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RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress

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Abstract *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae) shows high levels of variation in fruit color, fruit stripe pattern, seed coat color, and size. Thirty-eight accessions of *C. colocynthis* plants from different parts of semi-arid Rajasthan were collected and genetic diversity was assessed using random-amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. Out of 65 RAPD decamer primers, 50 primers produced 549 scorable bands of which 318 were polymorphic. Polymorphic banding patterns with the number of amplified fragments varied from 5 (OPA-08 and OPF-9) to 19 (OPT-20) in the molecular size range of 150–6000 bp. Percent polymorphism ranged from 22.2% (OPA-09) to 83.3% (OPE-12) with 55.14% polymorphism. Out of the 20 ISSR primers screened, 13 primers produced 166 amplification products, of which 99 were polymorphic. The number of bands amplified per primer varied between 9 (UBC-807, 802) and 16 (UBC-803, 812) with average band size between 250 and 4000 bp. Percent polymorphism ranged from 45.4% (UBC-815) to 73.3% (UBC-814) with 65.05% polymorphism. Dendrogram constructed on the basis of RAPD + ISSR polymorphism separated the accessions into four distinct clusters at 72% variation with Jaccard's similarity coefficient ranging from minimum 0.64 to 0.95. The matrices for RAPD and ISSR were also compared using Mantel's test and obtained correlation value ($r = 0.7947$). Discriminating power of RAPD and ISSR markers was assessed by calculating polymorphic information content, multiplex ratio, marker index, and resolving power. Approx. 50% RAPD and ISSR markers showed PIC value and heterozygosity (H) ≥ 0.50, indicating marker as informative. The primers that showed higher polymorphism had higher RP, MR, and MI values.

Introduction

Genetic variation and diversity are a prerequisite for the short- and long-term survival of the plant species in their natural habitat. The evaluation of genetic diversity of any species is very important for their conservation and gene manipulation (Khan et al. 2012).

Cucurbits are among the largest and most diverse plant families, cultivated worldwide in diverse environmental conditions, and have paid consideration globally because the fruits, seeds, and vegetables are traditionally, economically, and medicinally consumed in various ways. *C. colocynthis* (L.) Schrad. species belongs to genus *Citrullus*, family Cucurbitaceae. It is also known as "bitter melon", "bitter gourd", or "tumba" related to watermelon and is a creeping herb, small scarbid, non-hardy drought resistant, and perennial with prostate or climbing stem, bearing smooth spherical green and yellow-ripe fruits (Shah and Qadry 1985). The pharmacological properties of *C. colocynthis* are attributed to fruits. The white flesh of the fruit is

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associated with bitterness caused by Cucurbitacin E, glycoside, or colocynthine (Novot et al. 1990). It is more prominently used in anti-tumorous and antidiabetic drugs as it contains a mixture of Cucurbitacins (A, B, C, D, E, I, J, K, and L). Beside this, several important bioactive primary and secondary metabolites of this plant have been reported which attributed medicinal importance to *C. colocynthis* (Sturm et al. 2009). It is a drought-resistant desert plant with greater survival rate under severe stress and drought conditions (Dane et al. 2006). The plant is native to dry areas of North Africa, being common throughout the Sahara, areas of Morocco, Egypt and Sudan, eastward through Iran to India, Pakistan, and Afghanistan (Bhandari 1995).

C. colocynthis is the vegetation of desert and sand dunes and distributed mainly in desert areas of Rajasthan viz. Jaisalmer, Barmer, Bikaner, and Jodhpur, but it can also be seen excessively at Jaipur, Jhunjhunu, Churu, Ganganagar, Alwar, Hanumaangarh, and Udaipur in Rajasthan. The sand dunes vegetation is totally a different plant community with remarkable ability to tolerate a hostile environment of drought, nutrient deficiency, high winds, high temperatures, salts sprays, and sand blast.

C. colocynthis show high levels of variations within the species. The variations are more prominent in fruit color, fruit stripe pattern, seed coat color, and seed size. The fundamental source of biodiversity is genetic diversity—the total number of genetic characters contributing to variation within species. Genetic diversity described by the presence of various alleles in the gene pool and different genotypes within populations. Genetic variability on the other hand describes the tendency of the genetic traits within populations to vary (Laikre et al. 2009). Molecular phylogenetic and genetic diversity analysis can explain the taxonomic identity and evolutionary relationships amongst the wild species. Variability and genetic diversity are important factors in evolution and determine the response of a given organism to environmental stress, natural selection, and susceptibility to different diseases (Jain 2002). Finding and analysis of genetic diversity can help in understanding the molecular basis of various biological phenomena. The availability of different molecular markers and increased computing power has helped in the development of sophisticated techniques that have helped in understanding the various biological phenomena (Csillery et al. 2010).

Advances in the field of molecular biology provided many tools for studying genetic diversity at the genome level to investigate phylogenetic relationships among inter or intra-species. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the significant achievements in the field of molecular genetics. Molecular markers are reliable indicators of genetic diversity, because they are neutral to environmental influence and reveal differences at the whole genome level. Amid the several markers, no molecular markers are available that complete all the requirements needed. Different molecular markers used in genetic fingerprinting viz. restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), sequence characterized amplified regions (SCARs), sequence-based amplified polymorphism (SBAPs), and sequence tag sites (STSs) (Sharma et al. 2012), have their own advantages and disadvantages.

RAPD and ISSR are PCR-based markers, require only small amounts of DNA sample without involving radioactive labels, and are simpler as well as faster. RAPD has proven to be quite efficient in detecting genetic variations and used for diversity assessment as well as identifying germplasm in several plant species, bacteria, and microorganism (Kapteyn et al. 2002; Moghaieb et al. 2017). RAPDs are very quick and easy to develop due to the arbitrary sequence of the primers. It resolves most of the technical obstacle owing to its cost-effective and easy to perform (Williams et al. 1990). Therefore, RAPDs have been extensively used in assessing genetic relationship among various accessions of different plant species. ISSR has been shown to provide a powerful, rapid, simple, reproducible, and inexpensive means to assess genetic diversity and identify differences between closely related cultivars in many plant species, where the sequence information is limited; ISSR markers are easy to handle quick and more reproducible than other markers (Gonzalez et al. 2000). The nucleotide repeats (inter-simple sequence repeats) are distributed throughout the genome and has potential discriminatory power which has been seen in the clonal plant species for the assessment of genetic diversity. Earlier, these marker systems have been used in investigating genetic relatedness, for resolving intra- and intergenomic relationships, genetic diversity of plant populations, and cultivars (Verma et al. 2013; Khurana-Kaul et al. 2012; Velasco-Ramirez et al. 2014). Several other studies have been successfully effective in population genetics and in detecting genetic diversity in many species using RAPD or ISSR markers or both.

The natural habitat of a plant species determines its genetic diversity which is important for long-term survival and evolution under abiotic and biotic stresses. Diversity is influenced by various geographical factors which leads to development of subspecies, races, or ecotypes (Sreekumar and Renuka 2006). Genetic variation is required for the short- and long-term survival of plant species in the wild conditions. Identification of genetic diversity within and among widespread, restricted, and endangered species is necessary, prior to their conservation (Gitzendanner and

Soltis 2000) in situ as well as in vitro. The decline in genetic diversity has resulted from the loss of alleles which may be due to the reduction in population size as genetic drift can induce in genetic variation and thus affects the survival of natural population (Reed and Frankham 2003).

The present study is the first attempt on genetic variation among several accessions of *C. colocynthis* collected from different locations of Thar desert and other parts of Rajasthan using random-amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers. The study was aimed to characterize the morphological and molecular diversity employing both markers.

Materials and methods

Plant material and DNA extraction

To characterize the genetic diversity, a germplasm exploration survey was undertaken to identify *C. colocynthis* from the different locations of the University of Rajasthan, Jaipur, Agricultural Research Centre Durgapura, Jaipur, Ramgarh Dam, Jaipur, Amber, Alwar, Sikar, Jhunjhunu, Churu, and mainly dry areas of Rajasthan (Jaisalmer, baadmer, Bikaner, and Jodhpur; Table 1). The selection was made on the basis of phenotypic assessment of various characters viz. growth habitats, fruit color, fruit stripe pattern, and seed color.

A representative set of 38 accessions of *C. colocynthis* were collected from different regions of Rajasthan in the year 2009–2013 for assessing genetic diversity among them. Total genomic DNA was extracted from young leaves following the standard CTAB method as described by Doyle and Doyle (1990) with minor modifications.

C. colocynthis leaves (5 g) were ground in liquid nitrogen, then homogenized in 25 ml of extraction buffer (2% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris–HCl *p*H 8.0, and 1% β-mercaptoethanol) add 4 μl of RNAse incubated at 65 °C for 1 h. The supernatant was extracted twice with chloroform: isoamylalcohol (24:1 v/v). RNase A was again added and incubated at room temperature for 30 min. The DNA was pelleted with chilled isopropanol and washed twice with 70% ethanol. The washed pellet DNA was air-dried, dissolved in 500 μL TE buffer, and stored at −20 °C. DNA concentration was estimated by agarose (Himedia, India) gel electrophoresis using a known amount of λ DNA as standard and nanodrop (ND1000, Thermo Scientific, US) spectrophotometer.

RAPD amplification

A total of 65 random primers (Operon Technologies Inc., USA) were initially screened to assess genetic diversity

among 38 accessions of *C. colocynthis*. The composition of the PCR reaction mixture in 20 μL of RAPD reaction volume contains 2.5 μl DNA (25 ng), 2 μl Taq Buffer A (1×), 0.5 μl dNTP mix (100 μM each), 2 μl Primer (0.4 μM), 0.25 μl *Taq* Polymerase (0.25 U μl^{-1}), and Milli-Q water to complete the reaction. PCR reactions were performed at an initial denaturation at 94 °C for 5 min followed by 40 cycles at 94 °C for 1 min denaturation, annealing at 37 °C for 45 s. and 2 min extension at 72 °C with a final extension of 10 min at 72 °C using a DNA Engine (Bio-Rad, Germany). Amplified products were separated on 1.2% agarose (Himedia, India) gel in 1× TAE buffer by electrophoresis at 100 V, visualized with EtBr staining and photographed using Gel Documentation System (Bio-Rad, USA). The size of the amplification products was determined by comparison using 100 bp and 1 kb ladder (Bangalore Genei, India).

ISSR amplification

In the case of ISSR primers, the optimal annealing temperature was found to vary according to base composition of the primers. 20 ISSR primers (University of British Columbia, primer set no. 9, Vancouver, Canada) were initially screened to assess genetic diversity in 38 accessions of *C. colocynthis* out of which 13 primers gave amplification. PCR reaction was carried out in 20 μl reaction volume containing 25 ng genomic DNA (2.5 μl) as template, 2.5 μl MgCl$_2$, 2 μl Taq buffer B 0.5 μl of 100 μM dNTP, 2.5 μl of ISSR primers (4 μM), and 0.35 μl of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplifications were performed with the initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 92 °C for 1 min, 1 min at the annealing temperature (depending on the primer Tm), and 2 min extension at 72 °C with a final extension at 72 °C for 7 min using DNA Engine (Bio-Rad, Germany). The PCR products were separated on 1.5% agarose gel (Himedia, India) using 100 bp and 1 kb markers (Bangalore Genei, India) as the band size standard and photographed in a gel documentation system (Bio-Rad, Germany).

