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Assessment of Genetic Relationships among South Indian Chilli (*Capsicum annum* L.) Cultivars Using RAPD and ISSR Markers

Koona Subramanyam[1*] • Kondeti Subramanyam[2] • Pinnamaneni Rajasekhar[1] • Chirra Srinivasa Reddy[1]

[1] Department of Biotechnology, Sreenidhi Institute of Science and Technology (Autonomous), Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh 501301, India

[2] Plant Molecular Biology Laboratory, Department of Biotechnology, Bharathidasan University, Tiruchirappalli – 620024, Tamil Nadu, India

Corresponding author: * **subbubiochem2003@gmail.com**

ABSTRACT

The present investigation was carried out with the objective of evaluating genetic diversity in chilli (*Capsicum annum*. L). A total of 24 south Indian chilli cultivars including five commercial hybrids were characterized using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) analyses. Out of 50 primers employed to generate RAPD profiles, reproducible bands were obtained with 16, 15 (93.75%) of which could detect polymorphism. A total of 121 bands were scored, out of which 59 bands (48.76%) were polymorphic. RAPD primer SKURAPD18 exhibited the highest level of polymorphism (81.81%). ISSR analysis was carried out by using 13 ISSR primers, 10 of which produced reproducible amplified fragments, and 9 of which (i.e., 90%) showed polymorphic bands. From 9 ISSR primers 97 fragments were amplified, 61 (62.88%) of which were polymorphic. Primer SKUISSR8 showed maximum polymorphism (83.83%). A dendrogram was developed using Jaccard's coefficient of similarity and UPGMA with RAPD and ISSR data. The constructed dendrograms revealed that the commercial hybrid cultivars formed separate clusters. All 15 RAPD primers and 9 ISSR primers could distinguish all chilli cultivars. Only 9 ISSR primers were needed to generate sufficient information about genetic diversity, whereas 15 RAPD primers were required.

Keywords: *Capsicum annum*. L, dendrogram, genetic diversity, ISSR, RAPD, UPGMA

Abbreviations: AFLP, **a**mplified fragment length polymorphism; **ISSR**, inter simple sequence repeats; **RAPD**, randomly amplified polymorphism of DNA; **RFLP**, restriction fragment length polymorphism, **UPGMA**, unweighted pair group method with arithmetic average

INTRODUCTION

Chilli pepper (*Capsicum annum* L.) is an important vegetable and spice crop valued for its aroma, taste, pungency, and flavor (Sreelathakumary *et al*. 2004), which belongs to the family Solanaceae (Sanatombi and Sharma 2007). In addition to their importance as a vegetable, chillies have also received attention recently for their potential as nutraceuticals. Although used primarily for seasoning, it is now recognized that chilli pepper has played a major nutritional role in many cultures by supplying them with a primary source of vitamin C. *Capsicum*, also well known for many important chemicals such as steam volatile oil, fatty acids, capsaicinoids, carotenoids, vitamins, proteins and mineral elements (Bosland and Votana 2000) which play an important role in human health. Carotenoids present in chilli extract are known to have a synergetic anti mutagenic effect and *in-vitro* anti tumour promoting activity (Maoka *et al*. 2001). Topical capsaicin is effective against many painful conditions such as post herpetic neuralgia, diabetic neuropathy and osteoarthritis (Rains and Bryson 1995). Chilies also possess antifungal property against fungal species such as *Aspergillus* and *Fusarium* (De Lucca *et al*. 2006).

The evaluation of genetic diversity and construction of linkage maps would promote the efficient use of genetic variations in the breeding programmes (Paterson *et al*. 1991). DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann 1983). DNA based molecular markers have been used for cultivar identification, assessment of the genetic relationships between germplasm in many plant species and to study genetic relationship between individuals and species (Gepts 1993). Among the different DNA based molecular markers, RAPD and ISSR are very important to study the genetic variation among and within the species. Williams *et al*. 1991 developed the concept of RAPD and due to the speed and efficiency; RAPD markers were extensively used for the construction of high-density genetic maps in *Capsicum annum* L. (Cao 1994; Las Heras Vazquez *et al*. 1996; Wang *et al*. 1996; Ilan *et al*. 1998; Rodríguez *et al*. 1999; Baral and Bosland 2002; Sitthiwong *et al*. 2005; Adetula 2006; Dhanya *et al*. 2008; Makari *et al*. 2009; Maheshwari and Chandrashekhar 2011).

