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Assessment of genetic diversity in grapefruit (*Citrus paradisi* Macf) cultivars using physico-chemical parameters and microsatellite markers

Nimisha Sharma*[1], Anil Kumar Dubey[1], Manish Srivastav [1], Bikram Pratap Singh[2], Anand Kumar Singh[1], Nagendra Kumar Singh[2]

[1]Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110012, India
[2]National Research Centre on Plant Biotechnology, New Delhi, 110012, India

***Corresponding author: nims17sharma@gmail.com**

Abstract

Microsatellite markers and physico-chemical parameters were used to investigate whether variants of grapefruit (*Citrus paradisi* Macf) were originated genetically or they showed different behaviour due to the environmental factors. Twelve plantlets (6 varieties, 2 replicates and 4 variants) were chosen from grapefruit evaluation block of Fruits and Horticultural Technology Division, IARI, New Delhi. Important parameters like, maximum fruit weight (530.77g), fruit length (95.11mm), seeds/fruit (48.33), peel thickness (7.11mm) and acidity (1.10%) were observed in the Star Ruby. Whereas variables like, minimum fruit weight (304.10g), fruit diameter (83.11mm) and seeds/fruit (1) were observed in the Redblush. Out of 25 screened primers (ISSR and SSR), 23 primers exhibited clear, reproducible bands. A total of 50 out of 334 distinct bands were found polymorphic with an average of 23.85 bands per primer. The mean polymorphism information content (PIC) was found to be 0.248. Genetic similarity value among the grapefruit cultivars was 0.97 and among the variants it was 0.95. These values provided a clear indication that there was a low level of variation in the grapefruit. This study revealed that variants bear more similarity with Marsh Seedless and Redblush. Cluster analysis of two groups namely (1) seeded and (2) seedless, showed the good correlation between physico-chemical parameters and genetic data.

Keywords: *Citrus paradisi*; germplasm characterization; molecular markers; physico-chemical parameters.
Abbreviations: AFLP_amplified fragment length polymorphism; ISSR_inter simple sequence repeat; MI_marker index; PCA_ principal component analysis; PIC_polymorphism information content; SNP_single nucleotide polymorphism; SSR_simple sequence repeat; RAP_random amplified polymorphic DNA; RFLP_restriction fragment length polymorphism; UPGMA_unweighted pair group method with arithmetic average.

Introduction

Citrus is a large genus that includes several major cultivated species, including *C. sinensis* (sweet orange), *Citrus reticulata* (mandarin), *Citrus aurentifolia* (lime), *Citrus limon* (lemon), *Citrus grandis* (pummelo) and *Citrus paradisi* (grapefruit). The grapefruit (*Citrus paradisi*) is the fourth most economically important citrus fruit in the world. It is an excellent source of many nutrients, phytochemicals, vitamin C, and fiberpectin with pink and red hues that contain the beneficial antioxidant lycopene (Silver et al., 2011). There are many benefits of this fruit worth mentioning, (i) it helps in lowering cholesterol (Platt, 2000), (ii) peel and seed extract of grapefruit have antifungal properties (Okunowo et al., 2013) and also (iii) in animal cell studies, grapefruit powder, limonin and naringenin decrease growth and increase self-destruction of colon, mouth, skin, lung, breast and stomach cancers (Chidambara et al., 2011). It is a well-known fact that citrus varieties show diversity in their morphological traits such as size and shape of canopy, color, type, ripening season of the fruits and the number of seeds per fruit. Generally in plants, the diversity at the phenotypic level is much larger than at genetic level, as selectively neutral molecular markers are used to evaluate the extent of genetic variation. However, more and more nucleotide diversity within trait specific genes underlying adaptive traits are studied for signatures of selection at single sites.

