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RESEARCH ARTICLE

FINGERPRINTING INTRA-SPECIFIC DIVERSITY AMONG COCCINIA GRANDIS LANDRACES

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ABSTRACT

Comprehension of ecological and genetic evolution underlying the populace is a prerequisite for development of effective plant conversation, breeding strategies and trace crop varieties, as without phenome and genome variance, a population cannot acclimatize to its geo-edaphic milieu. The present study set sights on morphological and genetic differentiation pattern among 30 land races of *C. grandis* at intra- specific level, using 18 morphological, 15 RAPD and 10 ISSR markers. The values of fruit weight, girth and texture, along leaf shape displayed the major divergence among the 18 morphological traits. In RAPD and ISSR-PCR analysis of the 561 amplified products produced, 286 bands revealed polymorphism (50.98%). A marginal higher proportion of polymorphic bands were observed using ISSR (51.38%) than RAPD (50.10%) method. Mean PIC (polymorphism information content) for each of these marker systems (0.49 for RAPD and 0.62 for ISSR) suggested that both the marker systems demonstrated significant polymorphism. Jaccard's pairwise similarity coefficients ranged from 0.06 to 0.83 (RAPD) and 0.06 to 0.81 (ISSR) respectively. Cluster analysis based on RAPD, ISSR and their combined data clearly discriminated the cultivars into different clusters. Therefore, we conclude that these markers could be successfully used to assess genetic diversity with almost equal efficiency. The information provided here would contribute to breeding program as well as evolutionary study in *C. grandis*

INTRODUCTION

Coccinia grandis (L.) Voigt (Ivy gourd) is a dioecious, feral cucurbitaceous vegetal crop (Chun 2001). As an ethnic tribal plant it has potential therapeutic values as anti-diabetic, anti-ulcer, anti-inflammatory, anti-oxidant and anti-cancer properties (Vadivu 2008, Deokate 2011, Agarwal, 2011). The plant is native to central East Africa but has naturalized in Asia, Australia, Pacific Islands and Caribbean Islands. *Coccinia* comprises of 29 species of which only 5 have been completely characterized and only one species has been observed to be spread across India i.e. *C. grandis* (Holstein N. *et al.,* 2011). The crop is cyclically vegetatively propagated and a facultative apomictic and female flowers are observed to outcross (Shaina and Beevy, 2015), consequently multifarious phenome and perhaps genome may ensue. This also limits the scope of conventional breeding strategies. Thus genetic diversity assessments and linkage map construction is the call for hour to devise effective breeding programs for this vegetal species.

Intra-specific genetic and morphological variations of crop plants are increasingly acknowledged for their ease of appliance. (Fuller *et al*., 2013) Morphological traits are robust determinants of agronomic efficacy and taxonomical classification of plants **(**Cholastova and Knotova, 2012). They have been used to study divergence in crop plants like Alfalfa (Cholastova and Knotova, 2012), maize (Beyene *et al*., 2005), bitter gourd (Dalamu *et al*., 2012), cucumber and melon (Zhang *et al*., 2012). However they exhibit phenome plasticity, as they are environment and plant developmental stage dependent. Hence the clustering on basis of phenotypic markers is often *espoused* by genetic differentiators based on DNA sequence polymorphism (Dalamu *et al*., 2012). Among the different molecular markers, RAPD and ISSR markers are robust, efficant and simple markers. Random Amplified Polymorphic DNA (RAPD) markers, utilizing PCR amplification from single arbitrary primer (10-15 oligomer), were developed by Williams and his co-workers (Williams *et al*. 1990). Dominant RAPD markers is highly suitable for quick fingerprinting, tagging traits for marker assisted selection, identification of different plant species, as well as for assessing genetic diversity (Moreno, 1995; Behra *et al* 2008). Inter Simple Sequence Repeats (ISSR) markers based analysis involves gene amplification of a region between two inversely oriented microsatellites placed at an amplifiable distance (Lakshmanan *et al*., 2007). Both the marker systems permit assessment of genetic relatedness of cultivars at inter/ intra species level (Heikal 2008).

The present study was designed to assess the diversity among 30 incongruent *C. grandis* landraces using 18 morphological markers and molecular markers (15 RAPD and 10 ISSR) to discriminate among accessions of diverse origin, characterize

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genetic relationships among and between accessions, determine relative efficacy of these marker systems for population analysis and also to evaluate the correlation between phenotype and genetic distances. The present study is thus first time reporting the divergence among *C. grandis* using different pheno-geno-markers.