Inter simple sequence repeats (ISSR) are a type of DNA marker which involves the use of microsatellite sequences directly in the polymerase chain reaction for DNA amplifications (Gupta *et al*. 1994). Unlike simple sequence repeats (SSR), inter simple sequence repeat markers are generated using primers that amplify regions between SSR loci (Zeitkiewicz *et al*. 1994). To prevent incidental annealing of primers with in SSR, leading to smear formation, ISSR primers are anchored with one or two nucleotides at their 3′ end. These markers provide highly effective plant fingerprinting (Prevost and Wilkinson 1999; Arcade *et al*. 2000). ISSR primers are easier to design than SSR primers, as they do not require sequence knowledge. The repeatability of ISSR-PCR is better than SSR-PCR, RAPD-PCR, because ISSR primers are longer and hence have higher annealing temperatures (Kojima *et al*. 1998).

The present investigation was undertaken to develop the suitable RAPD and ISSR markers for the identification of DNA polymorphism in *C. annum* with the objective of determining the genetic distances, similarities between the different cultivars which were collected in different locations in south India.

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Original Research Paper

Table 1 List of chilli (*Capsicum annuum*.L) cultivars used in this study.

Cultivar	Collection center	Morphological characters
Hindpur	Anantapur, Andhrapradesh	Extremely pungent and red in color
Dhani	Bangalore, Karnataka	Tiny, green and color changed to bright red after maturation
Kanthari	Trivandrum, Kerala	Small, ivory white in color, extremely pungent
Indam Kaddi	IAHS, Bangalore, Karnataka	Slender, long, light green fruits with wrinkles, mild pungency
Indam Jwala	IAHS, Bangalore, Karnataka	Slender, light green fruits, medium pungency
Indam Dabba	IAHS, Bangalore, Karnataka	Light green, thick, medium to long, wrinkled, mild pungency
Indam 42	IAHS, Bangalore, Karnataka	Thick, green glossy fruits, light pungent, ripened fruit red in color
Indam 54	IAHS, Bangalore, Karnataka	Light green, pendent fruits, moderately high pungency
Pusa jwala	Bangalore, Karnataka	Long, slender, unripe fruit green and ripen fruit red in color
Arka sakthi	Trivandrum, Kerala	Long, slender, unripe fruit green and ripen fruit red in color
Naga Jolika	Bangalore, Karnataka	Long, conical, yellowish green- and ripen fruit red in color
Byadagi Ellachipur	Bangalore, Karnataka	When dried the skin is wrinkled red in color with aromatic odour
Warangal Chappatta	Warangal, Andhrapradesh	Short, dark red in color when dried with moderate pungency
Gundu Molzuka	Coimbatore, Tamilnadu	Roundish fruit, yellowish red in color, moderately pungent
Sanman	Khammam, Andhrapradesh	Dark red in color when in dried state with very hot pungency
TRBU04	Bhemavaram, Andhrapradesh	Dark red in color when in dried state with very hot pungency
Nakhara Local	Calicut University, Kerala	Thick, green fruits, moderate pungent, ripened fruit red in color
Nalcheti	Coimbatore, Tamilnadu	Long, red in color when dried and highly pungent
Jwala	Bangalore, Karnataka	Long, slender, unripe fruit green and ripen fruit red in color
AS2105	JNTU, Andhrapradesh	Long,, unripe fruit green and ripen fruit red in color and pungent
AS2104	JNTU, Andhrapradesh	Long with thick red skin with very high pungency
AS2103	JNTU, Andhrapradesh	Smooth shining skin, fleshy with dark red in color, highly pungent Long with thick red skin
Guntur	Warangal, Andhrapradesh	with very high pungency
Surya	JNTU, Andhrapradesh	Long, slender, unripe fruit green and ripen fruit red in color

MATERIALS AND METHODS

Genotypes

The experimental analysis comprised of about 24 south Indian chilli cultivars including five commercial hybrids (**Table 1**), collected in the form of seeds from different stations in south India, and germinated in department field research facility garden, Jawaharlal Nehru Technological University (OTRI), Anantapur, Andhra Pradesh during the appropriate season.