This adaptive genetic diversity constitutes important potential for future variety management and conservation purposes. Recently researchers (Amara et al., 2011; Ahmed et al., 2012; Jianfeng et al., 2012; Uzun and Yesiloglu, 2012), observed the substantial diversity among cultivated genotypes of *Citrus* genus in respect of morphological, physiological and agronomic traits but very little DNA variation has been detected using DNA-markers. In the similar studies microsatellite markers have been utilized to determine genetic diversity, characterization, phylogenetic relationships among the *Citrus* and related genera (Gulsen and Roose, 2001; Shahsavar et al., 2007; Uzun et al., 2009; Marak and Laskar, 2010; Golein et al., 2011). It was shown that the (i) ISSR markers involve the amplification of DNA segments between two identical microsatellite repeat regions, (ii) ISSRs have high reproducibility possibly due to the use of longer primers (16–25-mers) as compared to RAPD primers (10-mers), which permits the subsequent use of high annealing temperature (45–60°C) leading to higher stringency. This technique overcomes most limitations such as low reproducibility and high cost (Reddy et al., 2002). In the present study twelve plantlets of grapefruit were studied with the aim (a) to estimate genetic polymorphism and relationships among grapefruit accessions based on ISSR and SSR markers (b) to correlate the physico-chemical variation with the genetic diversity (c) to investigate whether variants

of grapefruit generated because of the environmental effects or it is genetically associated with studied cultivars?

Results

Characterization of physico-chemical parameters

Physicochemical parameters like; fruit weight, fruit size, seeds/fruit, peel thickness, juice content, total soluble solids and total acidity of six grapefruit cultivars and four variants were analyzed (Table 2). The significantly heaviest fruit (530.77g) was found in Star Ruby while Redblush had minimum fruit weight (304.10g) which was non-significant with GS-1 (464.47g). Other genotypes had intermediate fruits. Except Redblush, Marsh Seedless, GS-2 and GS-5, all other cultivars had fruit weight of more than 400.00 g. Similarly, the maximum fruit length was found in Star Ruby (95.11mm) and minimum fruit length was recorded in GS-2 (77.43 mm), which did not have significant difference with GS-5. However, highest fruit diameter was found in Foster (103.22 mm) which was statistically non-significant with Star Ruby (102.56 mm). Whereas, minimum fruit diameter was found in Redblush (83.11 mm) which was non-significant with GS-5 (86 mm).As we know that the number of seeds in a fruit is a very important parameter to differentiate seeded variety with seedless variety. In this study it was observed that number of seeds/fruit varied from 48.33 in Star Ruby to 1.00 in Redblush. From the results it is evident that Star Ruby (48.33), Foster (35.00), Duncan (40.00) and Imperial (34.00) come under highly seeded group. While, Redblush (1.00), Marsh Seedless (5.33) and all the variants come under seedless group. Peel thickness was also observed to be significantly different among the genotypes. The thicker peel was measured in Star Ruby (7.11mm) followed by Marsh Seedless (4.82 mm) which was non-significant with Duncan, Foster, GS-3 and GS-1. However, minimum thickness of peel was found in GS-2 (3.39 mm) which did not have significant edge over GS-5 (3.67 mm) and Redblush (3.87 mm). In another important observation it was found that genotypes had significant effect on juice content. The highest juice content was found in GS-5 (62.03%). Whereas the lowest was measured in Star Ruby (40.31%). It is important to mention that except Star Ruby, Foster and Duncan, all other genotypes had > 50% juice content. The highest total soluble solids (TSS) content measured in both Marsh Seedless and Redblush (8.50^0B) followed by Imperial which was non-significant with GS-1 and GS-3. Variation was also noticed in acidity contents among the grapefruit genotypes. The maximum acidity measured in both Star Ruby and Imperial (1.10%) which was non-significant with Foster (1.08%), GS-2 (1.08%), Redblush (1.07%), GS-3 (1.06%) and GS-5 (1.05%). However, the minimum acidity was measured in Marsh Seedless (0.94%). A dendrogram generated based on physico-chemical data grouped all the six cultivars and four variants into two major cluster A and B at genetic similarity of 0.48 as can be seen in Fig 1. Major cluster A is further sub-divided into one out group A1 and cluster A2 with genetic similarity value of 0.34. Cluster A2 consists of three cultivars namely Foster, Duncan and Imperial. Whereas, Star Ruby is presented as an out group A1. However, major cluster B is also sub-divided into two out group B1 and cluster B2 with genetic similarity value of 0.35. Cluster B2 comprised the cultivar Marsh Seedless along with four variants whereas, Redblush separated as an out group B1. In minor cluster B2.1, variant GS-1 and GS-3 showed 100% genetic similarity. It was observed that morphologically variants are more similar with Marsh Seedless and Redblush.