MATERIALS AND METHODS

Plant Accessions

The genotypes used for present study comprised of 25 indigenous and 5 exotic accessions of C. grandis (Table 2). The plant samples were identified and authenticated (Herbarium specimen no 16696) from Blatter Herbarium, Xavier's College, Mumbai, India. The landraces are maintained in the Departmental green house, School of Biotechnology and Bioinformatics, D Y. Patil University, India. The samples were thoroughly washed with tap water and rinsed with 70% alcohol and distilled water, blot dried and weighed prior analysis.

Morphological Characterization

Morphological variations were studied using Standard descriptor set for Cucurbitaceous (IPGRI) (http://www.bioversityinternational.org/). The following traits were studied for developing morphological markers. Shape, size, ribbing and color of leaves and fruits, texture of skin and flesh, length and width of fruit (mm) and its ratio, length and width of seed (mm) and its ratio. Qualitative traits were scored visually. Means across three replications were calculated for each character and randomized block design was applied for setting the experiments.

DNA extraction and PCR analysis

The DNA was extracted from all land varieties using *mCTAB* method described by Mala *et. al.* (2014) and was quantified using UV-Visible spectrophomoter (Pharmaspec, UV- Visible 1700, Shimadzu). Purity of the extracted DNA was estimated by the ratio of A_{260}/A_{280} and by resolving the DNA in 1% Agarose gel with ethidium bromide (analyzed on Gel Documentation System GeneSnap, SynGene). The extracted DNA (75ng/µl) was subjected to PCR amplification using 15 RAPD (10 oligomer) and 10 ISSR (18-20 oligomer) primers selected on the basis of primers used among *Cucurbitaceae* (Behera *et al.*, 2008, Zhang *et al.*,2012 and El-Adl A.M. *et al*.2012). Amplifications were performed according to William *et al* 1990 and Behera *et al* 2008, in a 25 µl reaction volume containing 75 ng of genomic DNA, $MgCl_2$ (6mM-RAPD & 1mM ISSR), 25 pM primer, 1x assay buffer, 800µM dNTPs and 1U of Taq DNA polymerase. Thermal cycler (Eppendorf) conditions involved initial denaturation at 94^0C for 4 mins and 30 cycles at 94^0C for 40 sec, 40 sec annealing temperature (table 4 & 5) and then 72°C for 1min, followed by 8 min of final extension at 72°C. Amplicons were separated on 2.5% agarose gel in 1X TAE buffer. The segregated bands were scored by illumination under UV light after ethidium bromide staining and documented using a Gel documentation and image analysis system (Syngene).

Data Analysis

Patterns of the studied genotypes using RAPD and ISSR primers were scored as presence (1) or absence (0), where each character state was treated independently. Only consistent, bright, reproducible bands were considered for analysis. Genetic similarity and cluster analyses were performed by subjecting character data to empirical examination using SPSS software, statistical analysis program, version 14.0 and Jaccard's similarity coefficient was calculated as (Behera 2008) Jaccard's coefficient $J_c = Nc/ N_a+ N_b- N_c$, Where, N_a= no of amplified fragments in sample A, N_b= no of amplified fragments in sample B and N_c = no of bands shared by sample A and B. The polymorphic information content (PIC) was calculated for each primer as PIC = 1-1/L Σ pi^2 where, L= Total no of Loci and p_i=Frequency of the i^{th} allele at the locus. The resolving power a primer (Rp) was calculated as Rp = Σ Ib_i, where Ibi describes the relative band informativeness and is calculated as Ibi= 1- (2X |0.5- pi|), p_i is the proportion of the accessions containing i^{th} band. The marker index (MI) was calculated as product of PIC and EMR (effective multiplex ratio), where EMR is the product of fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Saini M. *et al.*, 2010, Singh and Jawali 2012)

RESULTS AND DISCUSSION

Insight of molecular basis of the elemental biological phenomena is fundamental for the effective conservation, management, and efficient utilization of plant genetic resources (PGR). Molecular markers, along qualitative and quantitative morphocharacters represent a resilient and rapid tool for characterizing diversity within the target species.

Figure 1 Phylogram generated on morphological traits using UPGMA. The tree is rooted on CgM12.