Total genomic DNA isolation

The total genomic DNA was isolated from one-month-old *C. annum* field-grown plant leaves by following modified cetyl trimethyl ammonium bromide (CTAB) method described by Doyle and Doyle (1990). The isolated genomic DNA concentration was estimated at 260 nm by using a Chemito spectrophotometer and diluted the genomic DNA concentration to 20 ng/µl with sterilized TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0) for the RAPD and ISSR analysis.

RAPD-PCR amplification

50 arbitrary oligo nucleotide primers of 10 bp length (Sigma Genosys, Texas, USA) were used for the analysis of polymorphism in 24 different south Indian chilli cultivars. RAPD-PCR reaction was carried out by following the method described by Williams *et al*. (1990) with minor modifications. The reactions were carried out in a total reaction volume of 25 µl containing 35 ng genomic DNA, 0.2 mM each dNTP (Fermentas Life Sciences, Leon-Rot, Germany), 0.2 µM of random primer (Sigma Genosys, Texas, USA), 0.5 U of *Taq* polymerase (Fermentas Life Sciences) and 2.5 µl of 10X *Taq* polymerase buffer using PTC-100 thermal cycler (MJ Research Inc., Waltham, Mass, USA) programmed for an initial denaturation at 94°C for 6 min and 35 cycles at 94°C for 1 min, 35°C for 1 min, 72°C for 2 min which was followed by final extension at 72°C for about 7 min. The amplified products were separated electrophoretically on 1.6% agarose gel in 1X TBE buffer (100 mM Tris-HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) containing ethidium bromide at 100 V and photographed under ultraviolet light using a Gel Doc 2000 (Bio-RAD, Hercules, CA). The amplified fragments molecular weights were estimated by using λ DNA *Hind* III digest marker (Bangalore Genei, Bangalore, India). Each RAPD-PCR reaction was carried out in triplicate.

ISSR-PCR amplification

For ISSR-PCR, 13 ISSR primers were used to assess the polymorphism between 24 south Indian cultivated chilli varieties. The PCR reaction was carried out in 25 µl reaction containing 10 ng of isolated genomic DNA, 0.25 mM each dNTP, 0.3 µM ISSR primer, 1 U of *Taq* DNA polymerase and 2.5 µl of 10X *Taq* polymerase buffer. The amplification was carried out on a PTC-100 thermal cycler (MJ Research Inc.) programmed for 5 min initial denaturetion at 94°C followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 2 min with a 10 min final extension at 72°C. The PCR products along with the 100 bp ladder were separated on 2% agarose gel in 1X TBE buffer containing ethidium bromide at 150 V and photographed under ultraviolet light using Gel Doc 2000 (Bio-RAD, Hercules, CA). Each ISSR-PCR reaction was carried out in triplicate.

Data analysis

Reproducible DNA bands, i.e. bands present in all three repetitions of individual sample were scored manually. Weak bands with negligible intensity were excluded from final data analysis. Band profiles for each parent were scored in a binary mode with "1" indicating its presence and "0" indicating its absence. Using the binary data, a similarity matrix was constructed using the Jaccard's coefficient, which was further subjected to UPGMA clustering analysis and a dendrograms were generated using the soft ware package NTSYS – PC (version 2.02e).

RESULTS

RAPD polymorphism

Amplification of genomic DNA of the 24 south Indian chilli cultivars, including five commercial hybrids, using 50 primers for RAPD analysis, but only 15 primers (30%) yielded 121 reproducible intensely stained fragments that could be scored. All the chosen 15 primers amplified fragments across the 24 cultivars studied, with the number of amplified fragments ranging from three (SKURAPD23) to 12 (SKURAPD42), which varied in size from 125 bp (SKURAPD45) to 2,322 bp (SKURAPD18). Of the 121 amplified bands, 59 were polymorphic (48.76%), with an average of 3.93 polymorphic fragments per primer. The details of the primers producing polymorphic bands are presented in **Table 2**. Percentage polymorphism ranged from

Table 2 RAPD-PCR fingerprint pattern of the 24 chilli cultivars obtained by using 15 RAPD primers.

