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Molecular and functional diversity in *Capsicum* landraces of Andaman Islands

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The present study analyzed the diversity in 26 landraces of *Capsicum* from Andaman Islands using 20 morphological, 16 biochemical and 10 DNA markers. Significant differences were observed in tested landraces and 16 reference genotypes from mainland India. Biochemical markers grouped all the genotypes into eight clusters with inter-cluster distance of 0.5 to 1.9 while seven quantified morphological traits divided the test genotypes into three major clusters and seven sub-clusters with 0.1 to 1.6 inter-cluster distance value. The random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers assured the genetic nature of diversity in landraces. The similarity matrix from RAPD and ISSR markers revealed 48% diversity among 42 genotypes with polymorphism information content (PIC) values of 0.43 and 0.41, respectively. The correspondence in morphological and biochemical markers indicates their interdependence for observed traits. However, poor correlation between DNA profiles and functional markers suggest further screening of more number of markers.The study identified phytochemical rich landraces CA-334, SPG-7, CARI-1 and CCB-2. The information will be useful in chemo-taxonomic foot-printing of *Capsicum* landraces and devising apposite conservation and utilization strategies.

Key words: *Capsicum*, landraces, functional diversity, chemo-taxonomic diversity, DNA markers.

INTRODUCTION

The genus *Capsicum* belongs to family Solanaceae and have 27 species including five commonly cultivated species (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L. and *C. pubescens* Ruiz & Pav.). The most important species *C. annum* bears both pungent and sweet fruits having commercial value as spice and vegetable. Pungent chilli has diverse prophylactic and therapeutic uses such as antibacterial, antifungal, anticancer, anti-oxidant, anti-protozoal, hypocholesterolaemic, hypolipidemic, immunomodulatory and anti-mutagenic (Pawar et al., 2011). These properties are due to complex matrix of phytochemicals in fruits which includes flavonoids, phenolics and carotenoids. These compounds also acts as antioxidants and supplement the in built homeostasis mechanism of human body for inhibiting or neutralizing the free radicals (Nadhala et al., 2010). Some of these compounds showed strong correlation with antioxidant activity but, their concentration and capacity are influenced by genotypes, environment and estimation method (Singh et al., 2011). The screening of germplasm with these traits serves dual purpose of providing chemotaxonomic diversity (Goff and Klee, 2006) and information about phytochemical rich genotypes.

The archipelago of Andaman and Nicobar Islands (India) is consisted of 572 islands and located between 14° to 16° N and 92° to 94° E. It is recognized as one of the biodiversity hotspots (Mayers et al., 2000). The islands also have rich diversity of *Capsicum* with the existence of

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C. *annum*, *C*. *frutescens* and *C*. *chinense* (Abraham et al., 2008). It is presumed that these species were introduced during or after the second half of 19th century to meet up demand of settler communities. In period of 150 years, the introduced *Capsicum* germplasm faced natural evolutionary forces and got adapted to island conditions. This might have changed their genomic constitution at least to some extent but so far, no variety of *Capsicum* has been specifically bred for islands. Thus, assessment of diversity in the local germplasm was much needed to recognize the genetic relatedness in order to select the parents for breeding programme and also to avoid duplications in gene banks.

The limitation of morphological and biochemical markers can be reduced by the use of DNA markers as they are simple to use, cost effective, abundant in genome and independent to stage and environment. Thus, combined use of morphological, biochemical and DNA markers may generate sufficient information for authenticating the extent of diversity in germplasm. Among DNA markers, PCR based RAPDs and ISSRs are in common use for decoding the diversity in crop germplasm at preliminary stage when little is known about whole genome of the species (Singh et al., 2012). Therefore, the present study aimed to assess the extent of molecular and functional diversity in *Capsicum* landraces of Andaman Islands for understanding the extent of genetic distance between the landraces and identifying the potential genotypes for improvement of economic and phytochemical parameters.

MATERIALS AND METHODS

Collection of germplasm

Representative plant samples of 26 landraces of Capsicum were collected from different islands (Table 1). Sixteen elite genotypes were taken from all India Coordinated Research Project (vegetable Crops) as reference (Table 1). All the 42 sample genotypes were grown in randomized block design with three replications and managed with standard package of practices at Research Farm of Central Agricultural Research Institute, Port Blair during the dry season (December to April) of 2010-2011.

Chemicals and reagents

The analytical grade chemical reagents were used in the present study and 1,1-diphenyl 2 picrylhydrazyl (DPPH), gallic acid, anthrone reagent, aluminium chloride, formic acid, hexane, anthrone, dinitrosalicylic acid, 2, 6-dichlorophenol indophenols, ninhydrin, methyl orange, sulphuric acid, boric acid indicator, Davarda's alloy and leucine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tannic acid, Ascorbic acid, conc. HCl, sodium acetate buffer and sulphuric acid were purchased from Himedia (Himedia Laboratories Pvt. Ltd., Mumbai). Methanol, rutin, folin-ciocaulteu reagent, KCl, copper sulphate and sodium hydroxide were purchased from Merck (Merck, Darmstadt, Germany). Anhydrous sodium sulphate, potassium sodium tartarate, oxalic acid, sodium bicarbonate, citrate, potassium dichromate, ammonium thiocyanate, ferric chloride, ammonia, cal-cium chloride, potassium permanganate, magnesium oxide, sodium acetate, orthophosphoric acid, acetone and sodium carbonate solution were obtained from Rankem (RFCL Ltd., New Delhi, India).

