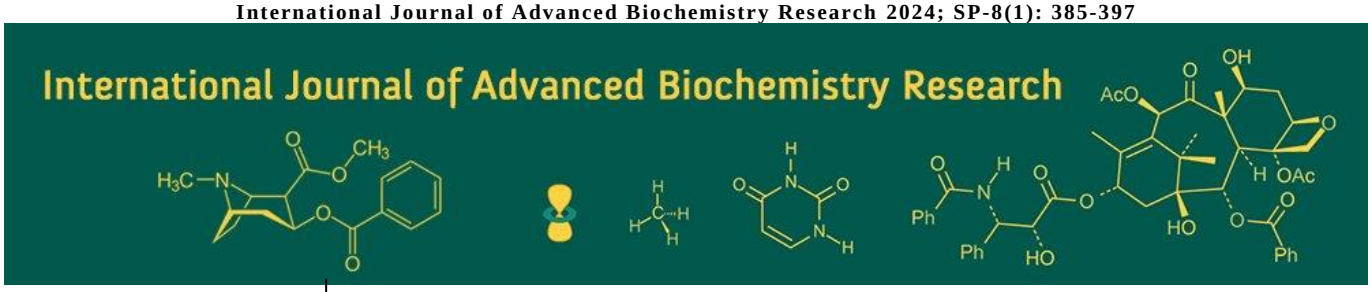

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 385-397
www.biochemjournal.com
Received: 20-11-2023
Accepted: 30-12-2023

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Assessment of genetic diversity in different Chilli (*Capsicum annuum* L.) genotypes using morphological, biochemical and molecular characters

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DOI: https://doi.org/10.33545/26174693.2024.v8.i1Sf.341

Abstract
Chilli (*Capsicum annum* L.) is an important vegetable and spice crop from *Solanaceae* family. Study on genetic diversity was conducted with twenty Chilli genotypes. Eleven quantitative characters *viz.*, plant height, fruit length, fruit width, fruit weight, no. of seeds, test weight of seed, ascorbic acid, total protein content, capsaicin, total chlorophyll content and total carotenoid content were taken into consideration. Based on combined analysis of morphological and biochemical data, highest genetic dissimilarity (36%) was observed between genotypes 2011/CHIVAR-7 and KTPL-19. RAPD analysis produced total 143 loci, out of which 104 loci were polymorphic (72.22%). The average Polymorphism Information Content value for RAPD markers were 0.88, thus considered as highly useful. From the cluster analysis of RAPD data, three clusters were formed. Based on our results, we can say that molecular study was found to be more precise in nature and gave clear picture of genetic relationship among studied Chilli genotypes.

Keywords: *Capsicum*, dendrogram, RAPD marker, genetic diversity

Introduction
Chilli is an important vegetable as well as spice crop in India, distributed world-wide and believed to be native of Tropical South America. The Chilli belongs to the *Solanaceae* family and the genus *Capsicum* with a wide genetic diversity, which is composed of 27 species, five domesticated and 22 semi-domesticated and wild-ones (Votava *et al*. 2002; Costa *et al*. 2006) [58, 9]. India is a major producer, exporter and consumer of Chilli. The area and production of Chilli in the country is 767 thousand ha and 1203 thousand tons respectively (FAOSTAT, 2014). Among the domesticated *Capsicum* species, pungent and non-pungent forms of *Capsicum annuum* L. (pepper) are most popular and have a worldwide commercial distribution. In India, hot pepper or Chilli is an important commercial crop which is cultivated for vegetable, spice and value-added processed product. Chilli consists of 12 chromosome pairs with a variable genome size from 3,200 to 5,600 Mb (Pakozdi *et al*. 2002). The genus *Capsicum* is often cross pollinated crop which accounts for considerable variation in terms of fruit and yield parameters (Hosamani and Shivkumar 2008) [23]. A wide range of variability is reported in this crop in India as well (Sreelathakumary and Rajamony 2004) [49]. To use genetic resources adequately, it is necessary to understand how the genetic variation is distributed and which environmental and species characteristics influence this distribution (Altieri and Merrick 1987) [2]. Thus, success in a crop improvement programme depends, chiefly on the availability of genetic variability in the crop.
Morphological markers are the conventional markers and used as fundamental methodology for studying genetic diversity followed by the use of biochemical markers. Morphological markers allow scoring of qualitative traits visually and are usually dominant or recessive. Morphological characters such as fruit weight, flower color, fruit shape, plant height *etc*., have been used to distinguish among Chilli genotypes and classify them into groups (Weerakoon and Somaratne 2010) [59]. Furthermore, seed proteins, used as genetic markers convey greater precision to measures of genetic diversity because they are the primary products of structural genes (Srivalli *et al*. 1999) [50]. Study on Proteomic in plants aim to detect precise proteins which are related to biotic and abiotic stress.