Primer name	Primer sequence	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
SKURAPD1	5' CAGCCTCGGC 3'	7	3	42.85
SKURAPD5	5' GGGACGTCTC 3'	8	4	50.00
SKURAPD11	5' CCGGTGTGGG 3'	7	5	71.42
SKURAPD16	5' CCCTGTCGCA 3'	6	1	16.60
SKURAPD18	5' GGTGACGCAG 3'	11	9	81.81
SKURAPD19	5' CTGAGACGGA 3'	5	2	40.00
SKURAPD21	5' TGCCCGTCGT 3'	8	3	37.50
SKURAPD22	5' CTCTCCGCCA 3'	9	5	55.55
SKURAPD23	5' CGCCCCACGT 3'	3	1	33.33
SKURAPD32	5' CCTTGACGCA 3'	7	4	57.14
SKURAPD35	5' ACGCACAACC 3'	11	5	45.45
SKURAPD37	5' GAGGATCCCT 3'	7	3	42.85
SKURAPD40	5' ACGGTACCAG 3'	6	2	33.33
SKURAPD42	5' GAGTCTCAGG 3'	12	6	50.00
SKURAPD45	5' TGGCCCTCAC 3'	10	6	60.00

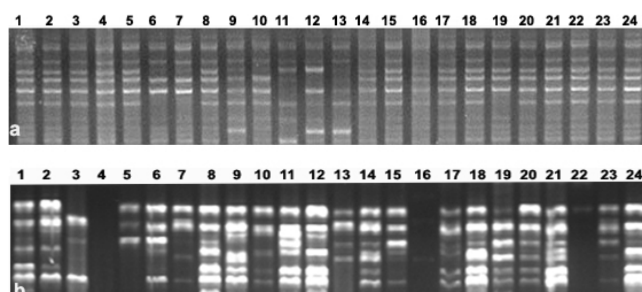

Fig. 1 Fingerprint patterns generated using RAPD and ISSR. a. RAPD polymorphism among the 24 South Indian chilli cultivars using SKURAPD18 primer. **b**. ISSR polymorphism among the 24 South Indian chilli cultivars using SKUISSR08 primer.

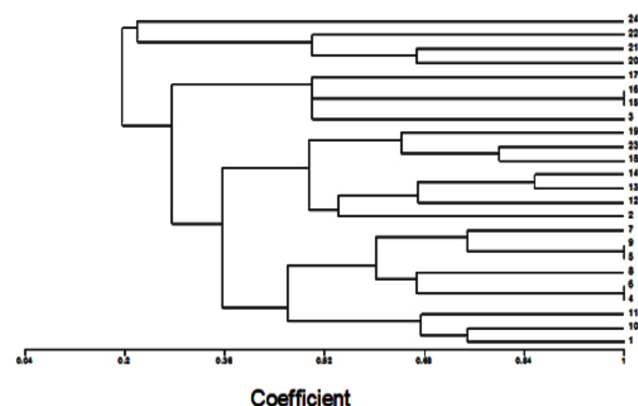

Fig. 2 Genetic relationships among 24 south Indian chilli cultivars based on 15 RAPD markers using Jaccard's coefficient of similarity and UPGMA clustering.

16.6% (SKURAPD16) to a maximum of 81.81% (SKURAPD18), with an average of 47.85% polymorphism. Only five out of 15 primers showed more than 55% polymorphism. **Fig. 1A** shows the RAPD profile of 24 south Indian chilli cultivars with primer SKURAPD18.

The dendrogram constructed based on RAPD analysis by using UPGMA (NTSYS-PC) is shown in **Fig. 2**. A dendrogram based on UPGMA analysis grouped the 24 cultivars into two main clusters, with Jaccard's similarity coefficient ranging from 0.04 to 1.00. Cluster I comprised of 4 cultivars ('Surya', 'AS2103', 'AS2104', and 'AS2105') where as cluster II had 20 cultivars. Cluster II could be further divided in to two groups at similarity coefficient of 0.36. Group I contained 4 chilli cultivars ('Nakara Local', 'TRBU04', 'Sanman', and 'Kanthari'), whereas group II had remaining 16 cultivars. Group II could be further subdivided in to two subgroups at similarity coefficient of 0.45. Subgroup I comprised of 7 cultivars ('Jwala', 'Guntur', 'Nalcheti', 'Gundu Molzuka', 'Warangal Chappatta', 'Byadagi Ellachipur', and 'Dhani'), where as subgroup II contained remaining 9 cultivars. In subgroup I, the cultivar 'Dhani' separated from the remaining 6 cultivars at similarity coefficient of 0.54. 'Pusa Jwala' and 'Indam Jwala' appeared to be closer to each other, with a 1.00 similarity coefficient. The differences among the subgroups gradually reduced. The clustering pattern was very random and not in consonance with the geographical distribution or morphological characters.