Morphological parameters

Twenty (20) morphological characters including seven quantified characters viz., leaf size, leaf shape, leaf colour, growth habit, stem colour, seed colour, number of flower/ axil, flower colour, mature fruit colour, ripe fruit colour, fruit shape, fruit end shape and fruit surface were recorded from five random plants of Capsicum landrace/genotypes using standard procedures.

Biochemical analysis

Estimation of phytochemicals

The polyphenol content in green fruits was estimated by Folin-Ciocalteau reagent method (10%, v/v) (Singleton and Rossi, 1965) with some modifications. The absorbance from samples was measured at 765 nm using UV-spectrophotometer (Elico SL-164, Pvt Ltd, Hyderabad, India). Gallic acid was used as reference and the results were expressed as mg of gallic acid equivalent (mg/100 g fresh weight). Flavonoid content in test genotypes was determined spectrophotometrically using standard protocol as described by Chang et al. (2002) and expressed as mg rutin equivalent (mg/100 g fresh weight). Concentration of anthocyanin in chilli fruits was determined by pH differential method as described by Sadasivam and Manickam (1996), and results were expressed as C_3GE (cyanidine-3-glucoside) mg/100 g fresh weight (Fuleki and Francis, 1968). The ascorbic acid was estimated using standard colorimetric method (Sadasivam and Manickam, 1996) and concentration was expressed as mg/100 g fresh weight. Total carotenoid and chlorophyll content in chilli fruit were determined through procedure given by Lichtenthaler and Buschmann (2001). Tannin content was estimated using AOAC method (1990) with tannic acid as standard and expressed as TAE mg/100 g fresh weight.

DPPH antioxidant activity

The antioxidant activity of methanol extract of fruit of chilli genotypes was determined by 1,1-diphenyl-2- picrylhydrazyl (DPPH) method (Wong et al., 2006). Stock solution of extracts were diluted to 20, 40, 60, 80 and 100 µg/ml and incubated for 2 h in dark chamber and absorbance readings were taken at 517 nm at 10 min interval. Sample extract (0.1 ml) were added to 3 ml of methanol solution of DPPH (0.001 M). The antioxidant activity (%) was calculated as $[(A_0 – A_E)/A_0]$ x100 (A_0 = absorbance without extract; A_E = absorbance with extract), whilst IC_{50} values were estimated from percent inhibition of DPPH free radicals against concentration sigmoidal curve, using a non-linear regression analysis.

Proximate components

The total carbohydrate was estimated according to the modified method of Hedge et al. (1962) and reducing sugar by Nelson-Somogyi method (Sadasivam and Manickam, 1996). The non-reducing sugar was calculated by subtracting the quantity of reducing sugar and multiplying with a conversion factor (0.95). The absorbance for extractable colour value in mature fruits was observed with acetone solvent at 450 nm with $K_2Cr_2O_7$ solution as reference. The colour value was determined by the formula: Colour value (ASTA units) = (Absorbance of chilli extract at 450 nm × 200)/Absorbance of $K_2Cr_2O_7$ solution at 450 nm (Sadasivam and Manickam, 1996). The antinutritional factors like phytate, oxalate, nitrate and saponin content in chilli fruits were determined using the

Table 1. Capsicum landraces and reference genotypes used in the study.

Accession	Specie	Source
CHIVAR-1-I	*Capsicum annum* L.	AICRP/IET (Mainland India)
CHIVAR-3-I	*Capsicum annum* L.	AICRP/IET (Mainland India)
CHIVAR-4-I	*Capsicum annum* L.	AICRP/IET (Mainland India)
CHIVAR-5-I	*Capsicum annum* L.	AICRP/IET (Mainland India)
CHIVAR-6-I	*Capsicum annum* L.	AICRP/IET (Mainland India)
LCA-334-1	*Capsicum annum* L.	AICRP/IET (Gutur, A.P., India)
KA-2	*Capsicum annum* L.	AICRP/IET (Kerala, India)
CARI-1	*Capsicum annum* L.	Neil Island, South Andaman
CARI-2	*Capsicum annum* L.	Neil Island, South Andaman
M-1	*Capsicum annum* L.	Mangultan, South Andaman
M-2	*Capsicum annum* L.	Mangultan, South Andaman
M-3	*Capsicum annum* L.	Mangultan, South Andaman
N-1	*Capsicum annum* L.	Nayasagar, South Andaman
G-1	*Capsicum annum* L.	Girgutan, South Andman
G-2	*Capsicum annum* L.	Girgutan, South Andaman
H-1	*Capsicum annum* L.	Humphreygunj, South Andaman
H-2	*Capsicum annum* L.	Humphreygunj, South Andaman
CHIVAR-1-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-2-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-3-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-4-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-5-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-6-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-7-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CHIVAR-8-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
LCA-334-II	*Capsicum annum* L.	AICRP/AVT-II (Mainland India)
CCB-1	*Capsicum pubescens*	Choldhari, South Andaman
CCB-2	*Capsicum frutescens*	Choldhari, South Andaman
CCB-3	*Capsicum frutescens*	Diglipur, North Andaman
CCW	*Capsicum frutescens*	Diglipur, North Andaman
CCO	*Capsicum frutescens*	Okrabraj, South Andaman
CCR	*Capsicum frutescens*	Rangat, Middle Andaman
CCLG	*Capsicum frutescens*	Hut Bay, Little Andaman
CCG	*Capsicum frutescens*	Guptapara, South Andaman
SPG-1	*Capsicum frutescens*	Garacharma, South Andaman
SPG-2	*Capsicum frutescens*	Hut Bay, Little Andaman
SPG-3	*Capsicum frutescens*	Sippighat, South Andaman
SPG-4	*Capsicum annum* L.	Sippighat, South Andaman
SPG-5	*Capsicum annum* L.	Sippighat, South Andaman
SPG-6	*Capsicum annum* L.	Calicut, South Andaman
SPG-7	*Capsicum annum* L.	Collinpur, South Andaman
LMCF	*Capsicum annum* L.	Haddo, South Andaman

method described by Sadasivam and Manickam (1996).