Genetic polymorphism among cultivars

Out of 16 screened ISSR primers, 14 primers were found to produce clear and reproducible bands (Supplementary Fig 1.). Only five primers *viz*; ISSR 2, 3, 10, 11 and 12 were found to be polymorphic (Table 3.). Out of 334 bands, 50 were polymorphic with 3.57 average polymorphic fragments per primer. A total of 12 alleles were detected with a mean number of alleles per locus of 1.33 from 9 SSR markers. All 12 alleles were determined as monomorphic. The amplified fragments size was found to be varying between 140 to 180 bp as shown in Supplementary Fig 2. The similarity value ranged from 0.87 to 1.00 with an average of 0.96. Genetic similarity value among the grapefruit cultivars and among the variants was 0.97 and 0.95, respectively. Genetic similarity value among Marsh Seedless, Redblush and all four variants was 0.96. The accessions were separated into one major cluster A and one out group B. These similarities indices were used to construct an UPGMA dendrogram (Fig 2.) and demonstrated that all eleven accessions except Imperial grouped together in major cluster A with a similarity level of 89%. The PIC value was 0.248. In the major grapefruit cluster A, accessions were not clearly separated. Cluster A is further subdivided into cluster A1 and out group A2. Grapefruit cultivars Star Ruby, Foster and Duncan showed 100% similarity (cluster A 1.2.1). Likewise variants GS-1 and GS-5 showed 100% similarity (cluster A1.2.2). Replicates of Marsh Seedless (MS-1 and MS-2) and Redblush (RB-1 and RB-2) showed 100% genetic similarity as shown in Fig 2. (cluster A1.2.2 and A1.3, respectively) which means seeds/ plant propagation material are pure and free from any mixture. According to only pulp color, variant GS-1 and GS-5 showed similarity with the cultivar Redblush. However, on the basis of physico-chemical parameters and genetic data they showed more closeness with Marsh Seedless rather than Redblush. In similar way GS-2 and GS-3 showed white pulp color like Marsh Seedless and on the basis of other physico-chemical parameters and genetic data they showed more closeness with Marsh Seedless, clearly indicating that they are the variants of Marsh Seedless. On the basis of number of seed/fruit, grapefruit cultivar is differentiated in two groups (i) seeded and (ii) seedless. Duncan, Imperial, Foster and Star Ruby came in seeded group whereas, Marsh Seedless, Redblush along with four variants GS-1, GS-2, GS-3 and GS-5 categorized in seedless group. However, genetically they also showed almost similar type of groups.

Principal Component Analysis (PCA) for physico-chemical parameters and genetic data

In a PCA generated with the genetic marker data, all the four variants were grouped in proximity with two other genotypes Redblush and Marsh Seedless (Fig 3.). While among other four genotypes, Foster and Duncan were also categorized near the variants group. It was observed that Star Ruby and Imperial genotypes did not clustered in any group and were placed very far from each other and even from the variants group. However, tree generated using genetic data showed that all cultivars and variants except Imperial separated in one major cluster A (Fig 2.). So the PCA results revealed that accessions of the same horticultural types tend to cluster together. The first three axis, respectively accounted 41.59%, 22.14% and 14.19% of the total variation. However, in case of the physico-chemical parameters first two principal components (83.36 and 15.89%, respectively) explained

Table 1. *Citrus paradisi* Macf cultivars and variants used in the study of germplasm characterization.