ISSR polymorphism

Thirteen ISSR primers were used to study the polymorphism in 24 south Indian chilli cultivars. Out of 13 ISSR primers, nine primers (69.23%) generated reproducible and polymorphic bands. Therefore, we used these nine primers for further polymorphic analysis in 24 chilli cultivars. The details of the primer and polymorphic bands are representing in **Table 3**. ISSR primers produced varying numbers of DNA fragments, depending on their SSR motifs. The number of bands produced with different selective nucleotide (CG) varied more with the poly (GA) primers than with the poly (AG) primers. The primers that were based on the poly (AG) motif produced more polymorphism on average (83.83%) than the primers based on the poly (GA) motifs (71.42%). The nine primers produced 97 bands across 24 cultivars, of which 61 were polymorphic, accounting for 62.88%. Number of bands varied from seven (SKUISSR16) to 13 (SKUISSR3 and SKUISSR5), with an average of 10.7 bands/primer. Maximum number of (10) polymorphic bands were obtained with the primer SKUISSR8 and minimum number (5) with primers SKUISSR5, SKUISSR12 and SKUISSR16. The percentage of polymorphic bands ranged from 38.46% (SKUISSR5) to 83.83% (SKUISSR8) with an average of 63.3% of polymorphism. The size of the amplified fragments ranged from 150 bp (SKUISSR3) to 700 bp (SKUISSR8). The primers based on (AG) and (GA) motifs produced high average polymorphism rates of 83.83

Table 3 ISSR-PCR fingerprint pattern of the 24 chilli cultivars obtained by using 9 ISSR primers.

Primer name	Primer sequence	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
SKUISSR3	[AC]8YG	13	9	69.23
SKUISSR5	[AC]8G	13	5	38.46
SKUISSR6	[AC]8YA	11	7	63.63
SKUISSR8	[AG]8YT	12	10	83.83
SKUISSR9	[CA]8GT	10	6	60.00
SKUISSR10	[GATA]4	11	6	54.54
SKUISSR12	[CT]8T	8	5	62.50
SKUISSR15	[CA]8YC	12	8	66.66
SKUISSR16	[GA]8YT	7	5	71.42

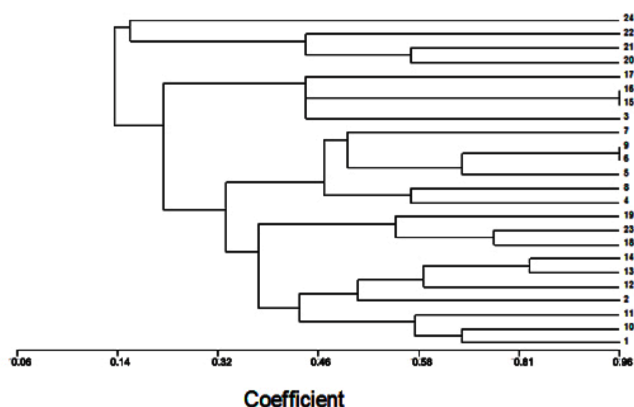

Fig. 3 Genetic relationships among 24 south Indian chilli cultivars based on 15 RAPD markers using Jaccard's coefficient of similarity and UPGMA clustering.

