2000.The use of random amplified polymorphic DNA to evaluate the genetic variability of Ponkan mandarin (*Citrus reticulata Blanco*) accessions. Genetics and molecular Biology 23(1): 169-172.

Coletta Filho, H.D., Machado, M.A., Targon, M.L.P.N., Moreira, M.C.P.Q.D.G. and Pompeu-Junior, J. 1998. Analysis of the genetic diversity among mandarins (*Citrus spp*.) using RAPD markers. Euphytica 102(1): 133-139.

Corazza Nunes, Machado, M.A., Nunes, W.M.C., Cristofani, M. and Targon, M.L.P.N. 2002. Assessment of genetic variability in grapefruits (*Citrus paradise Macf*.) and pummelos (*C.maxima (Burm.) Merr*.) using RAPD and SSR markers. Euphytica 126: 169-176.

Cristofani, M., Figueiredo, J.O., Targoan, M.L.P.N. and Machado, M.A. 2000. Differentiation of Lemon Varieties by Microsatellites. Proceedings of the Global Citrus germplasm Network. GCGN meeting, 7-8 December: P-163.

Das, A., Sarkar, J., Mondal, B. and Chaudhuri, S. 2004. Genetic diversity anlaysis of citrus cultivars and rootstocks of the North Eastern India. Indian Journal of Genetics and Plant Breeding 64(4): 281-285.

Dehesdtani, A., Kazemitabar, S.K. and Rahimian, H. 2007. Assessment of genetic diversity of navel sweet orange cultivars grown in Mazandaran province using RAPD markers. Asian Journal of Plant Sciences 6(7): 1119-1124.

Deng, A.N., Gentile, A., Nicolosi, E., Domina, F., Vardi, A. and Tribulato, E. 1995. Identification of *in vivo* and *in vitro* Lemon mutants by RAPD markers. J. Hort.Sci. 70(1): 117-125.

Dettori, M.T. and Palombi, M.A. 2000. Identification of *Feijoa selllowiana* Berg accessions by RAPD markers. Scientia Horticulturae 86:279-90.

Dominques, E.T., Teofilo Sobrinho, J., Pompeu Junior, J., Figueiredo, J.O.de. and Tulmann Neto, A. 2004. Characterization of sweet orange varieties with different harvest seasons. Laranja 25(1):139-170.

Gmitter, F.G., Grosser, J. W. and Moore, A.G. 1992. Citrus. In: Hammerschlag, F. and R. Litz (eds.), *Biotechnology of perennial Fruits Crops, pp.*335-69. CAB Int. Wakkubgford, UK.

Grattapaglia, D. and Sederoff, R. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo testcross: Mapping strategy and RAPD markers. Genetics 137:1121-1137.

Guerra, M. S. 1984. Cytogenetics of Rutaceae. II. Nuclear DNA content. Caryologia 37 (3): 219-226.

Gulsen, O. and Roose, M.L. 2001. Determination of genetic diversity and phylogenetic relation to citrus ancestors in lemons by DNA markers. Bahce 30(1/2): 53-63.

Hvarleva, T., Kapari-Isaia, T., Papayiannis, L., Atanassov, A., Hadjinicoli, A. and Kyriakou, A. 2008. Characterization of Citrus cutivars and clones in Cyprus through microsatellite and RAPD analysis. Biotechnology and Biotechnology.EQ.22/2008/3: 787-794.

Jaiswal, V. S. and Amin, M. N. 1992. Guava and Jackfruit In:Biotechnology of perrinial fruit crops (Eds.Hamerschag. F A and Litz R E).

C.A.B. International University press. Cambridge:421-431.

Motohashi, R., Matsuyama, T. and Akihama, T. 1992. DNA fingerprinting in citrus cultivars. Proc. Int. Soc. Citriculture, 1:221-224.

Murray, M. and Thompson, W. 1980. The isolation of high weight plant DNA. Nucleic Acids Research 8:4321-4325.

Rohlf, F.J. 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1 Exceter Software, New York, USA.

Scora, R.W. 1975. On the history and origin of citrus. Bulletin of the Torrey, Utah Botanical club, Lancaster 102: 369-375.

Siragusa, M., Pasquale, F. de., Abbate, L. and Tusa, N. 2006. Identification of sour orange accessions and evaluation of their genetic variability by molecular marker analyses. Hortscience 41(1):84-89.

Spiegel Roy, P. and Goldschmidt, E.E. 1996. Biology of Citrus. Cambridge Univ. Press. Cambridge.