Cultivar/variants	Abbreviation	Origin
Star Ruby	SR	Originated as a lower branch mutation bearing red-blushed fruits, noticed on a 'Foster' tree at San Benito, Texas, USA in 1930
Foster	FR	Originated as a branch sport of a selection called 'Walters' in Florida, USA in 1907
Duncan	DN	Originated as identical seedlings in Florida USA in 1830
Imperial	IL	Of unknown origin, originated in Florida USA in 1901
Redblush	RB-1	Originated as sports–lower branches–growing out of
	RB-2 (Replicate)	'Thompson' trees in USA in 1929
Marsh Seedless	MS-1	Originated as seedling trees on the property of a Mrs. Rushing
	MS-2 (Replicate)	near Lakeland, Florida, USA (1862)
Variant-1	GS-1	Not a released variety seems to be mutant
Variant-2	GS-2	Not a released variety seems to be mutant
Variant-3	GS-3	Not a released variety seems to be mutant
Variant-4	GS-5	Not a released variety seems to be mutant

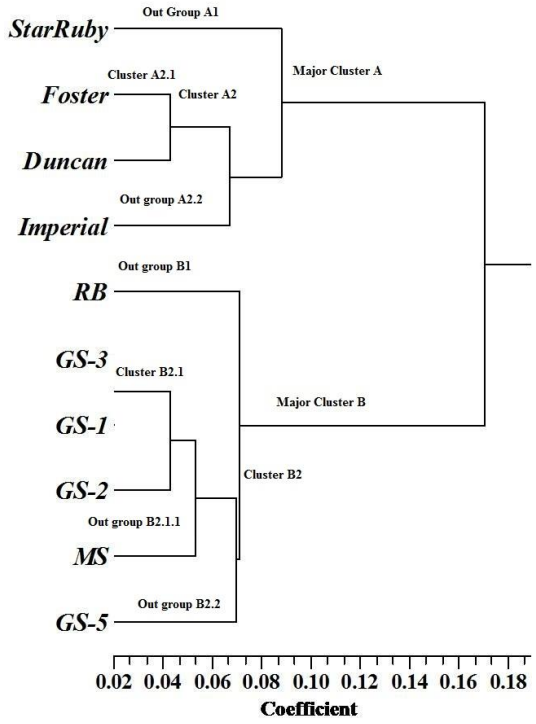

Fig 1. Dendrogram of grapefruits cultivars and variants based on physico-chemical parameters using the UPGMA method.

almost all variation (Fig 4.). PCA analysis unambiguously separated the grapefruit cultivars and variants. This result was supported by the differences in their origins.

Discussion

Molecular markers are powerful tools for elucidating genetic diversity, determining parentage, and revealing phylogenetic relationships among various *Citrus* species. However, accessions originated through spontaneous mutation are not easily distinguishable (Barkley et al., 2006). Grapefruit have a narrow genetic basis and its most morphological characters originated through mutations (Novelli et al., 2000; Targon et al., 2000; Bretó et al., 2001; Kahn et al., 2001; Yong et al., 2006).