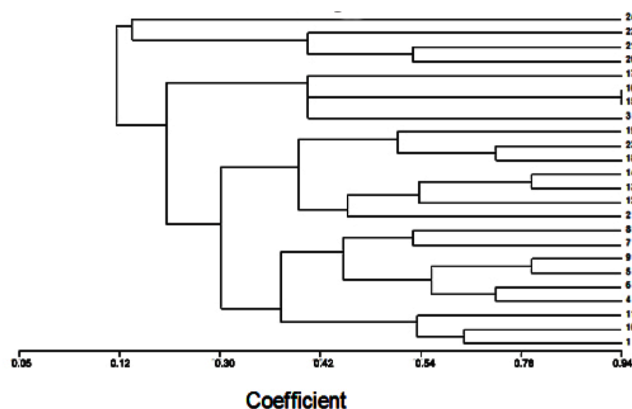

Fig. 4 Genetic relationships among 24 south Indian chilli cultivars based on RAPD and ISSR data using Jaccard's coefficient of similarity and UPGMA clustering.

and 71.42%, respectively. **Fig. 1B** shows the ISSR profile of 24 south Indian chilli cultivars with primer SKUISSR08.

The ISSR bands were scored for presence or absence among the genotypes and used for the UPGMA cluster analysis. A dendrogram based on UPGMA analysis with ISSR data is shown in **Fig. 3**. Jaccard's similarity coefficient ranged from 0.06 to 0.96. The 24 cultivars were separated into two main clusters. The cluster I comprised of 4 cultivars ('Surya', 'AS2103', 'AS2104', and 'AS2105'), where as cluster II contains remaining 20 cultivars. Cluster II were further divided in to two sub clusters at a similarity coefficient of 0.32. Sub cluster I comprised of 4 cultivars ('Nakara Local', 'TRBU04', 'Sanman', and 'Kanthari'), where as sub cluster II contained remaining 16 cultivar. Sub cluster II further divided in to two groups at a similarity coefficient of 0.38. Group I comprised of 6 cultivars ('Indam 42', 'Pusa Jwala', 'Indam Dabba', 'Indam Jwala', 'Indam 54', and 'Indam Kaddi'), whereas group II composed of remaining 10 cultivars. Group II inturn was further subdivided in to two sub groups at a similarity coefficient of 0.40. Subgroup I composed of 3 cultivars ('Jwala, Guntur', and 'Nalcheti') and sub group II composed of remaining 7 cultivars ('Gundu Molzuka', 'Warangal Chappatta', 'Byadagi Ellachipur', 'Dhani', 'Naga Jolika', 'Arka Sakthi', and 'Hindupur'). In subgroup II, the cultivar 'Dhani' separated from remaining 6 cultivars at a similarity coefficient of 0.50. 'TRBU04' and 'Sanman' were appeared to be closer to each other, with a 0.96 similarity coefficient. The differences among the subgroups gradually reduced.

RAPD and ISSR data

To decrease the inaccuracies of the independent techniques, a dendrogram was developed by pooling the data of both RAPD and ISSR. Two major clusters were observed in this UPGMA dendrogram with Jaccard's similarity coefficient ranging from 0.05 to 0.94 (**Fig. 4**). The 24 cultivars were separated into two main clusters. The cluster I comprised of 4 cultivars ('Surya', 'AS2103', 'AS2104', and 'AS2105'), where as cluster II contains remaining 20 cultivars. Cluster II were further divided in to two sub clusters at a similarity coefficient of 0.30. Sub cluster I comprised of 4 cultivars ('Nakara Local', 'TRBU04', 'Sanman', and 'Kanthari'), where as sub cluster II contained remaining 16 cultivar. Sub cluster II further divided in to two groups at a similarity coefficient of 0.38. Group I comprised of 7 cultivars ('Jwala', 'Guntur', 'Nalcheti', 'Gundu Molzuka', 'Warangal Chappatta', 'Byadagi Ellachipur', and 'Dhani'), whereas group II composed of remaining 9 cultivars. Group II sequentially was further subdivided in to two sub groups at a similarity coefficient of 0.45. Subgroup I composed of 6 cultivars ('Indam 54', 'Indam 42', 'Pusa Jwala', 'Indam Jwala', 'Indam Dabba', and 'Indam Kaddi') and sub group II composed of remaining 3 cultivars ('Naga Jolika', 'Arka Sakthi', and 'Hindupur'). 'TRBU04' and 'Sanman' were appeared to be closer to each other, with a 0.94 similarity coefficient. The differences among the subgroups gradually reduced.