Data analysis

The DNA fingerprint patterns obtained were converted into binary data matrices containing arrays of 0 and 1 s. The RAPD and ISSR bands were scored visually for the presence (1) or absence (0) of bands of various molecular weight sizes. Only polymorphic and reproducible bands were considered for the analysis. Data were analyzed using SIMQUAL route to generate Jaccard's similarity coefficient using NTSYS-pc version 2.02e22 (Numerical Taxonomy System) (Rohlf 1998). Similarity matrices were

Table 1 Details of *Citrullus colocynthis* germplasm collected from different locations in Rajasthan, India

S. no.	Accession code	Place	Location	Latitude and longitude	Collection site
1	A_1	Rajgarh	Alwar	27°14′09N 76°37′20E	Wild
2	A_2	Bahrod	Alwar	27°53′12N 76°16′51E	Wild
3	J_1	University of Rajasthan	Jaipur	26°53′06N 75°49′11E	Wild
4	J_2	Durgapura agriculture Inst.	Jaipur	26°50′22N 75°47′19E	Farm Land
5	J_3	Sekh Ki Dhani, Ramgarh	Jaipur	26°37′59N 76°16′00E	Farm land
6	J_4	Ragarh Dam	Jaipur	27°02′51N 76°03′22E	Farm land
7	J_5	Amber	Jaipur	26°58′59N 75°52′00E	Wild
8	S_1	Neem Ka thana	Sikar	27°44′14N 75°47′11E	Farm land
9	S_2	Rengus	Sikar	27°21′35N 75°33′59E	Farm land
10	S_3	Shri Madhopur	Sikar	27°28′00N 75°35′59E	Wild
11	Jh_1	Chidawa	Jhunjhunu	28°14′24N 75°38′44E	Farm Land
12	Jh_2	Bisaau	Jhunjhunu	28°14′41N 75°04′32E	Farm land
13	Jh_3	Surajgarh	Jhunjhunu	28°18′33N 75°43′57E	Wild
14	C_1	Ratangarh	Churu	28°04′47N 74°37′06E	Wild
15	C_2	Sujaangarh	Churu	27°41′59N 74°28′00E	Farm land
16	C_3	Churu outer area	Churu	28°18′02N 74°58′39E	Wild
17	H_1	Hanumangarh city	Hanumangarh	29°37′03N 74°17′20E	Wild
18	H_2	Bhadra	Hanumangarh	26°06′06N 75°10′16E	Wild
19	G_1	Anupgarh	Ganganagar	29°11′20N 73°12′34E	Farm land
20	G_2	Jaitsar	Ganganagar	29°19′27N 73°40′21E	Farm land
21	G_3	Ganganagar Canal Area	Ganganagar	30°07′31N 74°06′03E	Wild
22	B_1	Rasisar	Bikaner	27°44′56N 73°22′25E	Wild
23	B_2	Kolayat	Bikaner	27°50′08N 72°57′09E	Wild
24	B_3	Nokha	Bikaner	27°33′52N 73°28′33E	Farm land
25	B_4	Deshnokh	Bikaner	27°47′34N 73°20′16E	Farm land
26	Js_1	Sum	Jaisalmer	26°54′56N 70°54′30E	Wild
27	Js_2	Tanot	Jaisalmer	27°47′47N 70°21′14E	Wild
28	Js_3	Pokhran	Jaisalmer	27°05′41N 71°45′10E	Wild
29	Js_4	Jaisalmer canal Area	Jaisalmer	26°54′56N 70°54′30E	Farm land
30	B_1	Hodu	Baadmer	25°26′37N 71°45′28E	Farm land
31	B_2	Baadmer City	Baadmer	25°45′00N 71°22′59E	Wild
32	B_3	Balotra	Baadmer	25°49′51N 72°14′24E	Wild
33	Jd_1	Falodi	Jodhpur	27°07′57N 72°21′44E	Wild
34	Jd_2	kalda	Jodhpur	26°14′20N 73°01′27E	Wild
35	Jd_3	Jodhpur outer area	Jodhpur	26°15′17N 73°00′13E	Wild
36	U_1	Salumbar	Udaipur	24°08′05N 74°02′40E	Wild
37	U_2	Lasadia	Udaipur	24°34′16N 73°41′29E	Farm land
38	U_3	Kherwara	Udaipur	23°59′07N 73°35′46E	Wild

utilized to construct dendrograms independently for both the marker systems and on pooled marker data using UPGMA (Unweighted Pair Group Method with Arithmetic Average) algorithm and SAHN clustering (Sneath and Sokal 1973). Finally, a principal coordinate analysis was performed to highlight the resolving power of the ordination. A two- (2D) and three-dimensional (3D) principal component analyses were constructed to provide another means of testing the relationships among accessions using EIGEN program (NTSYS-PC). The robustness of each phenogram was evaluated by a bootstrap analysis of each data set using the computer program WINBOOT (Yap and Nelson 1996). There are two important measures of the quality or informativeness of a polymorphism as a genetic marker: heterozygosity (*H*) and polymorphic information content (PIC). To simplify the work of molecular studies, we used a useful online tool (http://www.genomics.liv.ac.uk/animal/pic.html) to facilitate the calculation of H and PIC values. This program, PIC Calculator Extra, can calculate these values from manually uploaded allelic

frequencies or from a given file containing binary data. Primer banding characteristics such as number of scored bands (NSB), number of polymorphic band (NPB), and percentage of polymorphic bands (PPB) were obtained. Polymorphism information content (PICi) and heterozygosity of a band were calculated according to Liu (1998)

$$PIC = 1 - \sum_{i=l}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2P_i^2 P_j^2$$

$$H = 1 - \sum_{i=l}^{l} P_i^2.$$

Informativeness of a band (BIi) was calculated as

$$BI_i = 1 - (2 \times |0.5 - p|)$$

where p is the proportion of the total genotypes containing a particular band. It is useful in calculating the resolving power (RP), which in turn enabled us to know the ability of a primer to distinguish various genotypes. Then, the resolving power (RP) of each primer was calculated as (Prevost and Wilkinson 1999)

$$RP = \sum_{i=1}^{n} BI_i.$$

Multiplex ratio was calculated as $MR = L/T$, where T = total number of primer combinations. The Marker Index (MI) was obtained by multiplying the average heterozygosity by Multiplex Ratio: $MI = Hav \times MR$ (Anderson et al. 1993).

Results

The characterization of different accessions selected on the basis of mainly fruit stripe pattern is presented in Fig. 1. The rind of *C. colocynthis* fruit can be striped or solid colored. The stripe patterns of *C. colocynthis* can be characterized using different stripe widths (narrow, medium, and wide), stripe colors, and background colors (dark green, medium green, and light green); besides stripe width, the stripe pattern can also have different characteristics. The stripe patterns are two sets of alternating light- and dark-colored stripes on the rind. The stripe pattern of fruit is an important factor to characterize genetic diversity. It might be possible that this pattern is controlled by some set of gene. Seed traits are important for characterizing diversity in *C. colocynthis*. Seed characters, such as seed size and seed coat color, may to a certain extent affect the diversity profile of *C. colocynthis*. In terms of seed coat color 'grey, dark grey, brown, light brown, and creamish seeds' often observed in the field but the observation regarding seed coat color, since light-colored or white seed

coat colors are seen in immature fruit. Seed coat color is also difficult to classify due to the shades of different colors. The characterization of seed coat color pattern in different accessions is presented in Fig. 2.

RAPD analysis

In the case of RAPD analysis, 65 RAPD primers were used for the initial screening of *C. colocynthis* genotypes, of which 50 primers revealed polymorphic banding patterns. The 50-decamer primers amplified DNA fragments across the 38 genotypes, with the number of amplified fragments varying from 5 (OPA-08 and OPF-9) to 19 (OPT-20) in the molecular size range of 150–6000 bp. A total of 549 bands were produced that could be scored, out of which 318 bands were polymorphic with an average of 6.36 polymorphic bands per primer. Percent polymorphism ranged from 22.2% (OPA-09) to 83.3% (OPE-12) with an average of 55.14% polymorphism (Table 2). The extent of polymorphism observed among the *C. colocynthis* genotypes as revealed by various RAPD primers is shown in Fig. 3a, b.

Similarity matrix values using Jaccard's coefficient ranged from 0.64 between U_1 and B_3 to 0.96 between Jh_1 and Jh_2 (Table 6). At 72% similarity, the accessions separated into three clusters (Fig. 5a). Cluster I comprised of most of the genotypes and is divided into two sub-clusters (Ia and Ib). Sub-cluster Ia comprised of the accessions A_1, A_2, Jh_1, Jh_2, Jh_3, C_1, C_2, C_3, Jd_1, Jd_2, and Jd_3. Sub-cluster Ib comprised of 13 accessions J_1, J_2, J_3, J_4, J_5, S_1, S_2, S_3, H_1, H_2, H_3, G_1, G_2, and G_3. Cluster II divided into two sub-clusters (IIa and IIb). Sub-cluster IIa comprised of the accessions U_1 and U_2 and sub-cluster IIb contains only one accession U_3. Cluster III divided into two sub-clusters (IIIa and IIIb). Sub-cluster IIIa comprised of four accessions B1, B2, B3, and B4 and sub-cluster IIIb comprised of the accessions Js_1, Js_2, Js_3, Js_4, Bd_1, Bd_2, and Bd_3. The result of principal coordinate analysis was comparable to the cluster analysis with minor deviations (Fig. 6a). Approx. 50% RAPD markers showed PIC value and heterozygosity ≥ 0.50 suggesting the informativeness of a marker. The highest PIC value (0.86) and heterozygosity (0.88) revealed by OPT 6, OPT 12, and lowest PIC value (0.11) and heterozygosity (0.22) shown by OPF 9 and OPT 7. The estimates of marker attributes for RAPD primers varied widely. The multiplex ratio (MR) was the highest for OPT-20 (19) and lowest for OPF-9 (5) and OPA-8 (5). Similarly, marker index (MI) value was highest for OPF-13 (13.94) and lowest for OPF-9 (1.10) and OPF-8 (1.10). The average value of MR and MI was 10.98 and 5.32 for RAPD markers. The values for average informativeness of a band (BIi) were observed maximal for OPA-9 (0.92) and minimal for OPT-4 (0.19). The resolving power (RP) is a

Fig. 1 a–t Different stripe patterns in fruit of *Citrullus colocynthis* (L.) Schard. collected from different locations in Rajasthan, India

parameter that indicates the discriminatory potential of the selected primers. The highest RP value was observed with the primer OPA-5 (5.11) and the lowest with the primer OPT-8 (0.63). The average informativeness of a band (BI_i) and resolving power (RP) was 0.45 and 2.98 for RAPD markers (Table 2).