Comparison of morphological and molecular characterization data is of immense importance to conclude the extent of genetic diversity present in the set of cultivars. In the present study although the correlation between the morphological and genetic data was found but it was low in the analyzed cultivars of *Citrus paradisi*. It is evident from the clustering pattern of the cultivars, where UPGMA dendrogram based on morphological data divided ten cultivars into two major clusters; whereas the dendrogram based on the ISSR and SSR marker divided them only into one major cluster and one out group. This shows that in spite of the wide phenotypic variations visible within the cultivars they had a very narrow genetic base. Genetic data based cluster analysis revealed that Imperial was the most distinct accession but morphologically it comes with Marsh Seedless and formed separate out-group. Genetically GS-1 and GS-5 showed 100% similarity and GS-2 and GS-3 showed 89% similarity. However, morphologically GS-2 and GS-5 were 100% similar whereas, GS-1 and GS-3 were distinct also genetically Star Ruby, Foster and Duncan showed 100% similarity while morphologically they are separated in different clusters. Morphologically Redblush separated as out group however, genetically it also showed variation with other cultivars. Similar findings were observed by Breto et al., 2001, where grapefruits showed considerable variation in morphological characters such as rind and flesh colour or fruit size. However, showed very low genetic variability using molecular markers. Genetic similarity value among the grapefruit cultivars was observed as 0.97 and among the variants was 0.95. Corazza-Nunes et al. (2002) also detected a similarity level ranging from 0.98 to 1.00 in 23 grapefruit cultivars, which is in good agreement with our results. Most grapefruits constituted an intensive group due to their low genetic variation concordantly with the dendrogram of the present study. Gulsen and Roose (2001) found similar results in lemons (*C. limon*) based on isozyme, SSR, and ISSR data. In present study grapefruit genotypes have low level of genetic diversity despite having high morphological variability suggesting that much of phenotypic variation may be because of somatic mutations. Similar findings have also been observed in grapefruit (Novelli et al., 2000; Bretó et al., 2001; Yong et al., 2006) and sweet orange (Malik et al., 2012). In the present study no polymorphic SSR marker was found to detect the low level of genetic diversity. Luro et al. (2001) also reported that the microsatellites could not distinguish mutation-derived species such as sweet and sour orange. However, in this study, we distinguished some grapefruit accessions with ISSR markers. So, this marker

Table 2. Physico-chemical parameters of grapefruit (*Citrus paradisi paradisi* Macf) cultivars and variants.

Cultivar/ Accession	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Seeds /fruit	Peel thickness (mm)	Juice (%)	TSS (°Brix)	Acidity (%)
Star Ruby	530.77^{a}±27.34	95.11^{a}±3.28	102.56^{a}±1.38	48.33^{a}±2.02	7.11^{a}±0.36	40.31^{c}±2.80	6.90^{d}±0.15	1.10^{ba}±0.12
Foster	491.20^{a}±17.38	88.02^{b}±0.66	103.22^{a}±1.78	35.00^{b}±3.00	4.57^{bc}±0.24	45.81^{c}±1.78	6.97^{d}±0.12	1.08^{bac}±0.02
Duncan	405.00^{bc}±22.17	84.26^{b}±1.29	93.63^{dc}±1.91	40.00^{b}±5.29	4.64^{bc}±0.63	46.08^{c}±2.83	7.30^{cd}±0.30	0.98^{d}±0.02
Imperial	410.03^{bc}±12.38	85.82^{b}±1.87	94.92^{bdc}±1.40	34.00^{b}±3.05	4.54^{cb}±0.12	51.20^{ba}±0.93	7.93^{b}±0.14	1.10^{ba}±0.01
Redblush	304.10^{d}±10.76	79.55^{cd}±1.75	83.11^{f}±0.45	1.00^{c}±0.00	3.87^{c}±0.47	57.08^{ba}±1.49	8.50^{a}±0.25	1.07^{bac}±0.01
Marsh Seedless	398.87^{bc}±26.99	85.11^{b}±1.97	96.01^{bdc}±3.56	5.33^{c}±0.33	4.82^{b}±0.20	53.45^{b}±2.29	8.50^{a}±0.23	0.94^{d}±0.00
Variant-1	464.47^{ba}±24.45	87.28^{b}±1.44	100.33^{ba}±1.84	3.67^{c}±0.33	4.53^{cb}±0.44	56.08^{ba}±3.59	7.63^{cb}±0.12	1.04^{c}±0.01
Variant-2	354.63^{dc}±25.46	77.43^{d}±1.74	91.67^{ed}±2.54	4.67^{c}±0.33	3.39^{cd}±0.10	57.30^{ba}±0.09	6.90^{d}±0.15	1.08^{bac}±0.02
Variant-3	421.40^{bc}±13.72	84.94^{b}±1.60	97.81^{bac}±1.20	4.33^{c}±0.66	4.62^{cb}±0.31	55.78^{ba}±2.51	7.67^{cb}±0.06	1.06^{bac}±0.02
Variant-5	358.83^{dc}±27.83	79.62^{cd}±1.41	86.95^{ef}±1.46	2.00^{c}±0.57	3.67^{cd}±0.15	62.03^{a}±5.60	7.40^{cbd}±0.11	1.05^{bc}±0.01
LSD (P ≤0.05)	68.63	5.15	5.67	6.91	0.54	6.46	0.56	0.05