Molecular markers

Genomic DNA isolation

The genomic DNA of 26 landraces and 16 elite genotypes of Capsicum were extracted leaves using the CTAB method with slight modifications. For this, the healthy leaves were collected, cleaned, surface sterilized and 3 g leaves were ground with 5 ml pre-warmed (65°C for 1 h) CTAB buffer. Further steps are similar to CTAB method of DNA isolation from plant tissues. Quantification and qualitative analysis of the DNA were performed using UV Spectrophotometer (ELICO Ltd., Hyderabad, India) and gel electrophoresis with 0.8% (w/v) agarose and ethidium bromide (3 µl/100ml gel

solution).

PCR analysis

The primer screening with 30 RAPD (Operon Technologies, Alameda, California; UB series, University of British Columbia, Vancouvur, BC Canada supplied by Bangalore Genei, Bangalore, India), and 37 ISSR markers (Sigma-Aldrich, St. Louis, Mo., USA) was done in 42 test genotypes. PCR analysis carried out in thermal cycler (G-STORM, Gene Technologies, United Kingdom) in a final volume of 20 µl containing 1 µl genomic DNA (25 ng), 1.5 µl dNTP mix (made up of 100 µM of each of the four dNTPs), 1 µl primer (RAPD/ISSR), 1.6 µl $MgCl_2$, 2 µl of 10X *Taq* buffer (10 mM Tris HCl pH 9.0, 50 mM KCl), 0.25 µl *Taq* DNA polymerase (0.5 U) (Banglore GeneI, Bangalore, India) and 12.65 µl sterile millipore water. A negative PCR was kept to test the PCR reactions.

The PCR programming for RAPD was done with steps of hot start (94°C, 5 min) and 39 cycles (denaturation at 94°C, 1 min; primer annealing at 36°C, 1 min and primer extension at 72°C, 1 min) followed by final extension (72°C, 10 min) and cooling (10°C, 1 h). The amplified products were taken out and kept at 4°C till electrophoresis. For ISSR markers, Touchdown PCR reaction (Don et al., 1991) was performed with minor modifications in PCR programming with steps of hot start (94°C, 5 min) and 8 down steps (94°C, 1 min; 45°C to 39°C, 1 min each; 72°C, 1 min) followed by 31 linear cycles (94°C, 1 min; 38°C, 1 min; 72°C, 1 min) followed by final extension (72°C, 10 min) and cooling (10°C, 1 h). Amplified PCR product (5 µl) were mixed in 6X bromophenol blue (5 µl) and separated by gel electrophoresis on 1.5% agarose gel stained with ethidium bromide and visualized with UVP MultiDoc-IT Digital Imaging System (UVP LCC, California).

Dendrogram construction and statistical analysis

The binary data of reproducible bands from RAPD and ISSR markers in 42 genotypes were subjected for construction of dendrogram through unweighted pair group method with arithmetic average (UPGMA) cluster analysis using software NTSYS-pc, version 2.02. Polymorphic information content (PIC) for each marker was calculated as PICi = 2fi (1-fi) as proposed by Roldan-Ruiz et al. (2000), where PICi is the polymorphic information content of i^{th} marker, fi is the frequency of the marker bands present, (1-fi) is the frequency of absent marker bands. While the dendrograms of test genotypes using seven morphological traits having quantitative data *viz*. plant height, seeds/fruit, fruit length, fruit width, pedicel length, fruits/plant and fruit yield/plant and 16 biochemical compounds were constructed using SAS 4.1 Enterprise software. The quantitative data for morphological and biochemical parameters were analysed for mean and standard deviation using Microsoft Excel 2007 software.

RESULTS AND DISCUSSION

Morphological diversity

The results from observations for 13 qualitative characters from test genotypes revealed that most genotypes had medium size (45.2%) and lanceolate leaves (40.5%) with horizontal orientation. Landraces had large leaves (53.0%) with intermediate growth habit (61.5%) while genotypes from mainland showed erect plant habit. The landraces showed variability in seed colour with predominance of cream (61.5%) and yellow (23.0%) while reference genotypes produced only cream colour seeds. Flower colour in germplasm was observed to be white, light white and purple. Only white flowers were observed in all reference genotypes while most of the landraces produced white (42.3%) and light white (42.3%) flowers and four landraces produced purple flowers.

Fruit colour showed great variation in landraces which was observed to be dark red (11), red (7), brownish red (4), light red (2) while one landrace (CCO) produced orange fruits. However, red fruit trait was predominant in reference genotypes. The fruit shape revealed great extent of diversity in landraces where small, medium, long, very long fruits were observed with fruit length of 1.1 to 7.5 cm length, maximum in CCB-1 while minimum in M-2. Two landraces CCB-1 and LMCF had round fruits while conical, conical long, curved, pointed, slightly curved, smooth, wrinkled, semi-wrinkled fruits were also observed.