Table 1 Morphological traits observed in 30 accessions of *C. grandis* and their standard descriptors

Sr.No	Characters	Abbs.	Standards description
1.	Weight (g)	FW	The weight of fruits at fully ripe stage
2.	Peduncle length (cm)	PL	The length from the base to fruit intersects peduncle
3.	Length (cm)	FL	The length from stalk end to the tip of the fruit
4.	Girth (cm)	FG	The diameter at the middle portion
5.	Shape	FS	Globular, flattened, oblate, elliptical, pyriform, ovate, acorn, elongated, and others
6.	Skin texture	FST	Smooth, waxy, rough, spiny, and others
7.	Color	FC	pale green, green, dark green, orange, red, and others
8.	Ribbing	Rb	Present or absent
	Seeds		
9.	Seed Length/ width ratio	SLWR	Ratio of the length of seed from end to tip of seed and girth at the middle portion
10.	Seed weight (mg)	SW	Weight of seeds obtained from fresh fruit
11.	Seed Color & Shape	SC/S	Oval, Crescent/ white, off-white and others
	Leaves		
12.	Leaf length (cm)	LL	The length from the tip to the leaf intersects petiole
13.	Leaf width (cm)	LW	The width at the broadest place of the leaf
14.	Leaf Petiole length (cm)	LPL	The length from the base of to the intersects petiole
15.	Leaf shape	LS	Orbicular, ovate, elliptical, reniform, cordate, triangular, entire, trilobite, pentalobate, and others
16.	Leaf margin	LM	Crenate, denticulate, entire, lobate, serrate, serrulate, spiny, undulate, and others
17.	Leaf color	LC	Light green, green, dark green, and others
18.	Leaf Texture	LT	Smooth, waxy, rough, hairy and others

Table 2 The values of quantitative traits of 30 accessions of *C. grandis*

Variety	Location	FW (g)	PL (cm)	FL (cm)	FG (cm)	SLWR	SW (mg)	LL (cm)	LW (cm)	LPL (cm)
CgVM1	Thane, Maharashtra	1.8	1.2	6.8	4.5	1.2	20	1.2	1.2	1.5
CgVM2	Bangalore, Karnataka	1.6	1.8	4.5	5.5	0.8	15	1	1.3	1.8
CgVM3	Kharghar, Maharashtra	2.1	1.3	3.4	3.5	0.85	18	0.8	1	3
CgVM4	Bharuch, Gujarat	1.6	1.5	6.8	12	0.5	20	1.1	1.18	3.1
CgVM5	Raipur, Chhattisgarh	2.2	1.2	6.6	6	0.9	20	1.1	1.09	1
CgVM6	Kolhapur,Maharashtra	2.8	1.3	5.6	4	1.25	19	1.2	1.08	1.2
CgVM7	Chennai, Tamilnadu	2.6	1.4	5.6	4	1.6	25	0.8	1.5	3.1
CgVM8	Ernakulum, Kerala	2.5	1.6	5.8	3	0.8	15	0.8	1.2	2.7
CgVM9	Sulapha / Kerala	NA	NA	NA	NA	NA	NA	0.9	1	2.6
CgVM10	Darjeeling, West Bengal	2.3	1.7	3.5	5.8	1	17	1	0.8	1
CgVM11	Central Horticultural Experiment Station, Aiginia, Bhubaneswar	NA	NA	NA	NA	NA	NA	1.2	1.1	1
CgVM12	Central Horticultural Experiment Station, Aiginia, Bhubaneswar	NA	NA	NA	NA	NA	NA	1.2	1.6	1
CgVM13	Mainpuri, Uttar Pradesh	1.7	1.5	4	3	0.8	18	0.6	0.8	1
CgVM14	Belapur Creek, Maharashtra	1.8	1.5	5.2	10.1	0.6	18	0.9	1.5	1.3
CgVM15	Kenya, Africa	1.5	1.3	5.5	3.6	1.1	20	1.1	1.3	2.2
CgVM16	Nirvana, Bhairawa- Nepal	2.3	1.8	6.1	4	0.7	15	0.8	1.4	2
CgVM17	Lumbini Garden, Nepal	1.9	1.3	5.2	2	0.8	16	1.5	1.1	1.8
CgVM18	Gorakhpur, Uttar Pradesh	2.5	1.8	4.8	4.2	1	20	1.1	1.4	1.5
CgVM19	Lucknow, Uttar Pradesh	2.8	1.6	4.8	2.8	0.6	20	1.5	1.8	1.6
CgVM20	Indore, Madhya Pradesh	2.7	1.3	4.2	4.5	0.5	17	1.2	1.4	1
CgVMS21	Kochi, Kerala	1.8	1.5	4.9	3.6	0.8	16	0.7	0.9	1
CgVMS22	Andheri, Maharashtra	1.6	1.5	5.6	2.1	0.9	16	0.9	1.2	1
CgVMS23	Chiplun, Maharashtra	2.1	1.7	6.5	1.8	0.7	17	1.3	1.5	1.6
CgVMS24	Pen, Maharashtra	2	1.8	5.7	3.6	0.65	18	1.1	1.3	1.5
CgVMP25	Haldia type I, Purba Medinipur, West Bengal	2	1.5	5.5	6.2	1.1	20	1.6	1.8	1.5
CgVMP26	Haldia type II, Purba Medinipur, West Bengal	1.5	1.1	4.3	5.3	0.82	16	1.1	1.4	1
CgVMP27	Uran, Maharashtra	1.8	1.3	4.7	5.2	0.7	16	1	1.3	1.1
CgVMP28	Gattaprabha, Goa	1.9	1.5	4	3.8	0.9	18	1	1.3	1
CgVMP29	Kolkata, West Bengal	2.1	1.6	5	2.9	0.6	18	0.9	1.4	1.3
CgVMS30	Satara, Maharashtra	2.3	1.7	5.1	4.5	0.7	15	1.1	1.5	2.1
Mean		1.82± 0.13	1.31± 0.08	4.65± 0.31	4.05±0.4	0.762± 0.06	15.7± 1.05	1.03± 0.04	1.24± 0.05	1.58± 0.12
S.D		0.73	0.49	1.78	2.48	0.34	5.874	0.232	0.252	0.688
Variance		0.54	0.25	3.1	6.2	0.11	35.54	0.07	0.09	0.47