Each data represent the mean value of five samples. Means with the same letters are not significantly differed at P ≤ 0.05

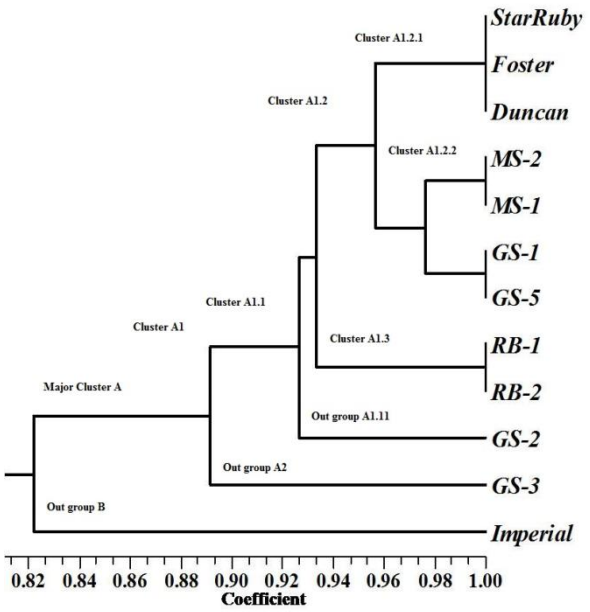

Fig 2. Dendrogram showing relationship among grapefruit cultivars and variants based on the ISSR and SSR markers using the UPGMA method.

system can be useful in detecting cultivars obtained by mutation. Studies indicated that ISSR markers were useful to determine genetic diversity of related citrus groups such as trifoliate oranges and their intergeneric hybrids (Hvarleva et al., 2014; Uzun et al., 2011). Similar results were obtained in lemons derived from clonal selections and in four of 12 lemon accessions distinguished using ISSR markers (Uzun et al., 2009). The ISSR procedure is an informative and suitable approach to the examination of the molecular polymorphism and the phylogenic relationships in the Indonesian Siam cultivars (Martasari et al., 2012). 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. paradisi*. These results can be further used to manipulate genetic determinants of horticulturally important traits and to characterize the basis of productivity of *C. paradisi* cultivars in India.

Materials and Methods

Plant materials

In this study we investigated 12 *Citrus paradisi* cultivars (Table 1). Leaf samples of all accessions were obtained from the orchard of Citrus Collection, Fruits and Horticultural Technology Division, IARI, New Delhi, India in the month of March, 2013.

Physico-chemical characterization

Morphological characterization of 12 cultivars of *Citrus paradisi* was performed. Data was recorded on yield and quality of fruits during 2012-13. Fruits were harvested at maturity and total yield were recorded. For quality analysis, twenty fruits were sampled from each cultivar, and quality characteristics were evaluated including seeds/fruit, peel thickness, fruit weight, fruit size and juice content. Juice was filtered through filter paper, thereafter, juice samples were examined to determine the following parameters; titratable acidity (% of citric acid) using N/10 NaOH and phenolphthalein as indicator, total soluble solids (TSS) using digital refractometer, and ascorbic acid (mg/100 ml of juice) using a dye (2, 6-dichlorophenol indophenol) according to the standard method (Rangana, 1986). Experiment was conducted in randomized block design with five replications. Data were subject for analysis of variation to one way ANOVA. Statistical analysis was performed using analysis of variance. P values ≤ 0.05 were considered as significant. All the eight physico-chemical characters were converted into bi- and multi-state code. A pair-wise similarity matrix was generated based on simple matching coefficient method using NTSYS ver. 2.10e software (Rohlf, 2000). A cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) based on simple matching coefficient in NTSYS software.