The morphological traits having quantified observations showed significant variations between landraces and reference genotypes and also within them. Length of mature green fruits in investigated landraces ranged from 1.1 (CCB-1) to 7.5 cm (M-2) while in reference genotypes, it ranged from 5.8 (CHIVAR-1-I, CHIVAR-2-II) to 11.4 cm (CHIVARI-5-I). Notably, number of seeds per fruit showed great variation in *Capsicum* landraces with the range of 17 (CCW) to 78 (M-2) while narrow range for seeds per fruit was observed in reference genotypes that is between 24 (KA-2) to 47 (CHIVAR-2-II). The fruit diameter in landraces ranged from 0.3 (SPG-1) to 1.6 cm (M-1) while it varied from 0.9 to 4.4 cm in reference genotypes. Individual fruit weight in genotypes ranged from 2.14 to 19.56 g.

Dendrogram based upon seven morphological traits (Figure 1) revealed three major clusters and seven subclusters with 0.1 to 1.6 inter-cluster values. Cluster-I, cluster-IIIa and cluster-IIId consists of seven, five and two genotypes, respectively and all representing the AICRP (VC) entries from mainland while Cluster-II and IIIc had 10 and 3 landraces of islands. Cluster-IIIb had 13 genotypes, mostly from islands except only one genotype from AICRP (VC).

The present study revealed great diversity among *Capsicum* landraces from islands and also showed genetic distance from reference genotypes of mainland India. The variation in some of the adaptive traits like leaf orientation and leaf size indicate that landraces in islands have been evolved with adaptive mechanisms to transpire more in tropical humid conditions of islands. The flower colour is Capsicum helped in understanding the relationship among the different species. The white flowers are produced by *C. annum* or *C. chinense*, whitish green by *C. frutescens* and purple by *C*. *pubescens*. Similar observations were made in the present study though some of the landraces showed yellowish white pattern in flower colour. The findings support the reports of Abraham et al. (2008) for the presence of species diversity in *Capsicum* genus in islands. However, they did not report *C*. *pubescens* in islands which might have

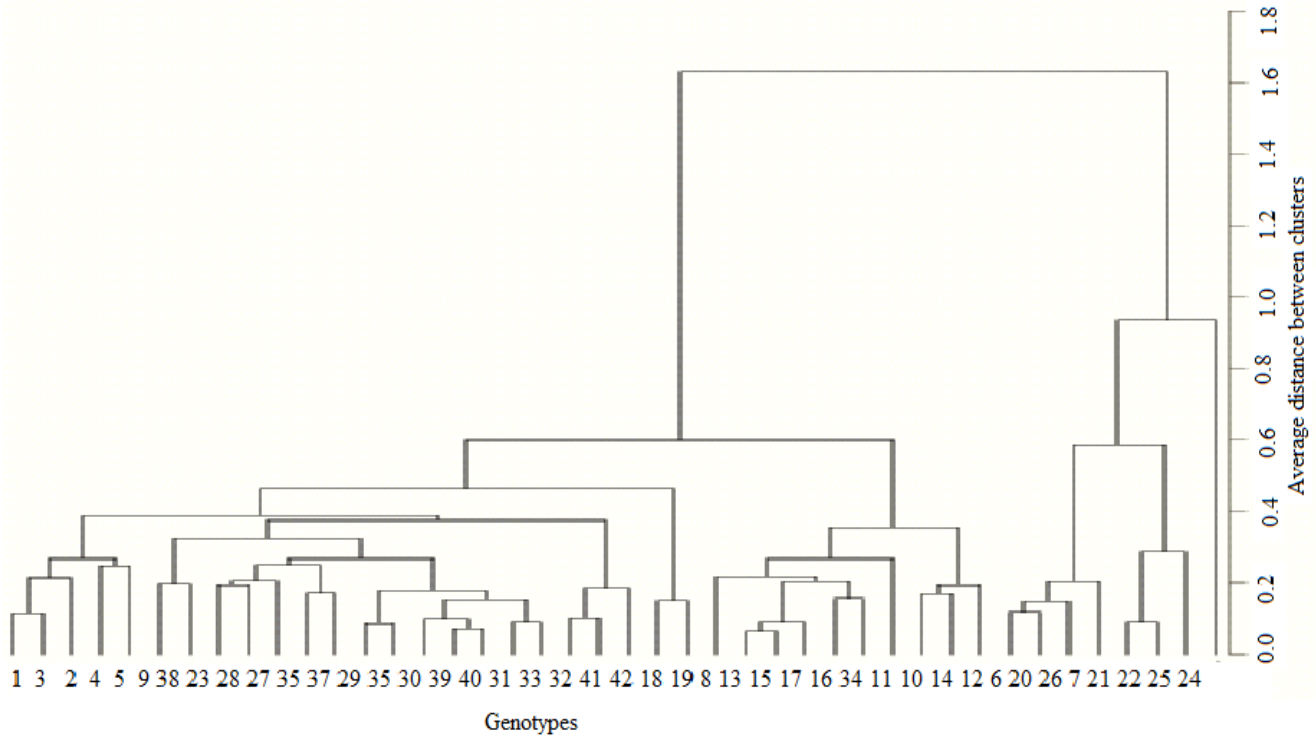

Figure 1. Cluster analysis of *Capsicum* genotypes with morphological parameters (S. No. 1-42 are genotypes as given in Table 1).

been brought after their survey period or remain unnoticed. The clustering pattern from morphological traits having quantitative observation showed distinctness of landraces from reference genotypes. This might be due to adaptive changes or 'in-house spread' of landraces among the islanders through their personal contacts or local seed vendors for thier homegardens or farms (Pandey et al., 2006).