Key: NA: information not available, S.D. : standard deviation

Two of these marker systems, RAPD and ISSR, along morphological traits were employed in the present study for detecting genetic diversity and relationships among 25 indigenous (22 wild+ 3 cultivated) and 5 exotic genotypes of *C. grandis.*

Variation and Cluster Analysis among morphodescriptors in C. grandis land races

Multivariate statistical analysis was used to group the accessions on the basis of their morphological similarity. A significant phenotypic variation was recorded among the 30 land races used in present study.

Table 3 Qualitative traits studied among *C. grandis* varieties

Variety	FS	FST	FC	Rb	LS	LM	LC	LT	SC/S
CgVM1	Ovate	Smooth Waxy	Pale Green	Ab	Triangular	Entire	Dark Green	Smooth & Waxy	Off White Pointed Ends
CgVM2	Short Stout	Smooth Waxy	Dark Green	White Thick	Trilobite	Serrate	Pale Green	Smooth	Off White Ovate
CgVM3	Pyriform Long	Wrinkled	Orange Red	White Faint	Triangular	Entire	Yellowish Green	Rough	Dusky Mango Shaped
CgVM4	Ovate Stout/thick	Smooth Waxy	Dark Green	Thick & Green	Trilobite	Entire	Pale Green	Smooth	Crème Pointed At Upper End
CgVM5	Elongated	Smooth Non-Waxy	Green	Ab	Triangular	Entire	Green	Smooth	Ovate
CgVM6	Oval Both Ends Pointed	Smooth Non-Waxy	Yellow/ Pistachio Green	White	Penta-Lobite	Dentate	Light Green	Rough	Mango Shaped
CgVM7	Oblong	Rough Non-Waxy Speckled	Pale Green	Faint White Non-Uniform	Triangular	Entire	Pale Green	Rough	Off White Round
CgVM8	Elongate Slender	Smooth	Dark Green	Thin White	Triangular	Entire	Dark Green	Smooth & Waxy	Off White Pointed Ends
CgVM9	NA	NA	NA	NA	Triangular	Entire	Green	Smooth	NA
CgVM10	Globular	Smooth	Dark Green	Thick White	Triangular	Entire	Green	Smooth/ Waxy	Dusky Ovate
CgVM11	NA	NA	NA	NA	Trilobite	Entire	Green	Smooth	NA
CgVM12	NA	NA	NA	NA	Triangular	Entire	Green	Smooth	NA
CgVM13	Ovate pointed ends	Speckled	Red	White	Penta-lobite	Entire	Green	Smooth	Ovate
CgVM14	Globular	Smooth/waxy	Dark green	Thick pale green	Triangular	Entire	Pale Green	Smooth	Off White Round
CgVM15	Elongated	Smooth	Green	Thin White	Triangular	Entire	Green	Smooth	Crème Pointed Ends
CgVM16	Elongated	Waxy	Pale Green	Ab	Trilobite	Serrate	Green	Smooth	Off White Ovate
CgVM17	Elongated	Smooth	Green	White Faint	Triangular	Entire	Green	Smooth	Crème Ovate
CgVM18	Ovate	Smooth	Green/ Red	AB	Triangular	Entire	Green	Smooth	Crème Pointed At Upper End
CgVM19	Elongated	Smooth	Dark Green	Thin White	Triangular	Entire	Green	Smooth	Crème Ovate
CgVM20	Ovate	Smooth	Dark – Light Green	White	Triangular	Entire	Light Green	Smooth	White Ovate
CgVMS21	Elongated	Rough	Pale Green	Faint White Non-Uniform	Triangular	Entire	Green	Rough	Off White Round
CgVMS22	Elongated	Smooth	Dark Green	Thin White	Triangular	Entire	Dark Green	Smooth & Waxy	Off White Pointed Ends
CgVMS23	Elongated	Smooth	Green	Ab	Triangular	Entire	Green	Smooth	White/ Flattened
CgVMS24	Elongated	Smooth	Dark Green	Thick White	Triangular	Entire	Green	Smooth/ Waxy	Dusky Ovate
CgVMP25	Globular	Smooth	Red	Ab	Trilobite	Entire	Green	Smooth	White flattened
CgVMP26	Globular	Smooth	Green	Ab	Triangular	Entire	Green	Smooth	White/ ovate
CgVMP27	Ovate with pointed tips	Rough	Green	White	Triangular	Entire	Green	Smooth	Ovate
CgVMP28	Oblong	Smooth	Green	White	Triangular	Entire	Green	Smooth	Off White Round
CgVMP29	Elongated	Rough	Green	Ab	Triangular	Entire	Green	Smooth	Ovate/ white
CgVMS30	Oblong	Smooth	Green	White	Triangular	Entire	Green	Smooth	Off White Round