ISSR analysis

Among the 20 ISSR primers screened, 13 primers amplifying polymorphic banding patterns were selected for further study (Table 3). ISSR primers produced different numbers of DNA fragments, depending upon their simple

Fig. 2 a–i Different seed coat color patterns in seeds of *Citrullus colocynthis* (L.) Schard. collected from different locations in Rajasthan, India

sequence repeat motifs. Thirteen primers produced 166 amplification products, and out of which 99 were polymorphic, with an average of 7.6 polymorphic bands per primer. The number of bands amplified per primer varied between 9 (UBC-807 and 802) and 16 (UBC-803 and 812) with average band size between 250 and 4000 bp. Percent polymorphism ranged from 45.4% (UBC-815) to 73.3% (UBC-814) with an average of 65.05% polymorphism (Table 3). The extent of polymorphism observed among the *C. colocynthis* genotypes as revealed by various ISSR primers is shown in Fig. 4a, b. Jaccard's similarity coefficient ranged from 0.58 between A_1 and Js_2 to 0.96 between B_2 and B_3 (Table 7). A dendrogram based on UPGMA analysis with ISSR data is shown in Fig. 5b. Dendrogram analysis separated the accessions into four clusters at 72% similarity. Cluster I comprised of five OTUs and is divided into two sub-clusters (Ia and Ib). Sub-cluster Ia comprised of A_1 and A_2. Sub-cluster Ib comprised of Jd_1, Jd_2, and Jd_3. Within sub-cluster 1a, A_1 and A_2 appeared to be closer to each other with similarity coefficient equal to 0.90 and accessions Jd_1, Jd_2, and Jd_3 appeared to be closer to each other with similarity coefficient equal to 0.93 0.96, and 0.69 in sub-cluster Ib, respectively. Accessions Jd_1, Jd_2, and Jd_3 had distinct OTU as in case of RAPD analysis. Cluster II divided into two sub-clusters (IIa and IIb). Sub-cluster IIa comprised of the accessions Jh_1–Jh_3 and C_1–C_3 and sub-cluster IIb contains accessions G_1–G_3, H_1, and H_2. Cluster III divided into two sub-clusters (IIIa and IIIb). Sub-cluster IIIa comprised of eight accessions J_1–J_5 and S_1–S_3, sub-cluster IIIb comprised of the accessions U_1, U_2, and U_3, and last IV cluster divided into two sub-clusters (IVa and IVb) which comprised of the accessions B_1–B_4,

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Table 2 List of RAPD primers and amplification details used for the study of genetic diversity of 38 *Citrullus colocynthis* accessions

S. no.	Primmer	Sequence (5′–3′)	NSB = L	NPB	NMB	PP	PIC	H	BIi	Rp	T	MR = (L/T)	MI = H × MR	Size range (bp)
1	OPA-1	CAGGCCCTTC	10	6	4	60.00	0.46	0.48	0.44	2.63	1	10	4.8	450–2000
2	OPA-2	TGCCGAGCTG	11	6	5	54.54	0.39	0.40	0.54	3.21	1	11	4.4	150–1000
3	OPA-3	AGTCAGCCAC	7	3	4	42.85	0.46	0.48	0.49	1.47	1	7	3.36	600–1500
4	OPA-4	AATCGGGCTG	11	6	5	54.54	0.46	0.47	0.68	4.11	1	11	5.17	200–1500
5	OPA-5	AGGGGTCTTG	16	12	4	75.00	0.64	0.65	0.43	5.11	1	16	10.4	250–2800
6	OPA-7	GAAACGGGTG	8	5	3	62.50	0.32	0.33	0.83	4.16	1	8	2.64	200–3500
7	OPA-8	GTGACGTAGG	5	4	1	80.00	0.11	0.22	0.57	2.26	1	5	1.10	150–1500
8	OPA-9	GGGTAACGCC	9	2	7	22.22	0.87	0.89	0.92	1.84	1	9	8.01	150–2000
9	OPA-10	GTGATCGCAG	15	11	4	73.33	0.34	0.35	0.45	4.95	1	15	5.25	350–3500
10	OPA-11	CAATCGCCGT	13	7	6	53.84	0.55	0.56	0.69	4.84	1	13	7.28	450–2200
11	OPA-14	TCTGTGCTGG	7	0	7	0.00	0.83	0.85	0.00	0.00	1	7	5.95	200–1700
12	OPA-15	TTCCGAACCC	10	4	6	40.00	0.46	0.47	0.55	2.21	1	10	4.70	500–2000
13	OPA-16	AGCCAGCGAA	9	5	4	55.55	0.23	0.24	0.60	3.00	1	9	2.16	300–1200
14	OPA-18	AGGTGACCGT	14	10	4	71.42	0.44	0.45	0.40	4.00	1	14	6.30	600–5000
15	OPA-19	CAAACGTCGG	14	9	5	64.28	0.55	0.56	0.27	2.47	1	14	7.84	230–1800
16	OPB-04	GGACTGGAGT	18	13	5	72.22	0.52	0.53	0.33	4.26	1	18	9.54	320–3500
17	OPB-08	GTCCACACGG	6	0	6	0.00	0.81	0.83	0.00	0.00	1	6	4.98	300–2500
18	OPE-12	TTATCGCCCC	12	10	2	83.33	0.46	0.47	0.38	3.84	1	12	5.64	450–2200
19	OPF-1	ACGGATCCTG	14	10	4	71.42	0.12	0.15	0.50	5.00	1	14	2.10	320–3200
20	OPF-4	GGTGATCAGG	11	7	4	63.63	0.16	0.18	0.52	3.63	1	11	1.98	200–3000
21	OPF-5	CCGAATTCCC	8	3	5	37.50	0.51	0.61	0.23	0.68	1	8	4.88	600–2000
22	OPF-6	GGGAATTCGG	6	4	2	66.66	0.42	0.49	0.50	5.00	1	6	2.94	200–1500
23	OPF-7	CCGATATCCC	15	10	5	66.66	0.36	0.37	0.33	3.26	1	15	5.55	250–4000
24	OPF-9	CCAAGCTTCC	5	3	2	60.00	0.11	0.22	0.58	1.74	1	5	1.10	200–1000
25	OPF- 10	GGAAGCTTGG	16	11	5	68.75	0.18	0.18	0.44	4.79	1	16	2.88	220–4200
26	OPF-13	GGCTGCAGAA	17	9	8	52.94	0.81	0.82	0.46	4.16	1	17	13.94	250–2000
27	OPF-14	TGCTGCAGGT	11	4	7	36.36	0.30	0.31	0.45	1.79	1	11	3.41	200–1700
28	OPF-17	AACCCGGGAA	11	5	6	45.45	0.68	0.69	0.53	2.63	1	11	7.59	450–2000
29	OPF-19	CCTCTAGACC	13	10	3	76.92	0.23	0.24	0.43	4.32	1	13	3.12	300–2250
30	OPF-20	GGTCTAGAGG	9	7	2	77.77	0.23	0.24	0.56	3.95	1	9	2.16	300–3000
31	OPJ-11	ACTCCTGCGA	13	5	8	38.46	0.59	0.60	0.85	4.26	1	13	7.8	350–2200
32	OPJ-12	GTCCCGTGGT	12	8	4	66.66	0.18	0.18	0.59	4.74	1	12	2.16	200–1500
33	OPK-19	CACAGGCGGA	14	5	9	35.71	0.29	0.30	0.38	1.89	1	14	4.2	200–1700
34	OPP-12	AAGGGCGAGT	12	7	5	58.33	0.23	0.24	0.46	3.21	1	12	2.88	400–3000
35	OPR-02	CACAGCTGCC	6	0	6	0.00	0.81	0.83	0.00	0.00	1	6	4.98	500–1200
36	OPS-08	TTCAGGGTGG	8	6	2	75.00	0.55	0.56	0.72	4.42	1	8	4.88	50–3500
37	OPS-13	GTCGTTCCTG	9	6	3	66.66	0.64	0.65	0.35	2.11	1	9	5.85	200–2100
38	OPT-1	GGGCCACTCA	11	7	4	63.63	0.71	0.72	0.62	4.32	1	11	7.92	300–2500
39	OPT-4	CACAGAGGGA	14	11	3	78.57	0.25	0.29	0.19	2.11	1	14	4.06	250–3500
140	OPT-6	CAAGGGCAGA	10	6	4	60.00	0.86	0.87	0.61	3.68	1	10	8.70	450–3000
411	OPT-7	GGCAGGCTGT	6	4	2	66.66	0.11	0.22	0.41	1.63	1	6	1.32	300–1800
412	OPT-8	AACGGCGACA	9	3	6	33.33	0.51	0.61	0.21	0.63	1	9	5.49	100–2000
413	OPT-9	CACCCCTGAG	11	6	5	54.54	0.39	0.40	0.39	2.37	1	11	4.40	350–3500
414	OPT-11	TTCCCCGCGA	8	2	6	25.00	0.59	0.61	0.56	1.16	1	8	4.88	450–2000
415	OPT-12	GGGTGTGTAG	9	3	6	33.33	0.87	0.88	0.58	1.74	1	9	7.92	150–2000
416	OPT-14	AATGCCGCAG	17	14	3	82.35	0.62	0.62	0.30	4.26	1	17	10.54	450–2700
417	OPT-15	GGATGCCACT	7	4	3	57.14	0.54	0.55	0.28	1.11	1	7	3.85	650–1800
48	OPT-16	GGTGAACGCT	12	7	5	58.33	0.71	0.72	0.53	3.63	1	12	8.64	400–2500

Table 2 continued

S. no.	Primmer	Sequence (5′–3′)	NSB = L	NPB	NMB	PP	PIC	H	BI$_i$	Rp	T	MR = (L/T)	MI = H × MR	Size range (bp)
49	OPT-19	GTCCGTATGG	11	5	6	45.45	0.71	0.72	0.44	2.21	1	11	7.92	100–1200
50	OPT-20	GACCAATGCC	19	13	6	68.42	0.38	0.38	0.32	4.21	1	19	7.22	350–6000
–		Total	549	318	231	2757.25	23.55	24.68	22.8	149	50	549	266.38	–
–		Average	10.98	6.36	4.62	55.14	0.47	0.49	0.45	2.98	1	10.98	5.32	–

NSB number of scorable bands, *NPB* number of polymorphic bands, *NMB* number of monomorphic bands, *PP* % polymorphism, *PIC* polymorphic information content, *H* heterozygosity, BI$_i$ average informativeness of a band, *RP* resolving power, *T* total no. of assays/primers combination, *MR* multiplex ratio, *MI* marker index
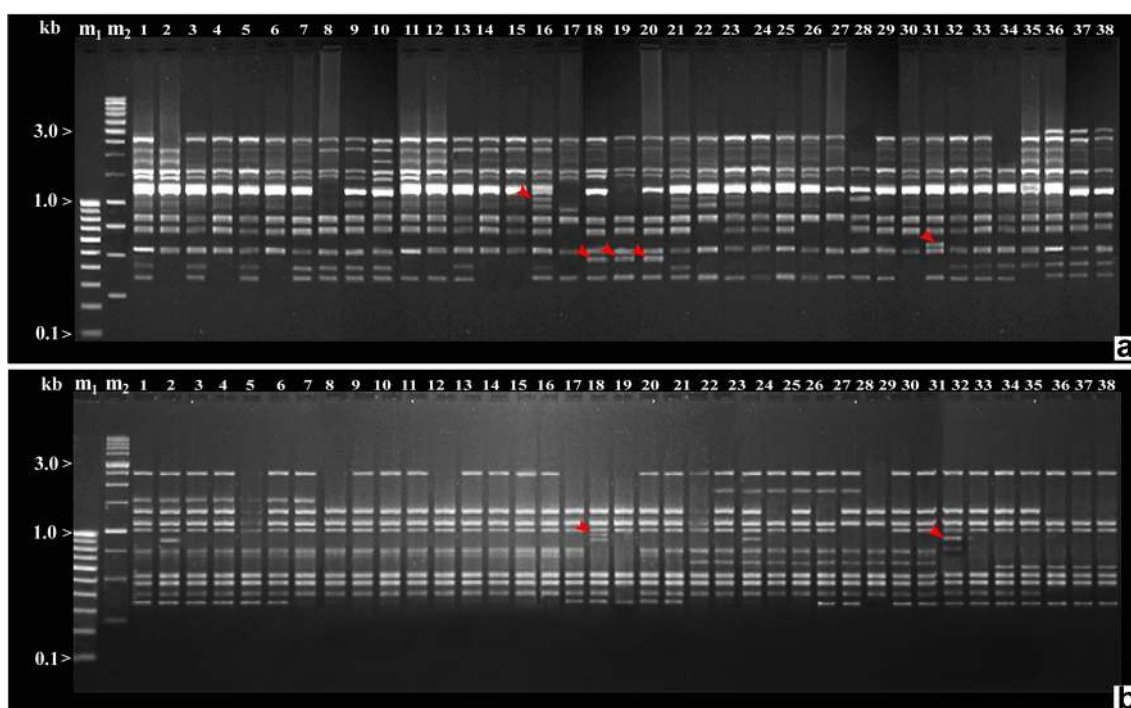