Biochemical markers (isozyme and alloenzyme) are proteins produced by gene expression which can be isolated and identified by electrophoresis and staining. Protein markers particularly isozymes have been used as biochemical markers. Biochemical characters such as ascorbic acid, capsaicin, carotenoids and capsaicin are usually used to distinguish among Chilli genotypes due to their valuable properties. Chilli provides a tremendous income opportunity to the farmers and also rich source of vitamin A and C. Chilli also contains considerable amount of minerals such as phosphorus, iron, potassium, Sulphur and calcium (Berke, 2002) [6].

However, morphological markers and protein marker studies are influenced by environment, stage specific, sex limited and their genome coverage is also low thus, lack adequate polymorphism (Singh *et al*. 2010) [48]. To solve this problem, more reliable markers are used, *i.e*., molecular marker. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Molecular markers are the DNA sequences which are readily detected and easily monitored for their inheritance. The used of these markers is based on naturally occurring DNA polymorphism. DNA markers are based on two different approaches *viz.*, Non-PCR based (RFLP) and PCR based (RAPD, SSR, ISSR, AFLP, etc.) techniques. The knowledge of genetic variability estimated from RAPD, AFLP, RFLP ISSRs and SSRs markers provide plant breeders with different levels of information that would cater for germplasm management and crop improvement programmes (Tam *et al*. 2005) [52].

The RAPD (random amplified polymorphic DNA) method is a widely used technique for molecular marker analysis and is based on the amplification of genomic DNA fragments by using primers of arbitrary nucleotide sequences; which in turn detects polymorphisms that can be employed as genetic markers without knowledge of previous genetic sequences (Williams *et al*. 1993) [62]. The technique has been successfully used to distinguish accessions, to evaluate genetic diversity among them, to recognize duplications in germplasm collections and for varietal identification (Virk *et al*. 1995; Daher *et al*. 2002; Teixeira-Cabral *et al*. 2002; Palomino *et al*. 2005; Singh *et al*. 2008) [57, 12, 53, 36, 46]. It can be used to elicit information on genetic differences among individuals of a population between lines or germplasm accessions or any breeding material (Welsh and McClelland, 1990; Williams *et al*., 1990) [61, 63]. In addition to being simple and fast this method is not affected by the prevailing environmental conditions, present in all plant, does not require radioactive markers and consumes minimum amount of DNA.

Therefore, in order to manage, preserve and improve different genotypes of Chilli, the present investigation was planned with the objective to examined genetic diversity in Chilli at morphological, biochemical and molecular level.

Materials and Methods

Biological material

The investigation was carried out using 20 different types of *Capsicum annuum* L. genotypes (Table 1), at Department of Plant Molecular Biology and Biotechnology, Navsari Agricultural University, Navsari (Gujarat), during year 2014-2015. Seeds for the experiment were collected from Department of Vegetable Science, ACHF, Navsari (Gujarat) and grown at Regional Horticulture Research Station (RHRS) farm, Navsari Agricultural University, Navsari (Gujarat).

Morphological characterization

Quantitative (plant height, fruit length, fruit width, fruit weight, no. of seeds per fruit and seed test weight) and qualitative (plant branching type, fruit color and seed color) morphological characters of 20 chilli genotypes were observed from plants, fruits and seeds according to methodology described in "Descriptors for *Capsicum* spp. - IPGRI" (IPGRI *et al*. 1995).

Biochemical characterization

Biochemical characterization was carried out using fresh Chilli fruits collected at intermediate stage of all 20 genotypes. Biochemical contents like Ascorbic acid content (Sadasivam and Balasubramanian 1987) [42], Total protein content (Lowry *et al*. 1915) [29], Total chlorophyll content (Hiscox and Israelstam 1979), Total carotenoid content (Wellburn, 1994) [60] and Capsaicin content by modified Bajaj method given by Gibbs and Garro (2004) [20] were quantified for biochemical characterization.