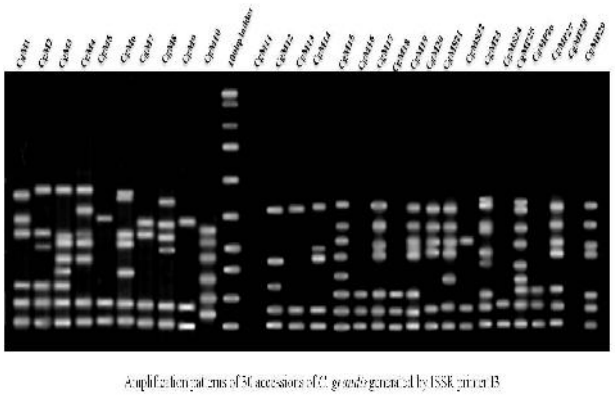

Figure 2 Amplification patterns of *C. grandis* generated by ISSR primer I3

The mean, standard error of difference (SE), standard deviation and variance values, along the quantitative and qualitative traits were evaluated (Table1, 2 & 3). A wide range of variation was observed for all characters except seed length: width ratio and leaf length and width, in which the variation was comparatively narrow. The morphotraits like fruit weight, length, girth and texture, leaf shape and texture displayed higher variation among the landraces. A reciprocal correlation was observed with fruit and leaf weight with their length and girth. Analysis of variance (One-way ANOVA) demonstrated differences among 30 accessions. A high variance was recorded in 10 of 18 traits screened in present study. However significant differences ($P \leq 0.01$) only in few traits were observed when morphodescriptors among indigenous (wild and cultivated) and exotic varieties were compared.

Table 4 Parameters signifying efficiency of 15 primers used for RAPD analysis in 30 accessions of *C. grandis*

Sr no	Marker/Codes	Sequence	Ta° C	TAB	PB	%	PIC	Rp	Jc	Mi
1	OPA-03R1	AGTCAGCCAC	34	18	6	33.34	0.73	15.25	0.16-0.51	08.26
2	OPA-04R2	AATCGGGCTG	34	22	13	59.09	0.82	16.35	0.08-0.52	13.22
3	OPA-09R3	GGGTAACGCC	33	20	9	45	0.32	10.75	0.26-0.63	8.46
4	OPA-18R4	AGGTGACCGT	33	26	12	46.15	0.28	9.28	0.42-0.82	9.07
5	OPA-19R-5	CAACGTCGG	33	25	14	56	0.3	9.6	0.38- 0.58	13.6
6	OPB-01R7	GTTTCGCTCC	33	10	4	40	0.69	11.25	0.06-0.47	7.23
7	OPB-17R8	AGGGAACGAG	33	25	14	56	0.63	10.25	0.1-0.58	13.6
8	OPAO-01R9	AAGACGACGG	33	28	14	50	0.29	6.24	0.28-0.83	13.1
9	OPC-20R10	ACTTCGCCAC	34	30	17	56.67	0.43	7.35	0.15-0.64	13.7
10	OPL-20R12	TGGTGGACCA	34	18	9	50	0.23	8.03	0.2-0.81	13.13
11	OPT-05R13	GGGTTTGGCA	33	30	13	43.33	0.69	11.25	0.09-0.53	08.23
12	OPU-02R14	CTGAGGTCTC	33	28	19	67.85	0.73	11.36	0.06-0.45	14.08
13	OPW-08R15	GACTGCCTCT	33	25	10	40	0.18	3.06	0.23-0.75	7.2
14	OPW-18R16	TTCAGGGCAC	33	30	12	40	0.52	7.23	0.11-0.56	7.25
15	OPA-19R19	CAAACGTCGG	33	22	15	68.18	0.56	7.54	0.092-0.6	14.6