Fig. 3 a–b RAPD profile of *Citrullus colocynthis* genotypes produced with different primers (**a** OPA-05, **b** OPA-10) m1-100 bp ladder (*Lane* 1), m2-1 kb ladder (*Lane* 2), 1–38 *Citrullus colocynthis* accessions

Js$_1$–Js$_4$, and Bd$_1$–Bd$_3$. The result of PCA was comparable to cluster analysis with minor deviations (Fig. 6b). Approx. 50% ISSR markers showed PIC value and heterozygosity ≥0.50 suggesting the informativeness of a marker. The highest PIC value (0.91) and heterozygosity (0.92) revealed by UBC 808 and lowest PIC value (0.18) and heterozygosity (0.20) shown by UBC 814. The estimates of marker attributes for ISSR primers varied widely. The multiplex ratio (MR) was the highest for UBC-803 (16) and lowest for UBC 803 and 807 (5). Similarly, marker index (MI) value was highest for UBC-808 (12.88) and lowest for UBC-809 (2.16). The average value of MR and MI was 12.76 and 5.86 for ISSR markers. The values for average informativeness of a band (BIi) were observed maximal for

UBC-803 (0.20) and minimal for UBC-803 (0.82). The highest RP value was observed with the primer UBC-803 (8.18) and the lowest with UBC-811 (1.56). The average informativeness of a band (BI$_i$) and resolving power (RP) was 0.46 and 4.10 for RAPD markers (Table 3).

Molecular markers specific to 17 accessions were identified (Table 4). These markers could be converted to SCAR primers for enhancing the reliability of the markers.

Combined RAPD and ISSR analyses

The combined RAPD and ISSR data showed that total 63 primers were used which produce 417 polymorphic bands

Table 3 List of ISSR primers and amplification details used for the study of genetic diversity of 38 *Citrullus colocynthis* accessions

S. no.	Primmer	Sequence (5′–3′)	Tm (°C)	NSB = L	NPB	NMB	PP	PIC	H	BI_i	Rp	T	MR = (L/T)	MI = H × MR	Size range (bp)
1	UBC 802	(AT)_8G	30	9	0	9	100	0.87	0.88	0.00	0.00	1	9	7.92	250–2500
2	UBC 803	(AT)_8C	30	16	10	6	62.5	0.50	0.59	0.82	8.18	1	16	9.44	300–2500
3	UBC 805	(TA)_8C	30	14	10	4	71.42	0.18	0.18	0.65	6.48	1	14	2.52	250–2000
4	UBC 807	(AG)_8T	51	9	5	4	55.5	0.23	0.24	0.41	2.86	1	9	2.16	600–4000
5	UBC 808	(AG)_8C	51.4	14	10	4	71.4	0.91	0.92	0.64	6.42	1	14	12.88	150–2500
6	UBC 809	(AG)_8G	51	13	9	4	69.2	0.55	0.56	0.32	2.84	1	13	7.28	250–1500
7	UBC 810	(GA)_8T	49	10	5	5	50	0.29	0.30	0.44	4.88	1	10	3.0	400–2000
8	UBC 811	(GA)_8C	50	13	7	6	53.8	0.39	0.40	0.20	1.56	1	13	5.2	450–3500
9	UBC 812	(GA)_8A	48.5	16	10	6	62.5	0.45	0.46	0.47	4.70	1	16	7.36	450–4000
10	UBC 813	(CT)_8T	48	13	9	4	69.2	0.27	0.28	0.40	3.58	1	13	3.64	450–2500
11	UBC 814	(CT)_8A	49	15	11	4	73.3	0.18	0.20	0.58	5.78	1	15	3.0	100–1000
12	UBC 815	(CT)_8G	50	11	5	5	45.4	0.51	0.53	0.61	3.06	1	11	5.83	150–2000
13	UBC 819	(GT)_8A	53	13	8	5	61.5	0.51	0.52	0.50	3.02	1	13	6.76	700–4000
–		Total	–	166	99	66	845.72	5.84	6.06	6.04	53.36	13	166	76.99	–
–		Average	–	12.76	7.6	5.07	65.05	0.44	0.46	0.46	4.10	1	12.76	5.86	–

NSB number of scorable bands, *NPB* number of polymorphic bands, *NMB* number of monomorphic bands, *PP* % polymorphism, *PIC* polymorphic information content, *H* heterozygosity, *BI_i* average informativeness of a band, *RP* resolving power, *T* total no. of assays/primers combination, *MR* multiplex ratio, *MI* marker index

out of 715 total bands with 60.09% polymorphism (Table 5). The RAPD and ISSR data were also combined for UPGMA cluster analysis. The UPGMA dendrogram thus obtained from the cluster analysis of RAPD and ISSR data is shown in Fig. 5c. Dendrogram constructed on the basis of RAPD + ISSR polymorphism separated the accessions into four distinct clusters at 72% variation (Fig. 5c) with Jaccard's similarity coefficient ranging from
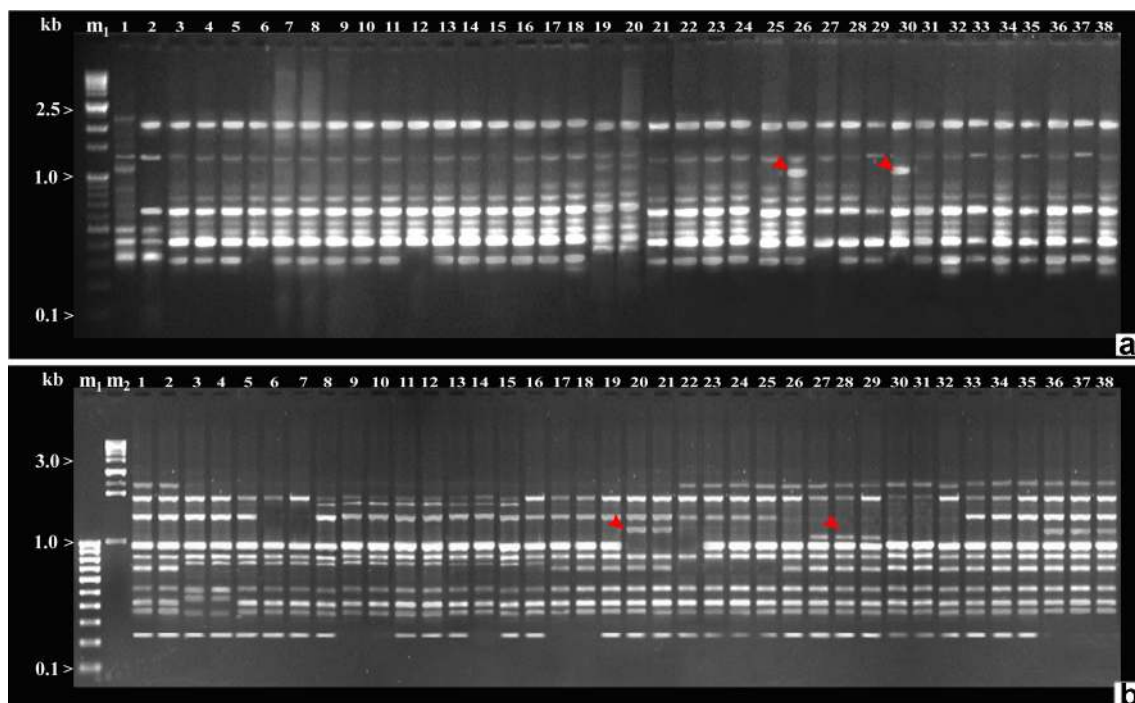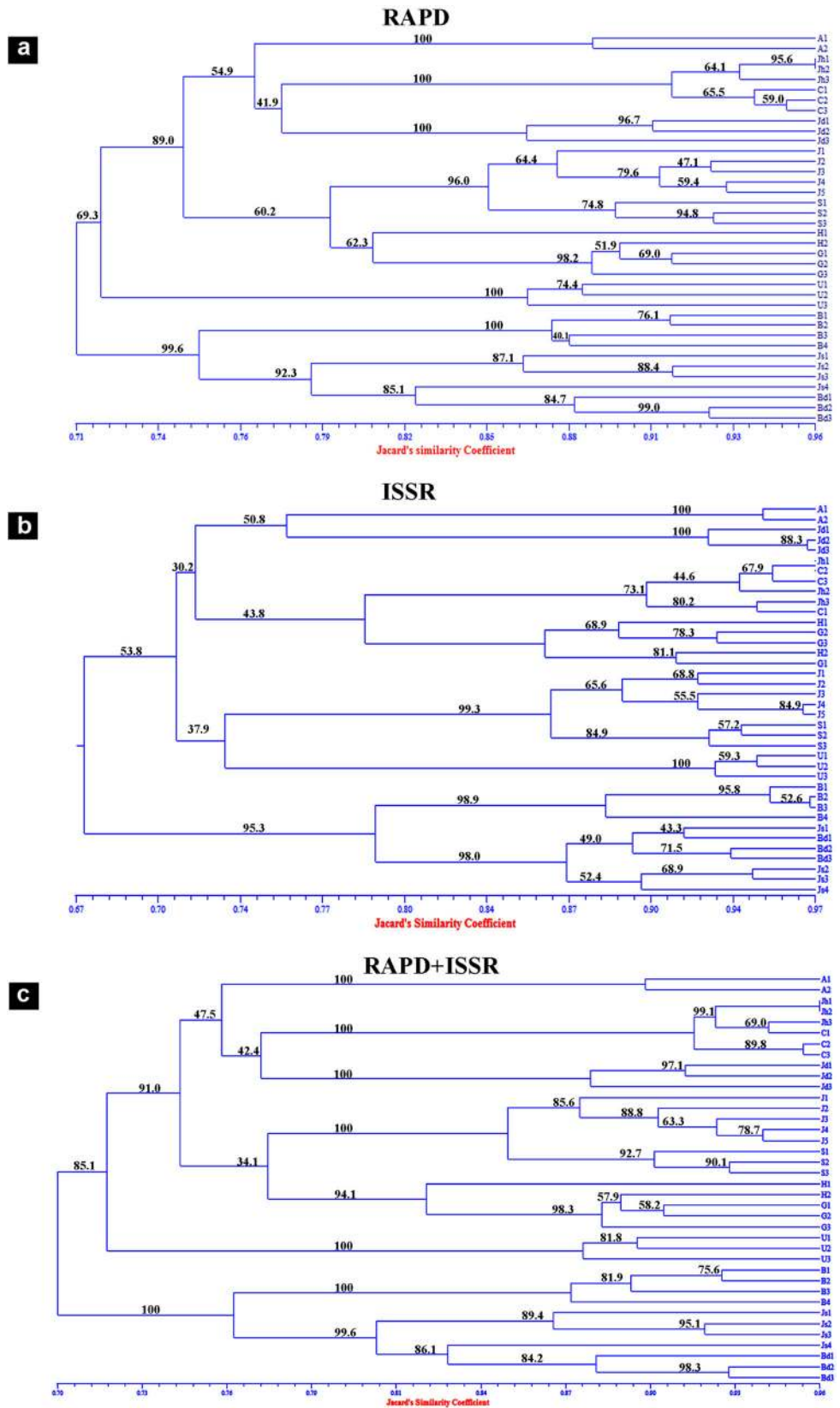