Molecular characterization

Genomic DNA isolation

The genomic DNA was extracted from fresh and tender leaves of Chilli genotypes using CTAB method of DNA extraction initially given by Murray and Thomson (1980) [33] and later on modified by Doyle and Doyle (1990) [15]. In brief it can be described as below: 100 mg of fresh leaf tissue was powdered and homogenized with 1 ml of pre-warmed extraction buffer and incubated at 65 ºC for 90 min followed by addition of equal volume of chloroform: isoamyl alcohol (24:1) and its inverted by mixing 4-5 times. The sample so prepared was spinned at 10,000 rpm, 4 ºC for 12 min and supernatant was collected and so repeated if necessary. Further for precipitation, supernatant was added with double volume of chilled absolute ethanol and 1/10th volume of 3 M sodium acetate (pH 5.2), mixed and incubated at -20 ºC for 2 hrs. After precipitation, it was spinned at 10,000 rpm, 4 ºC for 12 min and the pellet so formed was given a wash with 70% EtOH. The air dried pellet so formed was dissolved in 50 µl of TE buffer and extracted DNA was stored at -20 ºC. The samples with RNA impurities were treated with RNase and then stored. The integrity and size of DNA was checked on 0.8% agarose gel and was quantified using a Nano-spectrophotometer.

RAPD amplification

The RAPD analysis was carried out in twenty Chilli genotypes samples by using random 20 RAPD primers. Out of 20, 13 RAPD arbitrary primers were selected from those which showed reproducible amplification patterns on the agarose gel. Amplification of RAPD was carried out using 200 µl PCR tubes (Genaxy, India) in thermo cycler (Applied Biosystem, USA). PCR was performed in a 25-µl volume containing 35ng/ µl of genomic DNA, 250 nM of primer, 0.25 mMdNTPs, 1X reaction buffer (Thermo scientific, USA) and 5 unit Taq polymerase (Thermo scientific, USA). The reaction mixture was initially denatured at 94 ºC for 5 min, followed by 35 cycles of amplification at 94 ºC for 1 min, 36-38 ºC for 2 min, and 72 ºC for 1 min, and final extension at 72 ºC for 10 min in Thermal Cycler (Applied Bio-systems).

Electrophoretic analysis

PCR products of RAPD were fractionated on 2% agarose gel containing 0.5 µg ml^{-1} EtBr for visualization of bands. PCR amplified products, 8 µl and 1.5 µl 5X loading dye were mixed gently and loaded in to the wells of gel along with 100bp Plus DNA ladder as a reference. DNA bands were visualized by staining with ethidium bromide (EtBr) and photographed on a UV transilluminator using gel documentation system (Syngene, UK).

Data analysis

Morphological and Biochemical data analysis

The morphological and biochemical data obtained from study of different characters and contents of the 20 Chilli genotypes were observed in three replications and analyzed using randomized block design (RBD) in order to study the significance of variance.

Molecular data analysis

Differences in the DNA banding patterns were qualitatively scored from gel photographs for presence (1) and absence (0) of bands assuming that each band represents a unique genetic locus. Homology of bands among samples was based on the distance of migration in gel. The software used for the analysis of the scored data was SPSS version 15.0.0. The molecular weights of the PCR product were estimated by Alpha Ease FC4.0.0 software (Alpha Innotech Corporation, USA) for each primer to analyse allele (band) range. Formula described by Garcia *et al*. (2004) [19] was used to calculate Polymorphism Information content (PIC)

$PIC=1-\sum f^2$ Where "f" is the frequency of "i^{th}" allele.

Genetic relationship and cluster analysis

Coefficients of dissimilarity were calculated by using squared Euclidean distance, using the computer software SPSS 15.0.0 software. Relationships among the chilli genotypes in reference to morphological, biochemical and molecular characterization were expressed in the form of dendrograms and genetic dissimilarity matrix based on squared Euclidean distance value. The morphological and biochemical characters were combined for more effective study of genetic relationship among 20 chilli genotypes.