Williams, J.G.K., Kubelik, A.R., Livak, K.J. and Rafalski, J. A. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Table 1: Sweet Orange accessions selected for RAPD analysis

S.No.	Sweet orange Genotype	Short name	Origin
1.	Kodur Sathgudi	KS	Sathgur, TN
2.	Jaffa	JF	Palestine an Israel
3.	Bloodred Malta	BRM	South Europe
4.	Mosambi	M	Mozambique or East Africa
5.	Hamlin Sweet	HS	Florida
6.	Valencia	VA	London
7.	Valencia Late	VAL	London
8.	Mediterranean Sweet	MS	Florida
9.	Pineapple	PA	Florida groves
10.	HS-1	HS-1	Himakuntla
11.	HS-2	HS-2	Himakuntla
12.	HS-3	HS-3	Himakuntla
13.	HS-4	HS-4	Himakuntla
14.	TS-1	TS-1	Tirupati
15.	TS-2	TS-2	Tirupati
16.	TS-3	TS-3	Tirupati
17.	TS-4	TS-4	Tirupati
18.	TS-6	TS-6	Tirupati
19.	TS-8	TS-8	Tirupati
20.	TS-9	TS-9	Tirupati
21.	TS-10	TS-10	Tirupati
22.	TS-11	TS-11	Tirupati
23.	Nadempalli selection	NDS	Nadimpalli
24.	Ankalamma Gudur	AKG	Ankalamma gudur
25.	Ananthapur selection	ATP	Ananthapur
26.	CIP Sathgudi selection	CIP	Unkown
27.	Nandeli selection	NS	Nandeli

Table 2: Number of amplification fragments obtained and fragment size by using 52 decamer random primers.

Sl.No.	Primer code	Total number of bands	Polymorphic bands	Polymorphism (%)	Range of Fragment size (bp)
1.	OPA 01	12	6	50.00	290-3500
2.	OPA 02	10	6	60.00	410-1490
3.	OPA 03	9	5	55.56	470-3090
4.	OPA 04	11	5	45.45	390-2100
5.	OPA 05	8	5	62.50	660-2490
6.	OPA 06	10	6	60.00	470-3110
7.	OPA 07	9	7	77.78	570-2070
8.	OPA 08	9	5	55.56	290-2060
9.	OPA 09	12	5	41.67	250-1630
10.	OPA 10	14	10	71.43	310-2190
11.	OPA 11	9	3	33.33	380-2000
12.	OPA 12	7	5	71.43	260-1750
13.	OPA 13	13	6	46.15	290-2000
14.	OPA 14	3	1	33.33	740-1000
15.	OPA 15	12	9	75.00	310-2430
16.	OPA 16	6	3	50.00	670-2200
17.	OPA 17	9	5	55.56	370-2050
18.	OPA 18	12	4	33.33	300-1280
19.	OPA 19	9	5	55.56	190-2000
20.	OPA 20	11	6	54.55	260-1960
21.	OPM 01	11	7	63.64	350-1500
22.	OPM 02	8	7	87.5	250-1500
23.	OPM 03	11	6	54.55	250-1800
24.	OPM 04	9	5	55.56	400-2800
25.	OPM 05	9	7	77.78	400-1600
26.	OPM 06	11	7	63.64	400-2400
27.	OPM 07	12	4	33.33	300-1300
28.	OPM 08	7	7	100.00	300-1700
29.	OPM 09	10	9	90.00	350-6000
30.	OPM 10	7	4	57.14	500-2000
31.	OPM 11	14	8	57.14	375-3100
32.	OPM 12	19	11	57.89	300-3500
33.	OPM 13	13	11	84.62	280-1650
34	OPM 14	10	9	90.00	480-2500
34.	OPM 15	9	8	88.89	350-1870
36.	OPM 16	7	2	28.57	610-2600
37	OPM 17	8	8	100.00	310-1670
38	OPM 18	5	3	60.00	250-1750
39	OPM 19	4	0	0.00	500-1470
40.	OPM 20	10	3	30.00	250-2000
41.	OPB 04	4	3	75.00	560-2900
42.	OPB 07	11	8	72.73	500-2000
43.	OPB 15	2	0	0.00	740-1260
44.	OPE 08	5	5	100.00	700-1600
45.	OPE 09	7	3	42.86	700-1750
46.	OPE 12	3	2	66.67	760-1800
47.	OPE 14	12	9	75.00	375-1610
48.	OPH 04	7	6	85.71	490-1900
49.	OPH 11	6	4	66.67	730-1450
50.	OPH 15	5	4	80.00	1100-2750
51.	OPJ 09	9	7	77.78	730-2470
52.	OPJ 10	9	8	88.89	550-2400
	Total	469	292	62.26	
	Average	9.02	5.62	62.73	