Fig. 4 a–b ISSR profile of *Citrullus colocynthis* genotypes produced with different primers (**a** UBC-803, **b** UBS-805) m1-100 bp ladder (*Lane* 1), m2-1 kb ladder (*Lane* 2), and 1–38 *Citrullus colocynthis* accessions
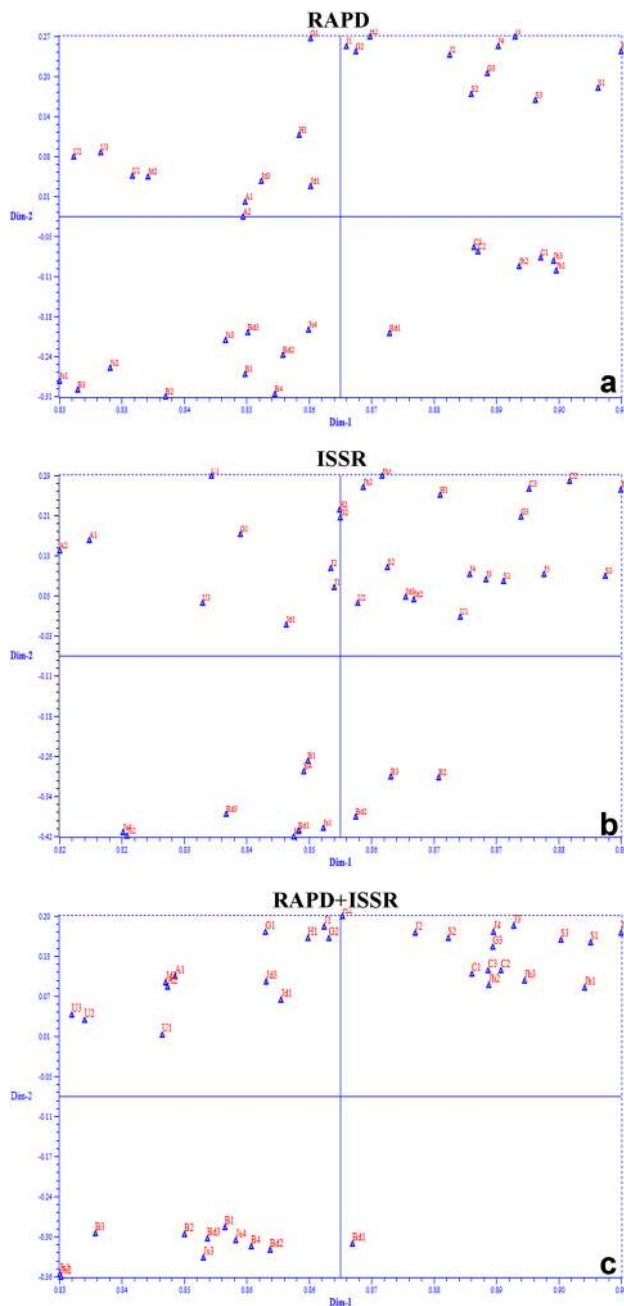
Fig. 6 Two-dimensional plot of 38 accessions of *Citrullus colocynthis* by principle coordinate analysis (PCA) using Jaccard's similarity coefficients

0.64 (Js_1 and H_1) to 0.95 (Jh_2 and Jh_2) (Table 8). A cluster consisting of 11 accessions and is divided into two sub-clusters (Ia and Ib). Sub-cluster Ia comprised of A_1 and A_2. Sub-cluster Ib comprised of Jh_1–Jh_3, C_1–C_3, and Jd_1–Jd_3. Cluster II was the largest cluster consisting 13 accessions divided into two sub-clusters IIa and IIb comprised of J_1–J_5, S_1–S_3, H_1–H_2, and G_1–G_3. Groups III and IV also divided into two sub-clusters (IIIa, IIIb and IVa, IVb) which consisting 14 accessions including U_1–U_3, B_1–B_4,

Js_1–Js_4, and Bd_1–Bd_3. The clustering pattern of the genotypes in the combined analysis remained akin to the ISSR dendrogram, while the RAPD-based dendrogram showed some variation in the clustering of genotypes.

The principal component analysis based on RAPD + ISSR polymorphism grouped the accessions into four major clusters and eight minor clusters (Fig. 6c). A few differences in clustering were observed with UPGMA clustering and principal component analysis. Principal component analysis showed three major groups consisting U_1–U_3, A_1–A_2, Jd_1–Jd_3, G_1–G_2, H_1–H_2, and J_1 in the first group, accessions J_2–J_5, S_1–S_3, C_1–C_3, Jh_1–Jh_3, and G_3 in the second group, and third group consisting accessions Bd_2–Bd_3, B_1–B_4, Js_1–Js_4, and U_1–U_3 in PCA. The matrices for two markers, RAPD and ISSR, were also compared using Mantel's test. The correlation value between the matrices is high ($r = 0.7947$) indicating a good correlation between the two molecular marker systems (Mantel, 1967).

The results of the relative efficiency of combined RAPD + ISSR markers for determining polymorphism in *C. colocynthis* showed PIC value and heterozygosity ≥0.50. The estimates of marker attributes for RAPD + ISSR showed slightly different results. The average value of MR and MI was 11.34 and 5.45 for combined RAPD + ISSR markers. The average informativeness of a band (BI_i) and resolving power (RP) was 0.45 and 3.21, respectively, for RAPD markers (Table 5).

Discussion

In the present study, the molecular diversity analysis was carried out using RAPD and ISSR markers to assess the phylogenetic relation at inter-specific level amongst 38 accessions of *C. colocynthis* from the different geographical zones of Rajasthan district, India. The 50 RAPD primers revealed polymorphic banding patterns amongst 38 accessions of *C. colocynthis* with 318 polymorphic bands out of 549 bands that covered the molecular size between 150 and 6000 bp. The amplified fragments ranged from five (OPA-08 and OPF-9) to nineteen (OPT-20) with an average of 6.36 polymorphic bands (PB) per primer (P). This average polymorphic band per primer is closely related with earlier studies as 6.83 polymorphic fragments per primer in *Ricinus communis* as (Gajera et al. 2010), 7.6 PB/P in *Pistacia khinjuk* (Karimi et al. 2012) and 7.6 PB/P in Iranian *Pistachios* (Aliakbarkhani et al. 2015). However, earlier observations also reflect less number of polymorphic band per primer in various plant species such as, 2.9 PB/P in bread wheat (Khaled et al. 2015), 3.58 PB/P in *Cucumis sativus* (manohar et al. 2013), 4.17 PB/P in *Citrullus lanatus* (Solmaz et al. 2010) and in barley 5.67 PB/P (Guasmi et al. 2012). Contrary to this,

Table 4 Accession-specific molecular markers for *Citrullus colocynthis*

S. no.	Genotype	Marker type	Primer identity	Amplicons size (bp)
1.	Js_1, Bd_1	ISSR	UBC-803, UBC-809	1100, 550
2.	G_2, Js_3	ISSR	UBC-805	1100, 1250
3.	C_3	ISSR	UBC-807	450
4.	Js_2	ISSR	UBC-809	550
5.	S_2	ISSR	UBC-813	1250
6.	Bd_3	ISSR	UBC-14	500
7.	H_1, Bd_2	RAPD	OPA-05	1000, 400
8.	H_2, Bd_3	RAPD	OPA-10	1000
9.	B_2	RAPD	OPT-04	1500
10.	G_2	RAPD	OPA-18, OPA-19, OPA-05	1750, 600, 550
11.	Js_4	RAPD	OPB-04	2200
12	Js_2, Js_3	RAPD	OPE-12	450
13	Jh_3	RAPD	OPF-1	600
14	J_1	RAPD	OPF-19	600
15	G_1	RAPD	OPF-19, OPT-14	600, 250
16	B_3, B_4	RAPD	OPT-04, OPR-02	2000, 800
17	H_2	RAPD	OPT-14	250

Table 5 Relative efficiency of molecular markers for determining polymorphism in *Citrullus colocynthis*

S. no.	Parameters for marker efficiency	Molecular marker system		
		RAPD	ISSR	RAPD + ISSR
1.	Number of individuals	38	38	38
2.	Total number of scorable bands (NSB = L)	549	166	715
3.	Total no. of assays/primers combination (T)	50	13	63
4.	Total number of Polymorphic bands (p)	318	99	417
5.	Multiplex ratio (MR) = (L/T)	10.98	12.76	11.34
6.	% Polymorphism	55.14	65.04	60.09
7.	Average polymorphism (bands primer^{-1})	6.36	7.60	6.98
8.	PIC average (PIC_{avg})	0.47	0.44	0.46
9.	Average heterozygosity (H_{avg})	0.49	0.46	0.48
10.	Marker index (MI) = $H_{avg} \times$ MR	5.32	5.86	5.45
11.	Average informativeness of a band (BI_i)	0.45	0.46	0.45
12.	Resolving power (RP)	2.98	4.10	3.21

deviations in polymorphic bands by RAPD markers were also reported extremely high values such as 13.87 PB/P in *Hordeum vulgare* (Eshghi et al. 2012) and 12.2 PB/P in *Citrullus lanatus* (Mujaju et al. 2010).