Results and Discussion

Morphological characterization

The standard morphological descriptors (quantitative) are useful to classify all 20 chilli genotypes in distinct clusters. The variable range of all characters is shown in Table 2. The standard morphological features of chilli genotypes were characterized genetically as described by Manju and Sreelathakumary (2002) [30]. The genetic characterization among the chilli genotypes based on standard descriptors helps to easily describe the morphological features of a genotype and makes diversity assessment easier. Plant height considered being a good indicator of growth and development is observed with a lot of variation in chilli plants (Hosmani 1982) [24]. Wide range of variability was observed among 20 chilli genotypes *i.e.* 41.93 to 97.33 cm (table 2). Fruit length in our study showed a broad range of variation among studied genotypes (4.19 to 9.44 cm) and was found to be comparable with studies revealed by Padda *et al*. (1970) [34] and Pillai and Bellukutty (1978) [38] for varietal variation in fruit length of chilli crops. Chilli fruit length is highly valuable character if it is in medium range because extra-large fruits are undesirable due to its lower productivity, irregular fruit shape and poor quality (Pochard 1966) [39]. Fruit width in this study ranged from 0.61 to 2.09 cm among 20 chilli genotypes and this variation helps a good selection index for fruit yield according to studies by Sharma *et al*. (1981) [44]. Fresh fruit weight was also found with significant variation (1.13 -5.18 g) among studied genotypes. Other scientist Dhaliwal *et al*. (2014) [14] stated that fresh fruit weight varies among the new chili genotypes. Consistent with this result, Hedge (1997) [21], noted that a comparable size in fruit weight characteristics of chili genotypes. Moreover, wide genetic variability is also observed in fruit colors (Fig. 1) of *Capsicum* species by Carvalho *et al*. (2003) [7] and Lannes *et al*. (2007) [27] (table 2). Some qualitative characters were also found to be variable among chilli genotypes, like intermediate type of Plant branching was found with highest frequency (40%) compared to other type, the green colored fruits were in majority (55%) rather light green or dark green and the seed color (Fig. 2) with maximum frequency (60%) was straw colored (Fig. 3). Changes in colors are a result of an increase in oxygenated carotenoids capsanthin, capsorubin and crypto-capsin of mature chilli genotypes (Matsufuji *et al*. 1998; Deepa *et al*. 2007) [11, 10].

Biochemical characterization

Carbohydrates, pigments, vitamins, proteins, volatile oil and minerals are the major constituent and tremendously present in the dry weight of spices (Subblakshmi and Naik (2002) [51]. Among them, Chilli is one of the most widely used condiments as coloring and flavoring agents in Asian counties (Jitbunjerdkul and Kijroongrojana 2007; Toontom *et al*. 2010) [25, 54]. All the biochemical traits examined in the present study were illustrated in table 3. Chilli is an extremely popular for the abundance content of ascorbic acid (vitamin C) larger than other vegetables and fruits commonly recognized as a source of this substance (Durust *et al*. 1997) [16]. The ascorbic acid (Vitamin C), a valuable nutritional component found in chilli fruits was found in range from 22.57 (2011/CHIVAR-1) to 388.89 mg 100 g^{-1} (2011/CHIVAR-8). This variation in ascorbic content might be due to decrease in moisture content in chilli fruits (Robi and Sreelatha Kumari 2004; Martinez *et al*. 2005) [40, 32]. According to previous research conducted by Antonious *et al*. 2006 [3]; Lee *et al*. 1995 [28]; Manju *et al*. 2002 [30], genotypes that showed significant difference in ascorbic acid content (ranged from 22.57 to 388.89 mg 100 g^{-1}) were characterized under hot peppers. Study by Kumar and Tata (2009) [26] stated that ascorbic acid content increased from green to red while, declined in red partially dried and red fully dried fruits. Similarly, chilli fruits studied for total protein content showed significant variation in the studied genotypes ranging from 16.83 (X-235) to 48.71 µg ml^{-1} (2011/CHIVAR-9) (table 2).

Variation in protein accumulation is regulated by numerous abiotic and biotic environmental factors and depends on how these factors affect photosynthesis and growth (Estrada *et al*., 1999) [17]. The capsaicin, an essential component for pungency in Chilli was found in range from 0.09 (2011/CHIVAR-9) to 0.73 mg g^{-1} (KTPL-19). Present results on capsaicin content were in agreement to other researcher Singh *et al*. (2009) [47]; Datta and Das (2013) [13]. The chlorophyll content is considered as the only primary compound for photosynthesis and energizing procedure for plants and fruits. Analyzed chlorophyll content (table 3) was observed in the range of 0.12 to 1.41 mg g^{-1} and was found maximum in genotype ACS-92-4, which revealed its

maximum capacity for photosynthesis. The results are in accordance with Manna *et al*. 2012 [31] (0.16 mg g^{-1}) and Yatung *et al*. 2014 [64] (0.49 mg g^{-1}). The Carotenoid content was found maximum (0.21 µg ml^{-1}) in KTPL-19 and thus this genotype can be used for quality improvement programme in chilli especially for carotenoid content as it is an important nutritional content. The carotenoid content increased from the fresh to the mature state, as expected accordingly to the natural biosynthesis of pigments as the fruit matures (Zhang and Hamauzu 2003) [65].