The pattern of clustering of the genotypes remained more or less the same like in ISSR and RAPD data, whereas the dendrogram based on RAPD showed some variation in the clustering of genotypes.

DISCUSSION

The evolution of varieties in different agro climatic zones demonstrates the significant level of variation in response to the selection pressure in the zones (Singh *et al.* 1998). Differences between cultivars with respect to agronomic characters, morphological characters, biochemical characters (e.g. storage proteins and isozymes) and molecular characters are either direct or indirect representations of differences at the DNA level and are therefore expected to provide information about genetic relationships. The assessment of genetic diversity is important not only for crop improvement but also for the efficient management and conservation of germplasm resources. For this purpose, 24 South Indian chilli cultivars including five commercial hybrids were analyzed by using fifteen RAPD and nine ISSR primers. In this study, we found that significant level of polymorphism among the 24 cultivars of chilli, in RAPD and ISSR were 47.85%, 62.88% respectively. Several reports proved that RAPD is a choice to assess the molecular polymorphism in pepper (**Table 4**). Makari *et al.* (2009) analyzed the genetic diversity in ten commercially available chilli varieties by using RAPD markers. Maheshwari and Chandrashekhar (2011) also successfully analyzed the genetic relationship among 45 accessions of chilli by RAPD markers. The success of our study in identifying polymorphism is due to the use of a number of randomly selected prescreened highly informative primers.

ISSR markers are more efficient than the RAPD markers as they detected 62.88% polymorphic DNA markers in *C. annum* compared with 47.85% of RAPD markers. The 15 RAPD and nine ISSR primers in the present study yielded about 120 polymorphic markers that unambiguously discriminated 24 cultivars into two clusters. The number of total polymorphic and discriminant fragments is higher for ISSR than RAPD. The ISSRs have a high capacity to reveal polymorphism and offer great potential to determine intra and intergenic diversity as compared to other arbitrary primers like RAPDs (Zietkiewicz *et al.* 1994). Geographically isolated population accumulates genetic differences as they adapt to different environment. Genetic variation among different cultivars of chilli based on RAPD and ISSR analysis could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes.

Table 4 Genetic polymorphism analysis in different chilli cultivars using RAPD markers by various research groups.

Research group	No. of cultivars tested	Total no. of RAPD primers used	Number of RAPD primers responded	Total number of bands	Number of polymorphic bands	% of polymorphism
Las Heras Vazquez *et al*. 1996	11	50	42	219	52	23.74
Ilan *et al*. 1998	34	21	19	158	34	22
Rodriguez *et al*. 1999	134	74	25	162	124	76.5
Baral and Bosland 2002	111	55	18	146	94	64.3
Adetula 2006	40	20	9	99	67	67.6
Maheshwari *et al*. 2011	45	25	16	99	63	63.3

The dendrograms did not indicate any clear pattern of clustering of 24 chilli cultivars according to the location in which they were collected. The different ability to resolve genetic variation among the cultivars may be partially related to the number of PCR products analyzed with each marker system (121 for RAPD and 97 for ISSR). The putatively similar bands originating from RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pair. This situation may lead to wrong results when calculating genetic relationships (Fernandez *et al*. 2002).

Hence, because of these problems we developed another dendrogram by pooling the data of both RAPD and ISSR. It clearly showed that all commercial hybrid cultivars (‘Indam Jwala’, ‘Indam Kaddi’, ‘Indam Dabba’, ‘Indam 42’ and ‘Indam 54’) formed separate cluster. It indicates, all the commercial hybrids may be originated from same parental line. When the dendrogram was correlated with the pedigree data, it was found that very few cultivars with common parents clustered together (‘TRBU04’ and ‘Sanman’). The dendrogram showed similar clustering pattern for the commercial hybrid cultivars with that of RAPD, but in the case of other cultivars, it showed variation with the dendrogram of both RAPD and ISSR.

ISSR analysis is more economical and reliable than that of RAPD. Earlier studies also reported that ISSR technique generates large number of polymorphisms (Collard *et al.* 2003). Thus, ISSR markers are useful in the assessment of chilli diversty, the detection of duplicate samples in germplasm collection and the selection of a core collection to enhance the efficiency of germplasm management for use in chilli breeding and conservation.

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