Similarly, 13 ISSRs markers out of 20 markers showed amplification profile in 38 accessions of *C. colocynthis* with 99 polymorphic bands out of 166 amplification bands with a molecular size between 250 and 4000 bp. The number of bands amplified per primer varied between 9 (UBC-807, 802) and 16 (UBC-803, 812) with an average of 7.6 polymorphic bands per primer (PB/P). This average result is similar to previous reports of 7.4 PB/P in sandalwood (Patel et al. 2016) and 8.0 PB/P in *Jatropha curcas*

(Khurana-Kaul et al. 2012). However, earlier observations of less average polymorphic band per primer from the present study were also reported in different plant species using ISSR markers viz. 2.8 PB/P in *Benincasa hispida* (Verma et al. 2007), 4.4 PB/P in sugarcane Germplasm (Khaled et al. 2015) and 5.12 PB/P in chickpea (Rao et al. 2007). Contrary to this, high average value of the present work has been reported in other plant species such as, 17.78 PB/P in *Primula obconica* (Nan et al. 2003), 16.87 PB/P in *Vigna radiata* (Singh et al. 2012), 15 PB/P in *Artemisia herba-alba* (Mohsen and Ali 2008), 14.4 PB/P in sugar beet (Izzatullayeva et al. 2014), 13 PB/P in *Miscanthus* (Hodkinson et al. 2002), 12.6 PB/P in *Citrullus*

Table 6 Jaccard's similarity coefficient of 38 *Citrullus colocynthis* accessions based on RAPD data analysis

	A_1	A_2	J_1	J_2	J_3	J_4	J_5	S_1	S_2	S_3	Jh_1	Jh_2	Jh_3	C_1	C_2	C_3
A_1	1.000															
A_2	0.885	1.000														
J_1	0.737	0.749	1.000													
J_2	0.742	0.742	0.870	1.000												
J_3	0.746	0.734	0.887	0.926	1.000											
J_4	0.741	0.736	0.858	0.890	0.926	1.000										
J_5	0.761	0.740	0.876	0.894	0.921	0.931	1.000									
S_1	0.762	0.741	0.836	0.853	0.884	0.873	0.908	1.000								
S_2	0.757	0.727	0.808	0.824	0.855	0.840	0.873	0.901	1.000							
S_3	0.767	0.737	0.804	0.824	0.846	0.845	0.868	0.885	0.927	1.000						
Jh_1	0.772	0.787	0.733	0.730	0.746	0.737	0.765	0.792	0.791	0.823	1.000					
Jh_2	0.770	0.773	0.715	0.720	0.744	0.734	0.762	0.786	0.780	0.812	0.961	1.000				
Jh_3	0.778	0.781	0.735	0.728	0.752	0.755	0.771	0.803	0.785	0.799	0.932	0.939	1.000			
C_1	0.771	0.778	0.745	0.742	0.762	0.752	0.773	0.796	0.764	0.796	0.922	0.925	0.939	1.000		
C_2	0.761	0.761	0.739	0.752	0.752	0.746	0.762	0.794	0.767	0.799	0.907	0.895	0.910	0.949	1.000	
C_3	0.760	0.760	0.734	0.747	0.751	0.733	0.761	0.793	0.770	0.793	0.910	0.898	0.903	0.932	0.951	1.000
H_1	0.728	0.739	0.749	0.746	0.762	0.761	0.760	0.758	0.727	0.737	0.784	0.786	0.786	0.800	0.781	0.797
H_2	0.729	0.729	0.803	0.819	0.812	0.816	0.825	0.804	0.772	0.782	0.725	0.735	0.731	0.733	0.719	0.730
G_1	0.696	0.711	0.800	0.794	0.810	0.804	0.827	0.788	0.769	0.775	0.707	0.717	0.713	0.722	0.709	0.708
G_2	0.706	0.698	0.796	0.797	0.800	0.827	0.831	0.806	0.773	0.783	0.713	0.715	0.732	0.717	0.711	0.714
G_3	0.741	0.720	0.790	0.814	0.826	0.839	0.863	0.822	0.812	0.827	0.757	0.755	0.751	0.736	0.734	0.733
B_1	0.706	0.722	0.683	0.695	0.703	0.710	0.730	0.748	0.729	0.727	0.762	0.752	0.757	0.737	0.723	0.718
B_2	0.718	0.738	0.676	0.684	0.689	0.687	0.706	0.720	0.701	0.704	0.762	0.748	0.748	0.741	0.732	0.735
B_3	0.718	0.722	0.671	0.691	0.680	0.686	0.693	0.703	0.696	0.703	0.734	0.728	0.724	0.730	0.720	0.715
B_4	0.722	0.726	0.683	0.711	0.703	0.706	0.726	0.731	0.729	0.727	0.758	0.744	0.752	0.741	0.748	0.738
Js_1	0.692	0.688	0.658	0.677	0.677	0.695	0.702	0.692	0.693	0.704	0.714	0.716	0.717	0.702	0.689	0.684
Js_2	0.674	0.671	0.681	0.692	0.708	0.707	0.721	0.707	0.682	0.688	0.718	0.712	0.724	0.721	0.697	0.688
Js_3	0.695	0.688	0.707	0.732	0.731	0.734	0.750	0.727	0.709	0.711	0.733	0.727	0.736	0.725	0.711	0.707
Js_4	0.682	0.682	0.730	0.744	0.748	0.742	0.767	0.765	0.728	0.726	0.754	0.747	0.743	0.749	0.747	0.746
Bd_1	0.698	0.709	0.726	0.756	0.751	0.776	0.766	0.764	0.741	0.747	0.753	0.742	0.759	0.752	0.751	0.749
Bd_2	0.699	0.691	0.687	0.732	0.719	0.722	0.746	0.735	0.725	0.723	0.741	0.739	0.736	0.741	0.731	0.734
Bd_3	0.700	0.693	0.697	0.733	0.729	0.728	0.748	0.733	0.710	0.717	0.723	0.725	0.729	0.735	0.717	0.724
Jd_1	0.766	0.778	0.720	0.733	0.737	0.732	0.751	0.757	0.731	0.749	0.787	0.785	0.785	0.790	0.785	0.792
Jd_2	0.759	0.742	0.696	0.733	0.721	0.715	0.735	0.741	0.710	0.720	0.759	0.760	0.765	0.762	0.773	0.763
Jd_3	0.764	0.787	0.729	0.726	0.742	0.721	0.740	0.738	0.744	0.750	0.797	0.794	0.782	0.783	0.774	0.776
U_1	0.682	0.690	0.694	0.711	0.723	0.721	0.733	0.743	0.737	0.752	0.741	0.731	0.739	0.717	0.703	0.718

Table 6 continued

	A₁	A₂	J₁	J₂	J₃	J₄	J₅	S₁	S₂	S₃	Jh₁	Jh₂	Jh₃	C₁	C₂	C₃
U₂	0.683	0.684	0.723	0.712	0.728	0.718	0.751	0.740	0.725	0.745	0.722	0.708	0.712	0.706	0.684	0.699
U₃	0.685	0.704	0.704	0.726	0.730	0.733	0.749	0.742	0.727	0.738	0.720	0.709	0.722	0.703	0.694	0.697

	H₁	H₂	G₁	G₂	G₃	B₁	B₂	B₃	B₄	Js₁	Js₂	Js₃	Js₄	Bd₁	Bd₂	Bd₃	Jd₁	Jd₂	Jd₃	U₁	U₂	U₃
A₁																						
A₂																						
J₁																						
J₂																						
J₃																						
J₄																						
J₅																						
S₁																						
S₂																						
S₃																						
Jh₁																						
Jh₂																						
Jh₃																						
C₁																						
C₂																						
C₃																						
H₁	1.000																					
H₂	0.852	1.000																				
G₁	0.801	0.899	1.000																			
G₂	0.796	0.890	0.912	1.000																		
G₃	0.790	0.888	0.870	0.896	1.000																	
B₁	0.686	0.699	0.692	0.711	0.756	1.000																
B₂	0.691	0.665	0.663	0.688	0.710	0.912	1.000															
B₃	0.675	0.664	0.657	0.663	0.690	0.853	0.899	1.000														
B₄	0.694	0.687	0.688	0.706	0.738	0.866	0.868	0.877	1.000													
Js₁	0.654	0.661	0.659	0.676	0.706	0.752	0.728	0.720	0.795	1.000												
Js₂	0.673	0.666	0.675	0.684	0.695	0.743	0.704	0.685	0.751	0.889	1.000											
Js₃	0.694	0.695	0.700	0.719	0.726	0.748	0.720	0.699	0.764	0.833	0.913	1.000										
Js₄	0.720	0.714	0.707	0.726	0.725	0.778	0.756	0.744	0.783	0.760	0.806	0.864	1.000									
Bd₁	0.693	0.726	0.719	0.738	0.746	0.795	0.764	0.739	0.795	0.789	0.796	0.815	0.860	1.000								
Bd₂	0.709	0.703	0.697	0.703	0.730	0.756	0.756	0.752	0.803	0.760	0.779	0.789	0.813	0.885	1.000							
Bd₃	0.703	0.712	0.706	0.704	0.723	0.737	0.729	0.742	0.771	0.758	0.777	0.770	0.801	0.873	0.925	1.000						

Table 6 continued

	H₁	H₂	G₁	G₂	G₃	B₁	B₂	B₃	B₄	Js₁	Js₂	Js₃	Js₄	Bd₁	Bd₂	Bd₃	Jd₁	Jd₂	Jd₃	U₁	U₂	U₃
Jd₁	0.782	0.740	0.742	0.737	0.735	0.690	0.695	0.686	0.694	0.676	0.698	0.698	0.696	0.700	0.705	0.726	1.000					
Jd₂	0.746	0.724	0.714	0.712	0.719	0.662	0.679	0.677	0.666	0.646	0.665	0.674	0.668	0.669	0.682	0.698	0.906	1.000				
Jd₃	0.767	0.741	0.743	0.718	0.737	0.687	0.692	0.676	0.695	0.684	0.681	0.680	0.670	0.701	0.695	0.689	0.873	0.852	1.000			
U₁	0.682	0.726	0.724	0.739	0.751	0.690	0.664	0.640	0.682	0.695	0.688	0.687	0.697	0.746	0.718	0.712	0.697	0.677	0.717	1.000		
U₂	0.690	0.719	0.725	0.719	0.743	0.679	0.654	0.652	0.679	0.654	0.677	0.680	0.702	0.702	0.707	0.705	0.698	0.677	0.676	0.882	1.000	
U₃	0.707	0.721	0.718	0.733	0.745	0.685	0.659	0.646	0.681	0.670	0.682	0.693	0.691	0.692	0.693	0.687	0.727	0.706	0.712	0.864	0.861	1.000

lanatus (Dje et al. 2010) and 11.6 PB/P in *Cucumis melo* (Stepansky et al. 1999).

Currently, the average value of percent polymorphism for ISSR markers was 65.05% which was higher than the average value of percent polymorphism for RAPD markers that was 55.14% amongst 38 accessions of *C. colocynthis*. This pattern of observation complies with findings by Levi et al. (2005) in which ISSR marker was more polymorphic compared to RAPD markers. This can be ascribed as ISSR markers are the class of microsatellite region, distributed throughout the genome, and having high mutation rate along with its may link with functionally important loci (Penner 1996), while RAPD markers are located in non-coding regions and are selectively neutral. Moreover, the practicing of ISSR technologies found to be more reproducible (Goulao and Oliveira 2001), more effective for uncovering polymorphism (Mahar et al. 2011; Gorji et al. 2011) and obtained more composite marker patterns than RAPD markers (Chowdhury et al. 2002). Previous observations have been mentioned that ISSR markers found to be more effective than RAPD markers and differentiated closely related cultivars in various plant species (Singh et al. 2012). Contrary to these reports, others enough observations that RAPD markers revealed more polymorphism than ISSR markers in different plant species (Farajpour et al. 2011; Patel et al. 2016). This may be possible that these divergences are due to the proportion of coding and non-coding sequences within the genome and differences in genome composition of species.