Table 3: Jaccard's similarity matrix among 27 sweet orange accessions based on RAPD data

	KS	JF	BRM	M	HS	VA	VAL	MS	PA	HS1	HS2	HS3	HS4	TS1	TS2	TS3	TS4	TS6	TS8	TS9	TS10	TS11	NDS	AKG	ATP	CIP	NS
KS	1.00																										
JF	0.94	1.00																									
BRM	0.93	0.97	1.00																								
M	0.94	0.93	0.94	1.00																							
HS	0.95	0.96	0.95	0.94	1.00																						
VA	0.92	0.93	0.93	0.94	0.94	1.00																					
VAL	0.94	0.94	0.94	0.94	0.96	0.94	1.00																				
MS	0.93	0.94	0.94	0.93	0.96	0.94	0.98	1.00																			
PA	0.92	0.93	0.92	0.92	0.94	0.91	0.94	0.94	1.00																		
HS1	0.94	0.93	0.92	0.93	0.96	0.92	0.95	0.95	0.96	1.00																	
HS2	0.94	0.94	0.92	0.93	0.96	0.92	0.95	0.95	0.96	0.98	1.00																
HS3	0.56	0.55	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.55	0.55	1.00															
HS4	0.79	0.77	0.77	0.80	0.78	0.80	0.79	0.79	0.78	0.78	0.78	0.64	1.00														
TS1	0.88	0.89	0.87	0.89	0.90	0.88	0.89	0.89	0.88	0.90	0.90	0.55	0.76	1.00													
TS2	0.91	0.92	0.91	0.90	0.95	0.90	0.92	0.94	0.92	0.94	0.94	0.55	0.77	0.91	1.00												
TS3	0.89	0.90	0.88	0.88	0.92	0.88	0.90	0.91	0.90	0.92	0.92	0.54	0.77	0.92	0.92	1.00											
TS4	0.91	0.90	0.89	0.91	0.92	0.91	0.93	0.94	0.91	0.93	0.93	0.54	0.79	0.87	0.90	0.88	1.00										
TS6	0.87	0.88	0.86	0.87	0.89	0.87	0.88	0.88	0.86	0.88	0.88	0.56	0.77	0.88	0.88	0.88	0.88	1.00									
TS8	0.90	0.91	0.90	0.91	0.91	0.90	0.90	0.89	0.88	0.90	0.89	0.55	0.78	0.86	0.88	0.87	0.87	0.86	1.00								
TS9	0.88	0.89	0.88	0.89	0.89	0.87	0.88	0.88	0.86	0.88	0.87	0.55	0.76	0.87	0.89	0.87	0.86	0.87	0.90	1.00							
TS10	0.91	0.92	0.91	0.93	0.93	0.92	0.92	0.92	0.94	0.92	0.93	0.54	0.79	0.87	0.90	0.87	0.89	0.86	0.90	0.89	1.00						
TS11	0.90	0.92	0.91	0.91	0.93	0.92	0.91	0.93	0.90	0.91	0.91	0.54	0.79	0.89	0.91	0.90	0.90	0.87	0.90	0.89	0.92	1.00					
NDS	0.87	0.88	0.87	0.88	0.89	0.88	0.88	0.89	0.87	0.87	0.88	0.52	0.77	0.83	0.85	0.84	0.88	0.84	0.88	0.86	0.90	0.90	1.00				
AKG	0.87	0.88	0.88	0.89	0.88	0.88	0.87	0.88	0.86	0.86	0.87	0.53	0.77	0.82	0.85	0.83	0.86	0.83	0.87	0.86	0.90	0.89	0.94	1.00			
ATP	0.79	0.78	0.78	0.80	0.78	0.79	0.78	0.78	0.79	0.77	0.77	0.57	0.78	0.74	0.75	0.74	0.77	0.75	0.80	0.76	0.81	0.78	0.82	0.84	1.00		
CIP	0.84	0.84	0.83	0.83	0.84	0.83	0.82	0.83	0.81	0.82	0.82	0.60	0.81	0.81	0.83	0.81	0.82	0.82	0.86	0.83	0.83	0.85	0.85	0.87	0.84	1.00	
NS	0.86	0.84	0.83	0.84	0.84	0.84	0.84	0.85	0.83	0.83	0.83	0.55	0.77	0.80	0.82	0.81	0.83	0.82	0.84	0.81	0.83	0.85	0.86	0.87	0.82	0.89	1.00

Fig 1: Dendrogram of 27 sweet orange accessions based on 52 RAPD primers (Cophenetic correlation (r) = 0.99)