Chemo-taxonomic diversity

The results for estimation of phytochemicals viz., polyphenol, flavonoid, tannin, anthocyanin, carotenoid, vitamin c and chlorophyll in 40 genotypes of *Capsicum* showed great extent of diversity, particularly in Islands landraces. Polyphenol content revealed significant variation among local landraces and reference genotypes of AICRP (VC). The highest polyphenol content was estimated in CA-334 (181.6 mg/100 g) while lowest in SPG-5 (53.1 mg/100 g). Some of the local landraces like CARI-1, CCO, M-1 and CCB-1 were also found to be rich in polyphenol content. The flavonoids content in test genotypes ranged from 41.01 to 791.0 mg/100 g, the highest was recorded in CARI-1 while lowest in M-1. The CHIVAR-6-I, CA-334, CHIVAR-5-I, CHIVAR-5-II and KA-2 of AICRP (VC) and CARI-1, G-2 and CCO from islands were found to be rich in flavonoids. The *Capsicum* landraces also showed variation for tannin content, the highest in CARI-1 (328.9 mg/100 g) and minimum in SPG-5 (113.3 mg/100 g). However, CHIVAR-4-II contained highest amount of tannin (415.6 mg/100 g) among the screened genotypes. Most of the genotypes were poor in anthocyanin content at mature green stage as its concentration was ranged from 0.9 to 41.7 mg/100 g with maximum in SPG-7 and minimum in CCB-1 and CCG. Capsicum genotypes were found to be rich in carotenoid content which was highest in SPG-7 (552.7mg/100 g) whilst lowest in H-1 (20.2 mg/100 g).

Chlorophyll content in mature green fruits ranged from 179.8 (CCB-3) to 579.5 µg/100 ml (CARI-1). The chlorophyll rich landraces were CARI-1, M-1, G-1, M-2, M-3, N-1 and CARI-2. The present study identified significant (p=0.05) differences among genotypes for ascorbic acid content which ranged from 43.3 (CHIVAR-7-II) to 140.0 mg/100 g (CHIVAR-1-I). The identified ascorbic acid rich genotypes were CHIVAR-1-I, CA-334, CHIVAR-4-I, CHIVAR-2-II, LCA-334 and SPG-6. The antioxidant activity of methanol extract of test genotypes ranged from 67.9 to 96.3%, the highest in H-1 and lowest in SPG-4.

The free amino acid content was highest in CCB-1 (747.1 mg/100 ml) while lowest in M-1 and SPG-7 (95.3 mg/100 ml). The Landraces CCB-2, G-2, CCW, CCG, G-1, CCLG and SPG-3 were identified to be rich in free amino acids. The level of sugar content also revealed diversity in Capsicum genotypes which ranged from 168.5 to 308.7 mg/100 g. Most of the genotypes rich in total sugar that is CHIVAR-3-I, CHIVAR-3-II, CHIVAR-5-II,