However, the presented experimental results on biochemical traits could be affected also by the various environmental conditions, such as water availability, temperature, humidity, as well as the type of soil, which of course are linked to the proximity of the diverse geographical areas (Troconis-Torres 2012) [55]. The study of various biochemical characters is also carried out by several researchers in order to select superior genotypes for breeding programme.

Genetic diversity analysis using combined morphological and biochemical characters

Cluster analysis allows refining and simplifying the complex genetic relationship among diverse population in which combination of morphological and biochemical characters can give more reliable relationship. The cluster analysis generated three clusters named; Cluster I, Cluster II and Cluster III (Fig. 4). The genotypes KTPL-19, O7 and ACS-2000-2 were included in Cluster I which might be due to their similar fruit and seed colores. The Cluster II included genotypes 2011/CHIVAR-8, Utkal yellow, DCL-352, 2011/CHIVAR-3, 2011/CHIVAR-7, 2011/CHIVAR-5, Pant chilli-3, 2011/CHIVAR-1, O5, 2011/CHIVAR-9 and 2011/CHIVAR-4. The Cluster III included genotypes 2011/CHIVAR-2, 2011/CHIVAR-6, JCA-283, SKAU-P-7, X-235 and ACS-92-4 (Fig. 4). Although, other two clusters (cluster I-II) were considered different because of their different morphological variations in Chiili genotype observation. From the study of genetic dissimilarity matrix, the genotype 2011/CHIVAR-7 and KTPL-19 were found more distant to each other and obtained with highest dissimilarity (36%) whereas the lowest dissimilarity (4%) was obtained between genotype 2011/CHIVAR-2 and 2011/CHIVAR-6 (Table 4). Chattopadhyay *et al*. (2011) [8]; Arunkumar *et al*. (2013) [4] also carried out genetic diversity studies among various chilli genotypes using morphological and biochemical characters together and found some distinct genotype.

RAPD pooled data analysis

Twenty primers were initially screened for their ability to produce polymorphic patterns and only 13 of them were selected which gave reproducible and distinct polymorphic amplified products (Table 5). The data collected from random amplification of polymorphic DNA (RAPD) with 13 arbitrary primers produced total 143 loci and with total 1864 bands. Out of 143 loci produced, 104 were polymorphic with the average polymorphism percentage 72.22%, which showed availability of polymorphism in chill genotypes. Williams *et al*. (1990) [63] detected that due to nucleotide substitutions and insertion or deletions polymorphism occurred between individuals. The RAPD analysis was also used by previous researchers to study genetic diversity in different chilli genotypes was found 80.95%, 44% and 77.6% by Uddin *et al*. (2012) [56]; Bahurupe *et al*. (2013) [5] and Sikora and Nowaczyk (2014) [45], respectively which revealed that wide range of polymorphism can be obtained using RAPD analysis.

The average polymorphism information content (PIC) produced by RAPD primers was 0.88. Highest PIC was revealed by the primer OPH-12 in the chilli genotypes. The PIC is an important parameter in studies related to diversity as it reveals the efficiency of a primer for finding the level of polymorphism (Rocha *et al*. 2010) [41]. The number of amplicons produced per primer varied from 8 to 14 with a mean of 11 bands per primer. Among the oligonucleotides tested, OPI-11 was the most polymorphic RAPD oligonucleotide generating a total 11 bands. The molecular size of the amplified PCR products ranged from 176 bp (OPI 06) to 2356 bp (OPG 12). The RAPD marker OPG 12 produced maximum number of 177 bands, while OPH 03 produced the minimum number of 92 bands. The RAPD primer OPG 09 showed lowest (33.34%) polymorphism. The lowest PIC value obtained was 0.82 for OPH 04 primer (Table 5 and Figure 5).