Key: T_a: Annealing temperature, TAB: Total Amplified bands, PB: Polymorphic bands, %: percentage polymorphism, PIC: Polymorphic information content, R_p: resolving power, J_c: Range of Jaccard's similarity coefficient range, MI: Marker Index

Table 5 Parameters signifying efficiency of 10 primers used for ISSR analysis in 30 accessions of *C. grandis*

S.N.	Code	Sequence	T_a (^{0}C)	TAB	PB	%	PIC	Rp	Jc	Mi
1	Cg I-1	$(CA)_8T$	48.5	25	14	56	0.62	17.25	0.06-0.51	1.26
2	Cg I-3	$(GA)_8$ YG	53.2	23	15	65.21	0.88	19.35	0.15-0.42	14.22
3	Cg I-4	$(AC)_8G$	52.8	18	8	44.45	0.54	6.75	0.36-0.73	6.86
4	Cg I -5	$(AG)_8$ G	52.8	15	5	33.34	0.58	7.28	0.092-0.62	5.83
5	Cg I-9	$(GTG)_5AT$	57.6	20	11	55	0.6	11.6	0.16- 0.58	12.45
6	Cg I-11	$(GTG)_5AG$	57.6	25	11	44	0.75	15.25	0.076-0.57	11.25
7	Cg I-12	$(GTG)_5$	53.6	30	17	56.67	0.83	18.25	0.06-0.42	13.54
8	Cg I-13	$(CCA)_6$ G	63.1	22	10	45.45	0.46	6.24	0.08-0.53	6.23
9	Cg I-15	$(CT)_8AC$	53.6	10	7	70	0.41	7.75	0.2-0.54	15.28
10	Cg I-19	CCTACCTACCTACCTA	50.4	16	7	43.75	0.53	8.3	0.22-0.81	3.06

Key: T_a: Annealing temperature, TAB: Total Amplified bands, PB: Polymorphic bands, %: percentage polymorphism, PIC: Polymorphic information content, R_p: resolving power, J_c: Range of Jaccard's similarity coefficient range, MI: Marker Index

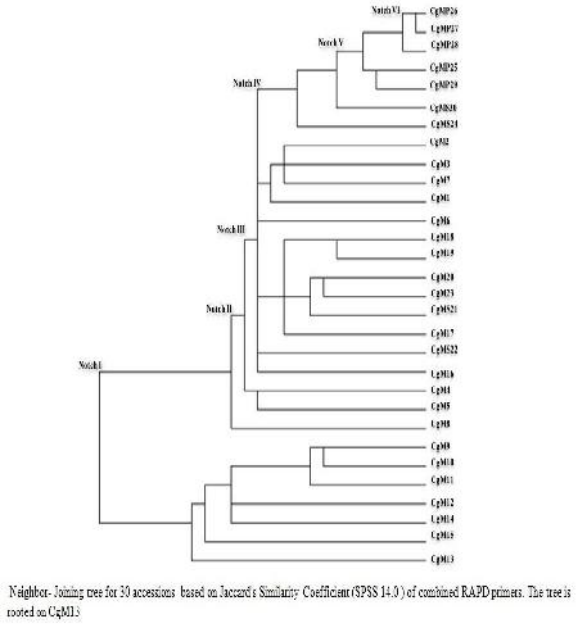

Figure 3 Neighbour joining tree based on Jaccard's Similarity Coefficient (SPSS 14.0) of combined RAPD primers. The tree is rooted on CgM13

A cluster analysis of 30 genotypes based on 12 quantitative traits was performed by UPGMA method (http://genomes.urv.cat/UPGMA/) and a dendogram was constructed as depicted in figure 2. The Cophenetic Correlation Coefficient was 0.9964 i.e. 99.64%, indicating the goodness of fit of the observed clustering pattern (http://people.revoledu.com/kardi/tutorial/Clustering/ Cophenetic.htm).