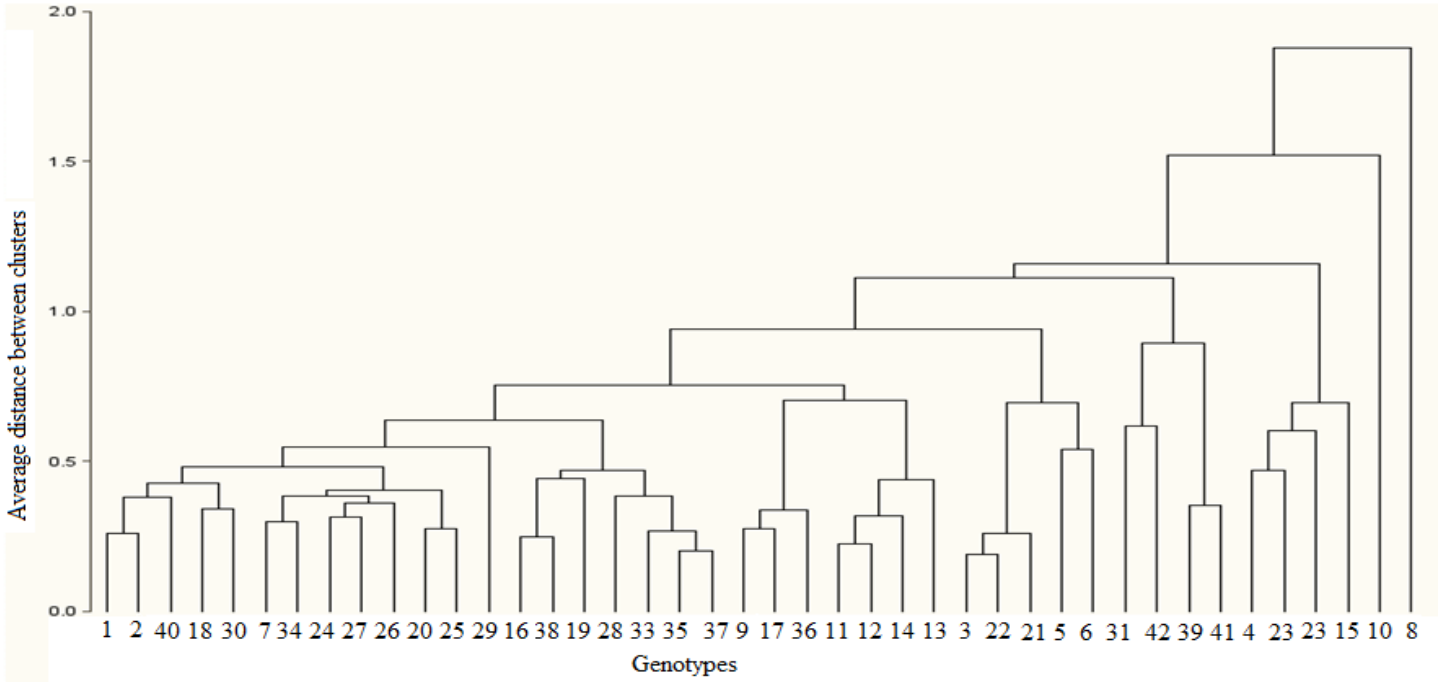

Figure 2. Cluster analysis of *Capsicum* genotypes with biochemical parameters (S. No. 1-42 are genotypes as given in Table 1).

CHIVAR-4-II and CHIVAR-2-II represented AICRP (VC) material while local landrace were low in total sugar content. Reducing sugar was estimated to be maximum in CCO (153.0 mg/100 ml) while the minimum was observed in M-3 (12.2 mg/100 ml). G-1, SPG-2, CCG, CCW, SPG-7 and CCO landraces were found to be rich in reducing sugar than AICRP (VC) genotypes. Non-reducing sugar content also showed significant (p=0.05) variations in capsicum genotypes which ranged from 61.1 (G-1) to 250.4 mg/100 g (CHIVAR-3-II). Among local landraces, the CCR (196.1 mg/100 g) and M-3 (188.9 mg/100 g) were found to be rich in non-reducing sugar. In the present study, significant (p=0.05) variations were also observed in capsicum genotypes for anti-nutrients such as nitrate content which ranged from 15.0 mg/100 g (SPG-2) to 83.8 mg/100 g (CHIVARI-2-II and CARI-1) (Figure 2). The phytate content was ranged from 25.9 mg/100 g (CARI-1) to 669.0 mg/100 g (LMCF). The highest oxalate content was observed in CHIVAR-3-I (10.1 mg/100 g) while minimum in CARI-1 (2.7 mg/100 g). The *Capsicum* genotypes were low in saponin content which ranged from 40.0 mg/100 g (M-1) to 95.0 mg/100 g (M-2). The methanol extract of green fruits of CHIVAR-1-I showed highest antioxidant activity (96.3 %) while lowest by another AICRP (VC) genotype CHIVAR-3-II (67.9 %). The highest colour values of mature green fruits *Capsicum* genotypes was observed for SPG-3 (214.0 ASTA Units) while the minimum in LMCF (14.9 ASTA Units).

The cluster analysis of 42 genotypes using 16 phytochemical parameters formed eight major clusters with intra-cluster similarly value of 0.5 to 1.9. Cluster-I had mixed representation from AICRP (VC) (CHIVAR-1-I, CHIVAR-3-I and CHIVAR-1-II) and landraces (CCW and SPG-6). Cluster-II represented AICRP (VC) genotypes from mainland while Cluster-III was constituted of local landraces. Cluster-IV, cluster-V, cluster-VII and cluster-VIII were predominated with local landraces while cluster-VI corresponded to the AICRP (VC) materials.

Chemosystematics helped in distinguishing the difference in capsicum varieties and species. Based on flavonoid content, Ballard et al. (1970) reported that *C. baccatum* var. *baccatum* and *C. baccatum* var. *pendulum* were representative of same species. The present study investigated the extent of diversity and characterized 26 *Capsicu* landraces from Islands by analyzing 16 phytochemicals in green fruits as markers. The green fruits of *Capsicum* are commonly used as vegetable, chutney, pickle or taste agent in various food items. The landraces were rich in carotenoids, chlorophyll, free amino acids, reducing sugar, nitrate, saponin while reference genotypes were comparatively rich in polyphenol, flavonoids, tannin, ascorbic acid, total sugar and non-reducing sugar contents. The findings for the phytochemicals in the present study were in parity with the findings of Ruanma et al. (2005) and Rodriguez-Burruezo et al. (2009). However, the slight differences might be due to variations in stage of samples, estimation methods, genotypes and environment. Now-a-days, the researchers are utilizing different set of markers to establish the relationship among the species or genotypes of crops (Singh et al., 2012). The study also identified phytochemical rich landraces/genotypes such as CCO, M-1, CCB-1, CCR and

Table 2. Amplification parameters of DNA markers in *Capsicum* genotypes.