Genetic diversity analysis using RAPD

The genetic dissimilarity matrix showed maximum dissimilarity (53%) between 2011/CHIVAR-1 and Pant chilli-3 genotypes and the most similar genotypes with least dissimilarity (19%) were X-235 and O7 as well as X-235 and ACS-2000-2 (Table 6). Cluster analysis with RAPD data also generated three clusters, *viz.*, Cluster I, Cluster II and Cluster III. Here the Cluster I included only a single genotype named 2011/CHIVAR-2. Cluster II was further subdivided as Cluster IIa and Cluster IIb. The genotype 2011/CHIVAR-3 was the only genotype included in Cluster IIa whereas; genotypes 2011/CHIVAR-1 and 2011/CHIVAR-6 were included in Cluster IIb. Thus it is reasonable that there are some fine differences between the two dendrograms based on an individual data set (Fig.6).

Further, Cluster III also had two subdivisions denoted Cluster IIIa and Cluster IIIb. The genotypes 2011/CHIVAR-7 and 2011/CHIVAR-8 were falling under Cluster IIIa and rest of the genotypes *viz.*, X-235, O7, Pant Chilli-3,ACS-2000-2, ACS-92-4, JCA-283, O5, Utkal Yellow, DCL-352, KTPL-19, SKAU-P-7, 2011/CHIVAR-4, 2011/CHIVAR-5 and 2011/CHIVAR-9 were included under Cluster IIIb. Maximum 16 genotypes could be grouped in a single cluster i.e. Cluster III (Fig. 6). This indicating that these 16 genotypes are more distantly related to the other and probably may be because of some unique repeat sequences. The dendogram reflect a good genetic analysis, which is based on amplification patterns from RAPDs, showing that it is a good marker to gauge the genetic relationships between chilli genotypes as previously researchers reported (Akbar *et al*. 2010; Peeraullee and Ranghoo-Sanmukhiya 2013) [1, 37].

Table 1: List of Chilli genotypes used in present study

Sr. No.	Genotypes	Sr. No.	Genotypes
1	2011/ CHIVAR-1	11	SKAU-P-7
2	2011/ CHIVAR-2	12	DCL-352
3	2011/ CHIVAR-3	13	KTPL-19
4	2011/ CHIVAR-4	14	ACS-2000-2
5	2011/ CHIVAR-5	15	ACS-92-4
6	2011/ CHIVAR-6	16	X-235
7	2011/ CHIVAR-7	17	JCA-283
8	2011/ CHIVAR-8	18	Pant chilli-3
9	2011/ CHIVAR-9	19	O5
10	Utkal yellow	20	O7

Table 2: Quantitative morphological characters of Chilli genotypes

Sr. No.	Genotypes	Plant height (cm)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	No. of seeds	Test weight of seed (g)
1	2011/ CHIVAR-1	88.20	5.08	0.79	1.73	32.00	0.67
2	2011/ CHIVAR-2	97.33	9.44	1.17	2.24	58.33	0.39
3	2011/ CHIVAR-3	62.87	4.84	0.67	2.67	57.67	0.50
4	2011/ CHIVAR-4	28.60	4.80	0.69	1.28	21.33	0.47
5	2011/ CHIVAR-5	61.60	4.19	1.10	2.65	114.67	0.58
6	2011/ CHIVAR-6	92.83	8.92	0.75	2.76	40.00	0.47
7	2011/ CHIVAR-7	82.83	7.31	0.98	1.13	24.00	0.54
8	2011/ CHIVAR-8	46.77	7.19	0.91	2.16	26.33	0.62
9	2011/ CHIVAR-9	92.20	6.79	0.84	2.56	51.00	0.29
10	Utkal yellow	61.27	5.60	0.61	1.30	38.33	0.36
11	SKAU-P-7	82.13	6.67	1.06	2.78	16.67	0.46
12	DCL-352	53.53	7.37	0.77	2.78	62.00	0.55
13	KTPL-19	41.93	7.83	2.09	5.18	21.00	0.36
14	ACS-2000-2	45.93	7.60	1.15	2.78	60.67	0.71
15	ACS-92-4	52.77	6.33	1.04	2.14	45.67	0.55
16	X-235	73.10	7.33	0.79	2.15	51.00	0.37
17	JCA-283	77.30	8.63	0.99	3.56	51.33	0.62
18	Pant chilli-3	60.80	7.27	1.77	2.18	146.67	0.46
19	O5	57.17	7.63	0.68	2.29	27.33	0.31
20	O7	46.97	9.43	1.17	3.27	32.33	0.49
	S.Em.	3.27	0.26	0.05	0.12	2.43	0.02
	C.D. (5%)	9.37	0.74	0.14	0.33	6.97	0.06
	CV%	8.68	6.44	8.50	8.10	9.62	7.55