It was observed that all the genotypes were resolved into two major clusters. Cluster I, comprised of only the cultivated varieties, among them CgM9 appeared to evolve from CgM11 and CgM12, though they belong to two different breeding stations in India. In cluster-II, 4 exotic lines were grouped together with indigenous lines. It was revealed from the dendogram and distance matrix that the exotic landraces were highly related to the indigenous wild varieties. In the present study using the morphodescriptors high genetic relatedness among the land accessions of diverse geolands are probably observed due to biome shifts among *C. grandis* (Holstein and Renner, 2011) from their native loci i.e. Africa. A high similarity among the phenomorphic traits is a corollary of, low degree of heterozygosis (Koffi *et al.* 2009), similar environmental and agronomical conditions (Kanwal *et al.*, 1983), ancestral homology (Flaconer, 1981). A similar unrestricted movement of seeds and close relatedness among distinct geological locations has been reported in other species of *Coccinia* i.e. *C. abyssinica* (Wondimu *et al.* 2014) and pointed gourd (Khan, 2006). Also the facultative apomixsis and vegetative propagation strategies impinge on the rate of germplasm diversity (Shaina and Beevy, 2015).

RAPD Morphovars and Cluster analysis

Out of 357 reproducible amplicons generated by 15 RAPD primers, 181 were polymorphic. The size of the amplified products varied from approximately 250 – 1300 bp. The number of amplicons per primer varied from 10 (R7) to 30 (R 10, R16), with an average of 23.6 amplicons per primer. The average number of polymorphic amplicons per primer was 12. The percentage of polymorphism ranged from 33.34% (R1) to

68.18 % (R19), with an average of 50.10 % (Table 5). The maximum number of polymorphic amplicons (19) was obtained with the primers R14 with an average of 12.06 per primer. The average polymorphic information content (PIC) value was 0.49 and ranged from 0.23 (I-15) to 0.98 (I-11). The primers R2, R7, R14 had the higher PIC values. The resolving power ranged from 3.06 (R15) to 16.35 (R2) with average of 9.65 The Jaccard's similarity coefficients ranged from 0.06 to 0.82, with an average of 0.42 (Table 4). Thus the fifteen primers used herein were very effective in differentiating the genotypes. Approximately 15-20 monomorphic loci were observed revealing for the first time putative conserved loci among these thirty cultivars of *C. grandis*. A collective phylogram generated from the similarity matrix characters using SPSS (14.0) software is presented in figure 3. The genotypes were grouped in six clades. The dendogram also revealed the relative magnitude of resemblance among different clusters and Cophenetic Correlation Coefficient was 0.98 i.e. 98%. All the cultivated genotypes were grouped in one cluster (Node I), along one exotic and few indigenous land races (similarity coefficient = 0.76). Three other exotic lines were congregated along wild indigenous lines in nodes II and III (average similarity coefficient = 0.62), while maximum number Indian wild genotypes were clustered in nodes V-VII (average similarity coefficient =0.57). The convening of the exotic lines along indigenous wild land races appends the observed assemblage pattern among the phenomorphic markers.

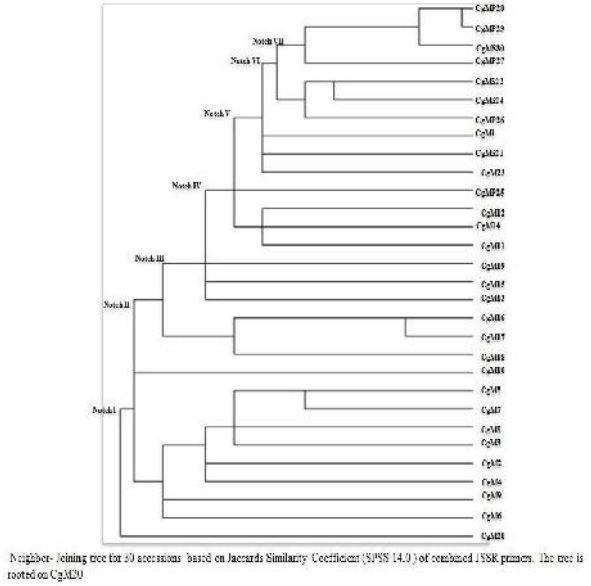

Figure 4 Neighbour joining tree based on Jaccard's Similarity Coefficient (SPSS 14.0) of combined ISSR primers. The tree is rooted on CgM20.