DNA marker	Marker sequence	Amplicon size (bp)	Total amplicons in genotype	Amplicons per genotype	PIC value
OPA3	AGTCAGCCAC	700-1400	138	12.55	0.35
OPA6	GGTCCCTGAC	1200-7000	198	24.75	0.35
OPA8	GTGACGTAGG	250-1200	136	9.71	0.33
OPD10	GGTCTACACC	300-1250	199	15.31	0.40
OPD13	GGGGTGACGA	250-1150	249	20.75	0.43
GC-32	$(GA)_8T$	400-1400	226	18.83	0.41
GC-37	$(CA)_8A$	100-1050	139	13.90	0.38
GC-47	$(CT)_8RC$	300-1350	292	29.20	0.31
GC-48	$(TC)_8G$	450-1400	148	14.80	0.36
GC-50	$(AC)_8YT$	300-1250	343	28.58	0.36

CCB-3 landraces for polyphenol, SPG-7, CCR, SPG-6, CCB-3, CCW, SPG-4, CCO, CCB-2 and M-1 for anthocyanin and SPG-7, CARI-1, CCO, SPG-5, MCF, SPG-2, CARI-2, CCR and H-2 for carotenoids. The significant differences in genotypes for colour value, free amino acids, anti-nutrients like phytate, oxalate, nitrate and saponin and sugars in test genotypes can be used in breeding programme. Overall, the study identified CARI-1, CCO, SPG-7 and G-1 as promising landraces from islands for quality breeding program in *Capsicum*.

Molecular diversity

Considerable genetic diversity was observed among the genotypes based upon RAPD and ISSR markers (Figure 3). Out of the 36 RAPD markers, five showed amplification in all the genotypes and the results are presented in Table 2. A total of 920 amplicons were scored in all the genotypes. OPD-13 produced maximum number of amplicons (249) with polymorphism information content value of 0.43. The amplicon size from five RAPDs ranged from 250 to 1400 bp. A of total 1148 bands were generated by five ISSR markers in 42 genotypes with average PIC value of 0.36 (Table 2). The highest numbers of bands were produced with ISSR-GC-50 (343), while minimum with ISSR-GC-48 (148). The amplicon size ranged from 100 to 1400 bp. The average similarity matrix from pooled data of RAPD and ISSR markers was used for generating a tree (Figure 4) for cluster analysis by unweighted pair group method with arithmetic average (UPGMA) using NTSYS 2.0 software package. Analysis revealed the overall similarity coefficient of 48% in 41 test genotypes of Capsicum. Except SPG-3 which was outlier with 54% intra-cluster similarity, the remaining 41 genotypes were divided into five clusters. Cluster-I had nine genotypes representing island landraces except CHIVAR-8-II. Cluster-II consisted of mixed genotypes from mainland (CHIVAR-3-II, CHIVAR-4-II) and islands (N-1, G-1, CCB-2). Cluster-III represented 11 genotypes mainly from islands (nine landraces) and only two CHIVAR-6-I and CA-334-1 from mainland. Cluster-IV also had mixed representation from mainland (six) and islands (four). Cluster-V predominantly represented the AICRP (VC) material except G-2 from islands.

The RAPD and ISSR markers are PCR based random markers which have been used in various studies for estimating the diversity in germplasm (Singh et al., 2012). Thul et al. (2012) also reported genetic similarities in Capsicum genotypes in the ranges of 23-88% and 11-96% with the RAPD and ISSR markers, respectively. Though, RAPD markers have limitations of reproducibility but precise regulation for PCR temperature and aliquot constituents can improve the reproducibility (Meunier and Grimont, 2012). The ISSR markers are rapid, simple, inexpensive, and highly reproducible due to their primer length and to the high stringency achieved by the annealing temperature. The study found good correspondence for diversity patterns generated with both DNA markers and functional markers viz. morphological and biochemical parameters within and between the species. Galvan et al. (2003) also reported good agreement between ISSR markers and morphological and biochemical. The polymorphic information content (PIC) value was measured to show the informativeness of used markers (Botstein et al., 1980). The amplified markers have considerable penetrance in the test genotypes which can be useful for further studies related to mapping of useful traits.

Correlation studies

The observation from different traits in three markers were assessed for correlation matrix and analyzed as pooled data. Three major set of markers showed significant diversity in *Capsicum* genotypes but no significant ($p>0.05$) correlation was observed among these markers. The r^2 value was 0.12 ($p>0.05$) for morphological and biochemi-cal markers while $r^2 = 0.08$ (p.0.05) for morphological and molecular markers. The biochemical and molecular markers showed $r^2=0.02$, indicating very poor correlation between them.

Similar analysis was conducted between biochemical traits and significant correlation was observed between

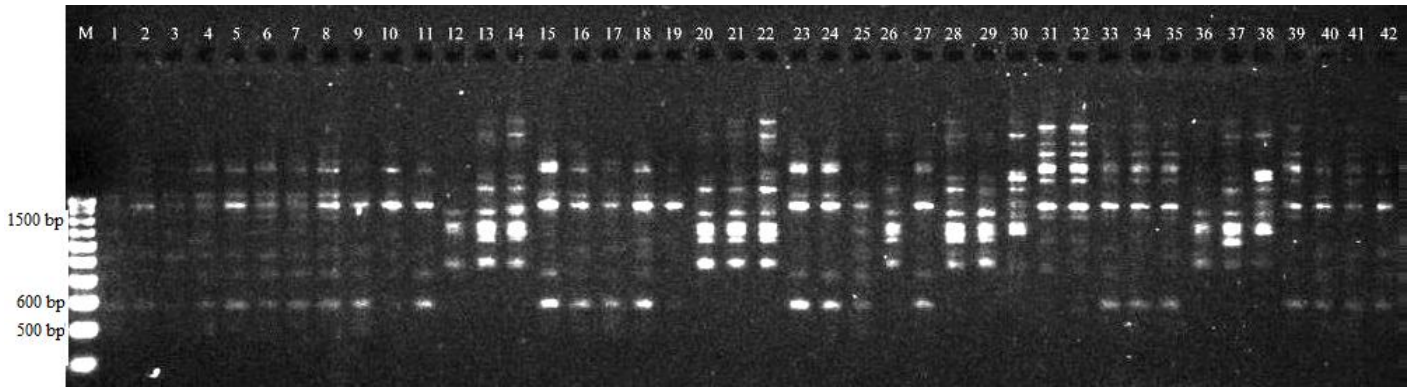

Figure 3. Diversity in 42 genotypes of *Capsicum* with RAPD-OPD10 (5' GGTCTACACC 3') marker. (S. No. 1-42 are genotypes as given in Table 1).