Table 3: Statistical analysis of biochemical characters

Sr. No.	Genotypes	Ascorbic acid (mg $100g^{-1}$)	Total protein (µg ml^{-1})	Capsaicin (mg g^{-1})	Total chlorophyll content (mg g^{-1})	Total carotenoid content (µg ml^{-1})
1	2011/ CHIVAR-1	22.57	31.69	0.19	0.32	0.06
2	2011/ CHIVAR-2	125.00	36.81	0.20	0.35	0.06
3	2011/ CHIVAR-3	274.31	29.51	0.15	0.30	0.07
4	2011/ CHIVAR-4	211.81	40.60	0.43	0.45	0.11
5	2011/ CHIVAR-5	263.89	29.17	0.30	0.38	0.07
6	2011/ CHIVAR-6	145.83	28.10	0.24	0.38	0.06
7	2011/ CHIVAR-7	250.00	33.84	0.36	0.28	0.09
8	2011/ CHIVAR-8	388.89	22.55	0.46	0.40	0.17
9	2011/ CHIVAR-9	180.56	48.71	0.09	0.12	0.02
10	Utkal yellow	125.00	33.45	0.49	0.33	0.11
11	SKAU-P-7	59.03	33.09	0.30	0.38	0.14
12	DCL-352	232.64	38.70	0.44	0.40	0.14
13	KTPL-19	34.72	37.18	0.73	0.52	0.21
14	ACS-2000-2	29.51	29.13	0.49	0.23	0.09
15	ACS-92-4	107.64	37.69	0.28	1.41	0.18
16	X-235	107.64	16.83	0.35	0.50	0.14
17	JCA-283	163.19	35.49	0.39	0.40	0.13

18	Pant chilli-3	178.82	42.43	0.17	0.44	0.13
19	O5	90.28	40.71	0.18	0.46	0.18
20	O7	71.76	42.25	0.49	0.19	0.07
	S.Em.	4.0	0.41	0.008	0.014	0.006
	C.D. (5%)	11.47	1.19	0.024	0.041	0.018
	CV%	4.53	2.10	4.38	6.09	9.79

Table 4: Genetic dissimilarity matrix using combined study of morphological and biochemical characters

	2011/ CHIVAR-1	2011/ CHIVAR-2	2011/ CHIVAR-3	2011/ CHIVAR-4	2011/ CHIVAR-5	2011/ CHIVAR-6	2011/ CHIVAR-7	2011/ CHIVAR-8	2011/ CHIVAR-9	Utkal yellow	SKAU-P-7	DCL-352	KTPL-19	ACS-2000-2	ACS-92-4	X-235	JCA-283	Pant chilli-3	O5	O7
2011/CHIVAR-1	0																			
2011/ CHIVAR-2	18	0																		
2011/ CHIVAR-3	15	13	0																	
2011/ CHIVAR-4	20	22	15	0																
2011/ CHIVAR-5	26	14	9	20	0															
2011/ CHIVAR-6	16	4	11	18	12	0														
2011/ CHIVAR-7	17	9	8	13	17	9	0													
2011/ CHIVAR-8	21	13	10	11	13	7	8	0												
2011/ CHIVAR-9	15	9	12	21	23	13	14	24	0											
Utkal yellow	18	12	13	10	12	10	9	7	19	0										
SKAU-P-7	8	8	13	16	20	8	11	11	11	10	0									
DCL-352	27	17	16	13	11	11	18	8	22	9	15	0								
KTPL-19	23	27	32	29	35	25	36	26	32	29	11	28	0							
ACS-2000-2	18	20	19	28	22	18	17	19	31	18	14	29	21	0						
ACS-92-4	17	11	16	15	13	11	16	10	22	11	9	12	16	19	0					
X-235	15	7	8	19	13	5	8	8	12	7	5	12	22	15	10	0				
JCA-283	17	7	12	19	15	5	10	8	14	13	7	10	18	15	8	6	0			
Pant chilli-3	21	13	16	21	11	15	24	22	12	17	15	10	28	31	14	16	14	0		
O5	8	12	15	12	24	12	15	15	9	14	6	15	17	22	11	13	9	13	0	
O7	18	16	21	16	28	14	19	17	17	20	8	19	11	18	19	17	11	19	10	0