DNA extraction, ISSR and SSR analysis

Genomic DNA was extracted from young leaves by the CTAB method as described by Doyle and Doyle (1990). A total of 16 ISSR (Gulsen et al., 2010) and 9 SSR primers (Barkley et al., 2006) were used for all cultivars (Table 3). PCR reaction components and PCR cycling parameters for ISSR and SSR marker were performed as described by Uzun et al. (2009) and Barkley et al. (2006), respectively. DNA

Table 3. List of primers, their sequence, numbers and percent polymorphism of the amplified fragments generated by ISSR and SSR primers in grapefruit.

Primer	*Sequence 5'- 3'	TmºC	TB	PB	%P	NOA	PIC	MI
ISSR-2	TCCTCCTCCTCCTCCAC	57.6	36	14	38.8	7	0.71	9.9
ISSR-3	AGAGAGAGAGAGAGAGCT	53.7	66	17	25.7	8	0.80	13.7
ISSR-4	CATGAGAGAGAGAGAGAT	51.4	24	0	0	2	0.37	0
ISSR-5	CACACACACACACACAAG	53.7	24	0	0	2	0.37	0
ISSR-8	GACAGACAGACAGACA	49.2	24	0	0	2	0.37	0
ISSR-10	TCCTCCTCCTCCTCCAC	57.6	36	3	8.3	6	0.74	2.2
ISSR-11	CCTACCTACCTACCTA	49.2	28	4	14.2	4	0.70	2.8
ISSR-12	GTGGTGGTGGTGGTG	53.3	12	12	100	2	0.37	4.50
ISSR-14	CGAGATAGATAGATAGATA	48	24	0	0	2	0.37	0
AG-14	F:AAAGGGAAAGCCCTAATCTCA	55.9	24	0	0	2	0.37	0
	R:CTTCCTCTTGCGGAGTGTTC	59.4						
AC-01	F:TTTGACATCAACATAAAACAAGAA	53.1	24	0	0	2	0.37	0
	R:TTTTAAAATCCCTGACCAGA	51.2						
CT-21	F:CGAACTCATTAAAAGCCGAAAC	56.5	24	0	0	2	0.37	0
	R:CAACAACCACCACTCTCACG	59.4						

*F = forward and R = reverse, TB-total no of bands, PB-no of polymorphic bands, %P-percent polymorphism, NOA-number of allele, PIC – Polymorphism information content, MI-Marker index