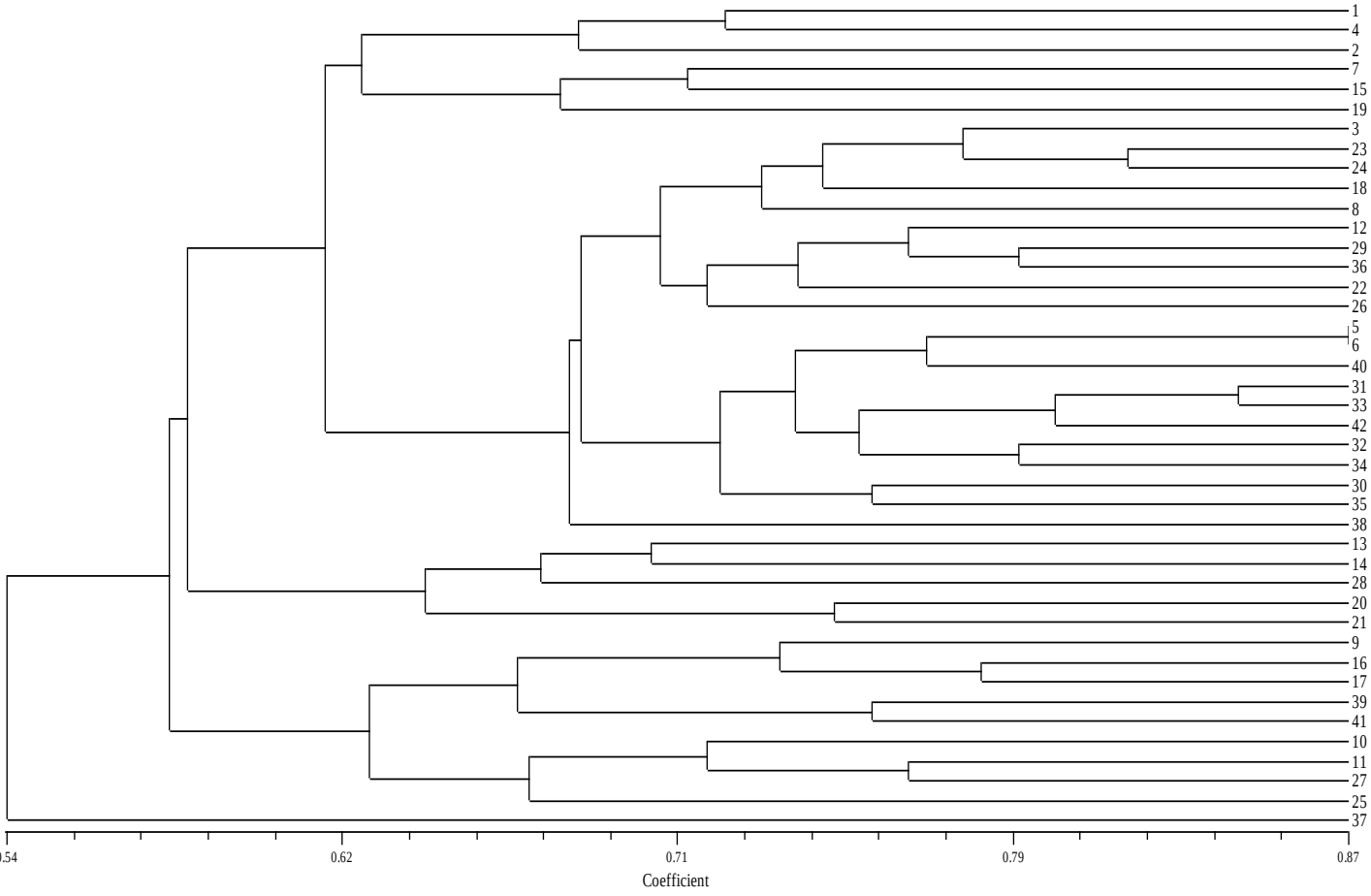

Figure 4. Dendrogram of *Capsicum* genotypes by UPGMA cluster analysis using DNA (RAPD and ISSR) markers similarity matrix (S. No. 1-42 are genotypes as given in Table 1).

antioxidant activity and polyphenol (r^2=0.33; p<0.05) and flavonoids (r^2=0.39; p<0.05). No correlation was observed between antioxidant and carotenoids, tannin, and ascorbic acid. However, positive correlation was observed between polyphenol and flavonoids (r^2 = 0.36; p<0.05) and flavonoids and tannin (r^2=0.570; p<0.01).

In conclusion, *Capsicum* landraces showed significant variation for morphological and biochemical parameters in islands. The local collections from islands showed significant difference over reference genotypes from

mainland India which may be due to adaptive changes in local collections. The morphological characteristics, bio-chemical and the molecular markers are found to be useful toward the delineation of the diversity in Capsicum landraces and identification of genetic stock. The distinct landraces Capsicum can be used in breeding program or collected and conservation in gene bank for further use.

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