The genetic diversity was also established amongst 38 accessions of *C. colocynthis* belonging to the different geographical zones of Rajasthan state (India) using RAPD, ISSR, and RAPD + ISSR markers. For the same, DNA fingerprinting employed using both markers to study the extent of genetic diversity across a set of germplasm or cultivars and group them into specific categories. Both the markers revealed above 50% percent polymorphism and almost comparable polymorphic information content (PIC). Therefore, the use of PIC analysis is required to identify most appropriate marker selection for genetic mapping and phylogenetic analysis (Powell et al. 1996). Enormous studies on the assessment of genetic diversity of the diverse group of plant species using molecular markers have been established a correlation between geographical distance and genetic similarity between individuals (Islam and Alam 2004). Beside PIC and heterozygosity, our study also evaluated the discriminatory power of RAPD and ISSR primers for genetic relationship studies through the marker index (MI), multiplex ratio (MR), average informativeness of bands (BI_i), and resolving power (RP); features that to the best of our knowledge have not yet been reported in other molecular studies in *C. Colocynthis*. The average PIC and heterozygosity values are higher for RAPD (0.47, 0.49)

Table 7 Jaccard's similarity coefficient of 38 *Citrullus colocynthis* accessions based on ISSR data analysis

	A$_1$	A$_2$	J$_1$	J$_2$	J$_3$	J$_4$	J$_5$	S$_1$	S$_2$	S$_3$	Jh$_1$	Jh$_2$	Jh$_3$	C$_1$	C$_2$	C$_3$
A$_1$	1.000															
A$_2$	0.949	1.000														
J$_1$	0.760	0.770	1.000													
J$_2$	0.781	0.778	0.922	1.000												
J$_3$	0.738	0.734	0.889	0.897	1.000											
J$_4$	0.754	0.736	0.874	0.915	0.930	1.000										
J$_5$	0.769	0.738	0.875	0.899	0.915	0.965	1.000									
S$_1$	0.769	0.752	0.844	0.852	0.851	0.883	0.916	1.000								
S$_2$	0.735	0.718	0.821	0.844	0.843	0.875	0.908	0.940	1.000							
S$_3$	0.733	0.715	0.835	0.843	0.842	0.874	0.907	0.923	0.931	1.000						
Jh$_1$	0.704	0.699	0.692	0.699	0.736	0.725	0.740	0.754	0.746	0.800	1.000					
Jh$_2$	0.699	0.682	0.649	0.682	0.705	0.708	0.710	0.710	0.702	0.754	0.948	1.000				
Jh$_3$	0.687	0.682	0.674	0.682	0.705	0.695	0.697	0.697	0.689	0.740	0.931	0.897	1.000			
C$_1$	0.672	0.654	0.659	0.642	0.690	0.667	0.669	0.682	0.674	0.724	0.897	0.879	0.946	1.000		
C$_2$	0.699	0.681	0.687	0.694	0.744	0.733	0.748	0.735	0.727	0.780	0.974	0.957	0.923	0.906	1.000	
C$_3$	0.689	0.697	0.702	0.697	0.748	0.723	0.738	0.712	0.718	0.756	0.948	0.914	0.914	0.864	0.957	1.000
H$_1$	0.721	0.717	0.674	0.681	0.715	0.705	0.719	0.707	0.712	0.723	0.838	0.808	0.808	0.779	0.846	0.852
H$_2$	0.681	0.676	0.681	0.676	0.711	0.688	0.691	0.679	0.683	0.706	0.780	0.750	0.819	0.803	0.788	0.792
G$_1$	0.664	0.660	0.676	0.671	0.706	0.696	0.686	0.674	0.667	0.688	0.761	0.731	0.785	0.756	0.756	0.759
G$_2$	0.685	0.669	0.674	0.693	0.715	0.717	0.719	0.695	0.688	0.710	0.784	0.767	0.754	0.713	0.791	0.795
G$_3$	0.690	0.686	0.703	0.710	0.746	0.735	0.750	0.737	0.717	0.741	0.831	0.786	0.786	0.744	0.824	0.829
B$_1$	0.632	0.638	0.691	0.674	0.709	0.686	0.701	0.701	0.681	0.704	0.664	0.636	0.636	0.599	0.660	0.674
B$_2$	0.634	0.641	0.693	0.676	0.711	0.688	0.703	0.715	0.696	0.719	0.679	0.650	0.638	0.613	0.674	0.676
B$_3$	0.626	0.621	0.671	0.667	0.701	0.691	0.705	0.717	0.698	0.721	0.681	0.652	0.641	0.615	0.676	0.667
B$_4$	0.620	0.626	0.667	0.650	0.697	0.674	0.676	0.689	0.669	0.692	0.652	0.635	0.672	0.644	0.647	0.650
Js$_1$	0.625	0.619	0.674	0.656	0.680	0.669	0.685	0.685	0.664	0.714	0.685	0.654	0.667	0.651	0.679	0.682
Js$_2$	0.583	0.588	0.629	0.612	0.659	0.662	0.664	0.664	0.644	0.693	0.677	0.672	0.634	0.606	0.672	0.674
Js$_3$	0.620	0.627	0.669	0.652	0.701	0.690	0.705	0.692	0.672	0.709	0.692	0.662	0.636	0.609	0.674	0.677
Js$_4$	0.615	0.646	0.677	0.646	0.696	0.672	0.674	0.662	0.641	0.677	0.662	0.618	0.618	0.603	0.644	0.646
Bd$_1$	0.639	0.634	0.690	0.672	0.698	0.698	0.701	0.688	0.667	0.704	0.674	0.656	0.643	0.615	0.656	0.659
Bd$_2$	0.637	0.632	0.688	0.695	0.696	0.709	0.711	0.711	0.677	0.714	0.698	0.654	0.654	0.638	0.679	0.669
Bd$_3$	0.609	0.603	0.656	0.652	0.706	0.664	0.667	0.679	0.647	0.695	0.679	0.649	0.649	0.646	0.674	0.664
Jd$_1$	0.733	0.742	0.672	0.679	0.662	0.679	0.681	0.693	0.674	0.722	0.744	0.714	0.714	0.712	0.739	0.729
Jd$_2$	0.761	0.758	0.699	0.693	0.652	0.693	0.708	0.721	0.701	0.750	0.773	0.729	0.729	0.727	0.754	0.744
Jd$_3$	0.771	0.767	0.720	0.701	0.667	0.701	0.704	0.716	0.696	0.746	0.769	0.725	0.738	0.736	0.750	0.740
U$_1$	0.692	0.700	0.746	0.727	0.752	0.727	0.742	0.756	0.748	0.760	0.770	0.724	0.724	0.722	0.750	0.754

Table 7 continued

	A₁	A₂	J₁	J₂	J₃	J₄	J₅	S₁	S₂	S₃	Jh₁	Jh₂	Jh₃	C₁	C₂	C₃
U₂	0.692	0.700	0.732	0.727	0.738	0.727	0.742	0.756	0.748	0.746	0.756	0.711	0.711	0.709	0.750	0.740
U₃	0.650	0.657	0.713	0.695	0.719	0.695	0.710	0.710	0.702	0.713	0.723	0.679	0.732	0.744	0.718	0.721

	H₁	H₂	G₁	G₂	G₃	B₁	B₂	B₃	B₄	Js₁	Js₂	Js₃	Js₄	Bd₁	Bd₂	Bd₃	Jd₁	Jd₂	Jd₃	U₁	U₂	U₃
H₁	1.000																					
H₂	0.878	1.000																				
G₁	0.830	0.913	1.000																			
G₂	0.880	0.850	0.885	1.000																		
G₃	0.901	0.870	0.850	0.930	1.000																	
B₁	0.718	0.702	0.697	0.743	0.774	1.000																
B₂	0.720	0.716	0.711	0.732	0.763	0.967	1.000															
B₃	0.722	0.706	0.701	0.722	0.764	0.936	0.968	1.000														
B₄	0.707	0.767	0.761	0.695	0.725	0.864	0.895	0.896	1.000													
Js₁	0.631	0.638	0.645	0.631	0.636	0.792	0.823	0.810	0.810	1.000												
Js₂	0.624	0.607	0.614	0.613	0.629	0.770	0.800	0.802	0.802	0.900	1.000											
Js₃	0.638	0.621	0.629	0.615	0.655	0.786	0.816	0.817	0.818	0.884	0.944	1.000										
Js₄	0.610	0.604	0.612	0.599	0.626	0.754	0.784	0.786	0.785	0.865	0.873	0.926	1.000									
Bd₁	0.621	0.628	0.659	0.633	0.638	0.768	0.798	0.786	0.815	0.917	0.873	0.908	0.889	1.000								
Bd₂	0.631	0.614	0.633	0.631	0.647	0.764	0.794	0.795	0.780	0.892	0.850	0.884	0.865	0.917	1.000							
Bd₃	0.627	0.621	0.629	0.638	0.631	0.758	0.787	0.789	0.774	0.901	0.842	0.843	0.825	0.874	0.936	1.000						

Table 7 continued

	H₁	H₂	G₁	G₂	G₃	B₁	B₂	B₃	B₄	Js₁	Js₂	Js₃	Js₄	Bd₁	Bd₂	Bd₃	Jd₁	Jd₂	Jd₃	U₁	U₂	U₃
Jd₁	0.761	0.719	0.702	0.736	0.729	0.693	0.719	0.721	0.706	0.729	0.682	0.684	0.679	0.705	0.756	0.764	1.000					
Jd₂	0.775	0.746	0.716	0.750	0.768	0.707	0.734	0.723	0.696	0.718	0.672	0.687	0.669	0.695	0.731	0.738	0.935	1.000				
Jd₃	0.772	0.743	0.725	0.746	0.765	0.703	0.730	0.719	0.704	0.713	0.667	0.682	0.690	0.703	0.727	0.708	0.918	0.967	1.000			
U₁	0.721	0.742	0.699	0.733	0.752	0.714	0.742	0.719	0.702	0.726	0.690	0.720	0.702	0.730	0.740	0.734	0.657	0.709	0.692	1.000		
U₂	0.733	0.716	0.686	0.733	0.752	0.689	0.716	0.706	0.677	0.698	0.664	0.693	0.688	0.702	0.726	0.706	0.657	0.684	0.679	0.946	1.000	
U₃	0.691	0.763	0.719	0.703	0.721	0.672	0.699	0.676	0.710	0.693	0.646	0.674	0.656	0.683	0.693	0.701	0.629	0.679	0.650	0.938	0.921	1.000

markers instead of ISSR (0.44, 0.46) showed that RAPD markers have higher polymorphism than ISSR. The marker index varied from 0.302 to 3.017 (average 1.575) and has been used to assess the informativeness of various markers in several crop species, soybean (Powell et al. 1996), wheat (Bohn et al. 1999), corn salad (Muminovic et al. 2004), and *jatropha* (Grativol et al. 2011), but in *C. colocynthis*, we observed that average MI value was 5.86 higher to RAPD 5.32. Resolving powers in our study were in the range of 0.63–5.11 (average 2.98) for RAPD and ISSR 1.56–8.18 (average 4.10) per primer. According to Fang and Roose (1997) and Naik et al. (2017), ISSR markers were found to be more effective in diversity study than RAPD markers. The present study clearly supports the view. Average MI value of both the markers is more or less similar (5.32–5.86), but average RP value (4.10) of ISSR primers was more than RAPD primers (2.98). Such variation shown by ISSR markers is due to selective amplification. They amplify conserved regions present between the microsatellite repeat sequences, but RAPD markers are not selective; rather, they amplify any regions within the entire genome (Zietkiewicz et al. 1994). The values of multiplex ratio (MR), marker index (MI), average informativeness of a band (BI$_i$), and resolving power (RP) for ISSR marker proved that these markers are more informative to detect polymorphism. Furthermore, our results represent that both RAPD and ISSR markers have the similar potential for phylogenetic relationships in 38 accessions of *C. colocynthis* with a moderate amount of similarity exists among the both marker systems. Therefore, arbitrary primers have enormous potential for resolving intra- and intergenomic relationships and different marker technologies target the different portions of the genome. While UPGMA-based dendrograms revealed geographic similarity and some differences in the area magnitude were also reported among the different accessions with a particular type of molecular markers technology. Moreover, high reproducibility in dendrogram topologies was obtained for both RAPD and ISSR markers with a few differences between the species and different accessions of *C. colocytnhis*. These differences may be attributed to marker sampling error and/or the level of polymorphism detected, reinforcing again the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al. 1996). Therefore, the evolution of varieties in distinct agro-climatic zones also demonstrates significant levels of variation in response to the selection pressure in the zones (Singh et al. 1998). Likewise, a huge number of studies also evaluated to genetic relationship establishments in a variety of cultivars and plant species, such as in *Curcuma longa* (Singh et al. 2012), *Jatropha curcas* (Khurana-Kaul et al. 2012), *Cajanus cajan* (Yadav et al. 2014), *Dioscorea*