ISSR Morphovars and Cluster analysis

Out of 204 reproducible amplicons generated by 10 ISSR primers, 105 were polymorphic. The size of the amplified products varied from approximately 300 bp to 1.5 Kbp (figure 2). The number of amplicons per primer ranged from 10 (I15) to 30 (I12), with an average of 20.4 amplicons per primer. The average number of polymorphic amplicons per primer was evaluated as 10.5. The percentage of polymorphism ranged from 33.34% (I5) to 70 % (I15), with an average of 51.38 % (Table 4). The maximum number of polymorphic amplicons (17) was obtained with the primers I12. The average polymorphic information content (PIC) value was 0.62 and ranged from 0.23 (I-15) to 0.98 (I-11). The primers I1, I3, I11, and I12 had the higher PIC values. The resolving power ranged from 6.24 (I13) to 19.35 (I3) with average of 11.8 The Jaccard's similarity coefficients ranged from 0.06 to 0.81, with an average of 0.41 (Table 5). Thus the ten primers used herein were very effective in differentiating the genotypes. Approximately 10 monomorphic loci were observed revealing for the first time putative conserved loci among these thirty cultivars of *C. grandis*. A collective phylogram generated from the similarity matrix characters using SPSS (14.0) software is presented in figure 4. The genotypes were grouped in seven clades. The dendogram also revealed the relative magnitude of resemblance among different clusters (Figure 4) and Cophenetic Correlation Coefficient was 0.973 i.e. 97.3%. All the cultivated genotypes were observed to be assorted along the wild genotypes in nodes I and IV (average similarity coefficient = 0.68). The exotic lines were congregated together separately on node III (similarity coefficient = 0.73), while maximum number of Indian wild genotypes were clustered in nodes II, V-VII (average similarity coefficient =0.59). Thus the ISSR markers were observed to be efficient to segregate the accessions on the basis of their geological milieu.

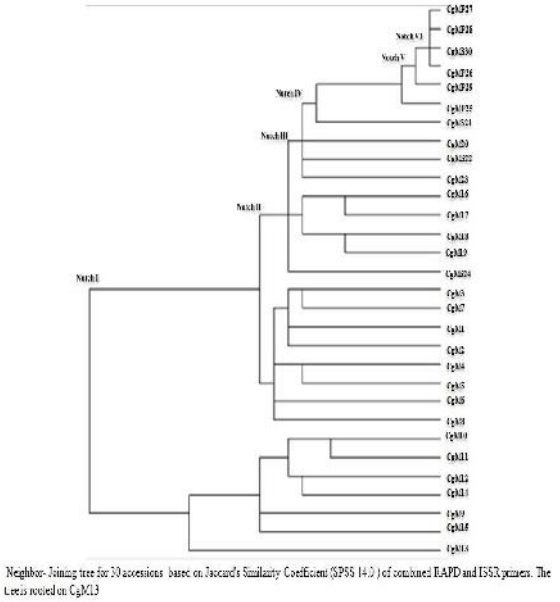

Figure 5 Neighbour joining tree based on Jaccard's Similarity Coefficient (SPSS 14.0) of combined RAPD and ISSR primers. The tree is rooted on CgM13.

Cluster analysis based on combined genetic markers

Collective phylogram was generated from the similarity matrix characters using SPSS (14.0) software using RAPD and ISSR markers figure 5. The genotypes were grouped in six clades. The dendogram also revealed the relative magnitude of resemblance among different clusters and Cophenetic Correlation Coefficient was 0.9933 i.e. 99.33%. All the cultivated genotypes were observed to be constellated together along few wild genotypes and one exotic type in nodes I (average similarity coefficient = 0.81). The other three exotic land races were observed to be clustered along a number Indian wild land types on node II, (similarity coefficient =0.73). The

node III- VII comprised of exclusively the Indian North-Eastern wild genotypes, indicating common distribution, breeding and agronomical environment. Thus the combined genetic markers were observed to be efficient to segregate the accessions on the basis of their geological milieu and common breeding lines.

The genetic descriptors used in present study were efficient to distinguish the land accessions at the intra- varietal level exhibiting conserved and unique gene loci. The RAPD markers complement the observations of phenomorphic markers, as they are distributed across genome and are based on multiple loci (Williams *et al.*, 1990), of which some loci may code for agronomically important phenotraits (Pandey *et al.*, 2008). The ISSR markers exhibited finer differentiation among land accessions on the basis of their geographical origin, as these marker systems are based on microsatellite repeats in genome (Behera *et al.*, 2008). RAPD (29.5% polymorphism) analysis was found to be less effective than ISSR (65% polymorphism) markers in water-melon by (Levi *et al.* 2001b) in assessing cultivar variation.

Similarity Correlation matrices data obtained from the two genetic differentiators in thus study thus revealed the following: a) North-Eastern Indian wild germplasms represent a divergent lineage from others ;b) Exotic landvars are observed to be assorted along Indian land races, indicating small genomic divergences among geologically distant plants; c) commercial cultivars are diverse in their geographic origins and genetic compositions from wild types and d) the integrity and confinement of some cultivars exhibit low gene flow among them as, *C. grandis* is preferably vegetatively propagated.

We thus conclude that the state of genetic diversity in *C. grandis* cultivars may be better described when different markers are exploited in a complementary mode. The data can be utilized for local crop development, or exchange of varieties, their maintenance and utilization, their enhancement seed multiplication, processing and storage, along for varietal identification.

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