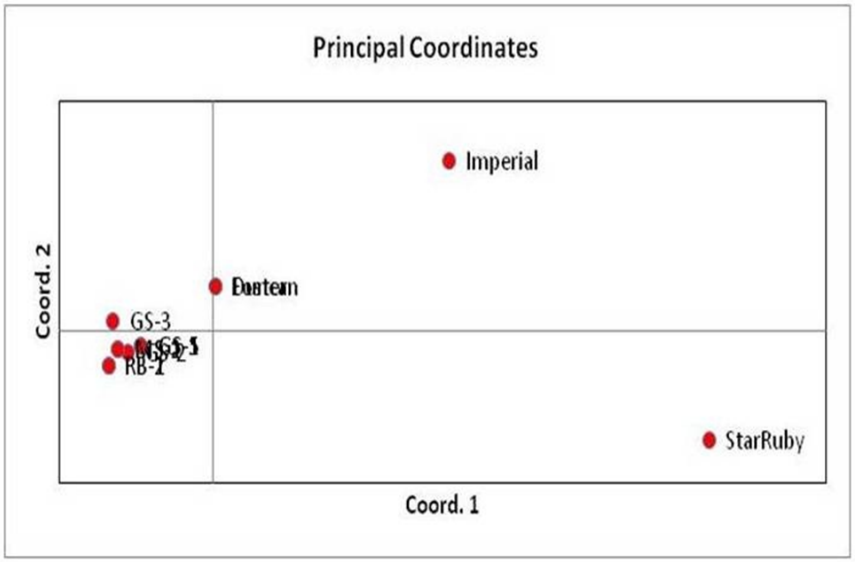

Fig 3. Principal Component Analysis (PCA) of the ten cultivars and variants of *Citrus paradisi* with genetic data generated by ISSR and SSR markers.

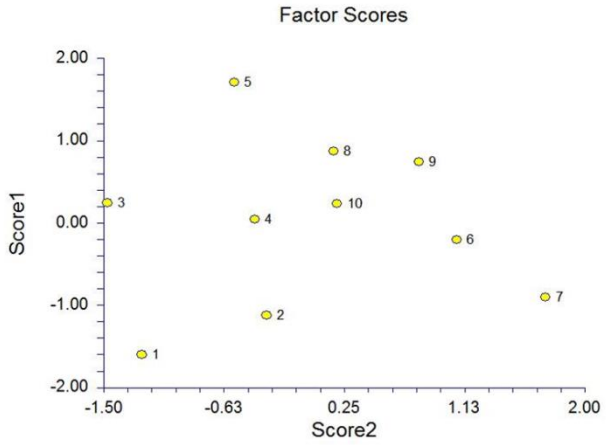

Fig 4. Principal Component Analysis (PCA) of the ten cultivars and variants of *Citrus paradisi* with physico-chemical parameters.

thermal cycler (Biorad DNA-Engine Gradient Cycler, Hercules, CA, USA) was used and cycling parameters included 3 min of denaturing at 94°C, following 35 cycles of these steps: 30 s of denaturing at 94°C, 30 s of annealing temp (41°C to 50°C), 1 min of elongation at 72°C, and lastly one cycle at 10 min at 72°C. PCR products were separated on 1.5% agarose gel for ISSR and 4% metaphor agarose gel (Amresco SFR, OH, USA) for SSR in 1X TBE buffer (89mM Tris, 89mM Boric acid, 2mM EDTA) at 115 V for 2.5–3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp DNA ladder (Gene Ruler, Fermentas) was used for SSR and ISSR analysis.

Data analysis

Each band was scored as present (1) or absent (0), and data was analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.1) software package **(**Rohlf, 2000). The similarity matrix was used to construct a dendrogram using the Unweighted Pair Group Method Arithmetic Average (UPGMA) to determine genetic relationships among the germplasm studied. Polymorphism Information Content (PIC) provides an estimate of the discriminatory power of a locus by taking into account not only the number of alleles that are expressed but also the relative frequencies of those alleles. Using the algorithm for all primer combinations as performed by Smith *et al.* (1997). PIC values were calculated. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies). Marker Index (MI) was determined as the product of PIC and the number of polymorphic bands per assay unit (Powell et al., 1996). Principal Components Analysis (PCA) of all accessions genotyped with ISSR and SSR markers were performed using GenAlEx 6.5 (Peakall and Smouse, 2012) while PCA of physico-chemical parameters was done by NCSS 2007 v 07.1.18 (Hintze, 2007).

Conclusion

Present study indicated that genetic diversity in *C. paradisi* was found to be very low, despite having high morphological variability, which could be elucidated by the fact that much of the phenotypic variation witnessed may be due to somatic mutations. Further based on morphological and molecular analysis, GS-1, and GS-5 which had red pulp colour like Redblush seems to be mutants of Marsh Seedless.

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