Table 8 Jaccard's similarity coefficient of 38 *Citrullus colocynthis* accessions based on RAPD + ISSR data analysis

	A$_1$	A$_2$	J$_1$	J$_2$	J$_3$	J$_4$	J$_5$	S$_1$	S$_2$	S$_3$	Jh$_1$	Jh$_2$	Jh$_3$	C$_1$	C$_2$	C$_3$
A$_1$	1.000															
A$_2$	0.899	1.000														
J$_1$	0.742	0.754	1.000													
J$_2$	0.751	0.750	0.882	1.000												
J$_3$	0.744	0.734	0.887	0.919	1.000											
J$_4$	0.744	0.736	0.861	0.896	0.927	1.000										
J$_5$	0.763	0.739	0.876	0.896	0.920	0.939	1.000									
S$_1$	0.764	0.744	0.838	0.853	0.876	0.876	0.910	1.000								
S$_2$	0.751	0.725	0.811	0.829	0.852	0.848	0.882	0.910	1.000							
S$_3$	0.759	0.732	0.811	0.829	0.845	0.852	0.877	0.894	0.928	1.000						
Jh$_1$	0.756	0.767	0.723	0.723	0.744	0.734	0.759	0.783	0.780	0.818	1.000					
Jh$_2$	0.753	0.752	0.699	0.711	0.735	0.728	0.750	0.767	0.761	0.798	0.958	1.000				
Jh$_3$	0.757	0.758	0.721	0.717	0.741	0.741	0.754	0.778	0.762	0.785	0.932	0.929	1.000			
C$_1$	0.748	0.750	0.725	0.718	0.745	0.732	0.748	0.768	0.743	0.779	0.917	0.914	0.941	1.000		
C$_2$	0.747	0.742	0.726	0.738	0.750	0.743	0.759	0.780	0.757	0.794	0.922	0.909	0.913	0.939	1.000	
C$_3$	0.743	0.745	0.726	0.735	0.750	0.731	0.756	0.773	0.757	0.784	0.919	0.902	0.905	0.917	0.953	1.000
H$_1$	0.726	0.734	0.730	0.730	0.750	0.747	0.750	0.745	0.723	0.734	0.796	0.791	0.791	0.795	0.796	0.809
H$_2$	0.718	0.716	0.772	0.782	0.787	0.783	0.790	0.772	0.749	0.763	0.738	0.738	0.751	0.749	0.735	0.744
G$_1$	0.688	0.699	0.769	0.762	0.784	0.777	0.790	0.759	0.743	0.753	0.720	0.720	0.730	0.730	0.720	0.720
G$_2$	0.701	0.691	0.764	0.770	0.779	0.798	0.802	0.777	0.750	0.764	0.730	0.727	0.737	0.716	0.730	0.733
G$_3$	0.728	0.712	0.768	0.787	0.806	0.813	0.833	0.800	0.787	0.805	0.775	0.762	0.759	0.738	0.755	0.755
B$_1$	0.688	0.702	0.685	0.690	0.705	0.704	0.723	0.736	0.717	0.722	0.738	0.723	0.726	0.703	0.708	0.708
B$_2$	0.698	0.714	0.680	0.682	0.694	0.688	0.705	0.719	0.700	0.707	0.742	0.724	0.721	0.710	0.718	0.720
B$_3$	0.695	0.697	0.671	0.685	0.685	0.687	0.696	0.707	0.697	0.707	0.721	0.709	0.704	0.701	0.709	0.703
B$_4$	0.697	0.702	0.679	0.696	0.702	0.698	0.714	0.721	0.714	0.719	0.732	0.717	0.733	0.718	0.723	0.717
Js$_1$	0.676	0.673	0.661	0.672	0.678	0.689	0.698	0.690	0.686	0.706	0.708	0.702	0.705	0.691	0.687	0.684
Js$_2$	0.653	0.652	0.669	0.674	0.697	0.696	0.708	0.698	0.673	0.689	0.709	0.703	0.703	0.695	0.691	0.685
Js$_3$	0.677	0.674	0.698	0.712	0.724	0.724	0.740	0.719	0.700	0.710	0.724	0.712	0.713	0.698	0.703	0.700
Js$_4$	0.666	0.674	0.718	0.720	0.736	0.725	0.745	0.740	0.707	0.715	0.732	0.716	0.714	0.714	0.722	0.722
Bd$_1$	0.684	0.692	0.718	0.736	0.738	0.757	0.751	0.746	0.723	0.737	0.735	0.723	0.732	0.720	0.728	0.728
Bd$_2$	0.684	0.678	0.687	0.723	0.716	0.719	0.738	0.730	0.714	0.721	0.731	0.719	0.717	0.717	0.719	0.719
Bd$_3$	0.679	0.672	0.687	0.714	0.713	0.713	0.728	0.720	0.695	0.712	0.713	0.707	0.711	0.714	0.707	0.710
Jd$_1$	0.759	0.769	0.709	0.720	0.717	0.719	0.734	0.742	0.717	0.742	0.777	0.768	0.769	0.772	0.774	0.777
Jd$_2$	0.759	0.746	0.697	0.723	0.708	0.710	0.728	0.736	0.708	0.727	0.762	0.753	0.757	0.754	0.768	0.759
Jd$_3$	0.765	0.782	0.727	0.720	0.729	0.716	0.731	0.733	0.732	0.749	0.790	0.778	0.772	0.773	0.768	0.768
U$_1$	0.685	0.693	0.706	0.714	0.729	0.723	0.735	0.746	0.740	0.754	0.748	0.729	0.736	0.718	0.714	0.726

Table 8 continued

	A_1	A_2	J_1	J_2	J_3	J_4	J_5	S_1	S_2	S_3	Jh_1	Jh_2	Jh_3	C_1	C_2	C_3
U_2	0.685	0.687	0.725	0.715	0.730	0.720	0.749	0.744	0.731	0.745	0.730	0.708	0.712	0.706	0.699	0.708
U_3	0.676	0.693	0.707	0.718	0.727	0.723	0.739	0.734	0.721	0.732	0.720	0.702	0.724	0.713	0.699	0.702

	H_1	H_2	G_1	G_2	G_3	B_1	B_2	B_3	B_4	Js_1	Js_2	Js_3	Js_4	Bd_1	Bd_2	Bd_3
A_1																
A_2																
J_1																
J_2																
J_3																
J_4																
J_5																
S_1																
S_2																
S_3																
Jh_1																
Jh_2																
Jh_3																
C_1																
C_2																
C_3																
H_1	1.000															
H_2	0.858	1.000														
G_1	0.808	0.902	1.000													
G_2	0.817	0.879	0.905	1.000												
G_3	0.817	0.883	0.865	0.905	1.000											
B_1	0.694	0.699	0.694	0.719	0.760	1.000										
B_2	0.698	0.677	0.675	0.699	0.723	0.925	1.000									
B_3	0.687	0.674	0.668	0.678	0.708	0.873	0.916	1.000								
B_4	0.697	0.706	0.706	0.704	0.735	0.865	0.875	0.882	1.000							
Js_1	0.649	0.656	0.656	0.665	0.689	0.761	0.749	0.740	0.798	1.000						
Js_2	0.662	0.652	0.660	0.667	0.679	0.749	0.725	0.711	0.762	0.891	1.000					
Js_3	0.681	0.677	0.683	0.693	0.708	0.756	0.741	0.726	0.777	0.844	0.919	1.000				
Js_4	0.693	0.686	0.683	0.693	0.700	0.772	0.763	0.754	0.783	0.782	0.820	0.877	1.000			
Bd_1	0.676	0.702	0.705	0.712	0.719	0.788	0.772	0.750	0.799	0.816	0.812	0.835	0.866	1.000		
Bd_2	0.691	0.681	0.681	0.685	0.709	0.758	0.764	0.762	0.798	0.787	0.794	0.809	0.824	0.892	1.000	
Bd_3	0.685	0.690	0.687	0.688	0.700	0.742	0.742	0.753	0.771	0.787	0.790	0.785	0.806	0.873	0.927	1.000

Table 8 continued

	H₁	H₂	G₁	G₂	G₃	B₁	B₂	B₃	B₄	Js₁	Js₂	Js₃	Js₄	Bd₁	Bd₂	Bd₃	Jd₁	Jd₂	Jd₃	U₁	U₂	U₃
Jd₁	0.777	0.735	0.732	0.736	0.734	0.691	0.701	0.695	0.697	0.688	0.695	0.695	0.692	0.702	0.717	0.735	1.000					
Jd₂	0.753	0.729	0.714	0.721	0.731	0.673	0.692	0.689	0.673	0.662	0.666	0.677	0.668	0.675	0.693	0.708	0.913	1.000				
Jd₃	0.768	0.742	0.739	0.725	0.743	0.691	0.701	0.686	0.697	0.691	0.678	0.680	0.675	0.702	0.702	0.693	0.883	0.878	1.000			
U₁	0.691	0.730	0.718	0.738	0.751	0.696	0.682	0.658	0.687	0.702	0.688	0.694	0.698	0.743	0.723	0.717	0.687	0.684	0.711	1.000		
U₂	0.700	0.718	0.715	0.723	0.745	0.681	0.668	0.665	0.679	0.664	0.674	0.683	0.699	0.702	0.711	0.705	0.688	0.679	0.676	0.896	1.000	
U₃	0.703	0.731	0.718	0.726	0.739	0.681	0.668	0.653	0.688	0.676	0.674	0.689	0.683	0.690	0.693	0.690	0.703	0.700	0.697	0.881	0.875	1.000

spp. (Velasco-Ramirez et al. 2014), *Cymbopogon flexuosus* (Debajit et al. 2015), and sandalwood (Patel et al. 2016).

In conclusion, the present study was intended to assess molecular characterization, marker polymorphisms, and genetic diversity analysis amongst 38 different accessions of *C. colocytnhis* from the distinct geographical zone. Likewise, RAPD and ISSR fingerprinting technologies were employed for genetic analysis which provides a powerful tool for the generation of potential diagnostic of markers for cultivar analysis. Fifty RAPD markers and thirteen ISSR markers revealed clear amplification in 38 different *C. colocytnhis* with percent polymorphism 55.14 and 65.05%, respectively. Furthermore, genetic diversity was established through dendrogram construction amongst all the different accessions of *C. colocytnhis* using RAPD, ISSR, and RAPD + ISSR marker technologies. Both the markers indicate an important level of genetic differences amongst 38 distinct accessions of *C. colocytnhis* in relation with their different geographical distributions and some genetic differences were also obtained from different types of marker technologies. Thus, the study provides an evidence for selecting of marker technologies further for breeding, integration into the marker-assisted selection and comparative genetic studies.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

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