Table 5: Numerical data as obtained from PCR amplification by RAPD primers in Chilli genotypes

Sr. No	Primer Id	Sequence 5'–3'	GC content (%)	Molecular size range (bp)	Total no. of ample icons	No. of loci	No. of Polymorphic bands	Percent Polymorphism (%)	PIC value
1	OPE 09	CTTCACCCGA	70	242-2286	154	11	8	72.72	0.889
2	OPG 09	CTGACGTCAC	60	387-1810	142	9	3	33.34	0.872
3	OPG 12	CAGCTCACGA	60	326-2356	177	14	10	71.42	0.910
4	OPH 03	AGACGTCCAC	60	351-2080	92	9	8	88.89	0.853
5	OPH 04	GGAAGTCGCC	70	258-1660	94	8	5	62.5	0.820
6	OPH 12	ACGCGCATGT	60	238-2284	206	12	7	58.34	0.913
7	OPH 14	ACCAGGTTGG	60	230-2161	125	11	8	72.72	0.867
8	OPI 06	AAGGCGGCAG	70	176-1671	122	10	8	80	0.877
9	OPI 11	ACATGCCGTG	60	203-2234	155	13	12	92.30	0.903
10	OPI 13	CTGGGGCTGA	70	528-2680	169	12	9	75.0	0.903

11	OPI 14	TGACGGCGGT	70	388-2117	164	12	8	66.67	0.898
12	OPI 16	TCTCCGCCCT	70	396-2661	124	10	9	90.0	0.880
13	OPI 20	AAAGTGCGGG	60	223-2236	140	12	9	75.0	0.880
Total					1864	143	104	938.9	11.46
Average				303-2172	143.38	11	8	72.22	0.881

Table 6: Genetic dissimilarity matrix based on RAPD analysis

	2011/CHIVAR-1	**2011/ CHIVAR-2**	**2011/ CHIVAR-3**	**2011/ CHIVAR-4**	**2011/ CHIVAR-5**	**2011/ CHIVAR-6**	**2011/ CHIVAR-7**	**2011/ CHIVAR-8**	**2011/ CHIVAR-9**	**Utkal yellow**	**SKAU-P-7**	**DCL-352**	**KTPL-19**	**ACS-2000-2**	**ACS-92-4**	**X-235**	**JCA-283**	**Pant chilli-3**	**O5**	**O7**
2011/CHIVAR-1	0																			
2011/ CHIVAR-2	41	0																		
2011/ CHIVAR-3	34	49	0																	
2011/ CHIVAR-4	32	49	34	0																
2011/ CHIVAR-5	40	35	48	34	0															
2011/ CHIVAR-6	30	37	32	38	34	0														
2011/ CHIVAR-7	48	41	52	46	40	40	0													
2011/ CHIVAR-8	37	42	45	41	37	33	35	0												
2011/ CHIVAR-9	43	40	47	35	41	39	49	40	0											
Utkal yellow	39	46	45	29	29	39	43	36	30	0										
SKAU-P-7	49	38	47	37	37	39	45	44	38	34	0									
DCL-352	45	40	45	35	35	35	45	38	38	32	30	0								
KTPL-19	41	40	41	33	37	29	49	30	36	30	32	28	0							
ACS-2000-2	38	35	44	26	28	32	44	37	35	23	37	25	23	0						
ACS-92-4	46	43	42	36	40	38	42	35	43	31	39	31	31	20	0					
X-235	45	36	45	37	43	37	47	38	34	30	38	24	34	19	21	0				
JCA-283	35	38	43	35	41	31	39	26	44	32	40	32	32	29	21	26	0			
Pant chilli-3	53	44	49	45	43	43	43	40	46	34	40	38	40	33	25	22	28	0		
O5	38	49	46	36	44	44	50	35	41	27	41	31	35	34	28	33	21	29	0	
O7	42	47	44	40	42	40	44	33	41	23	43	35	33	24	24	19	21	21	26	0

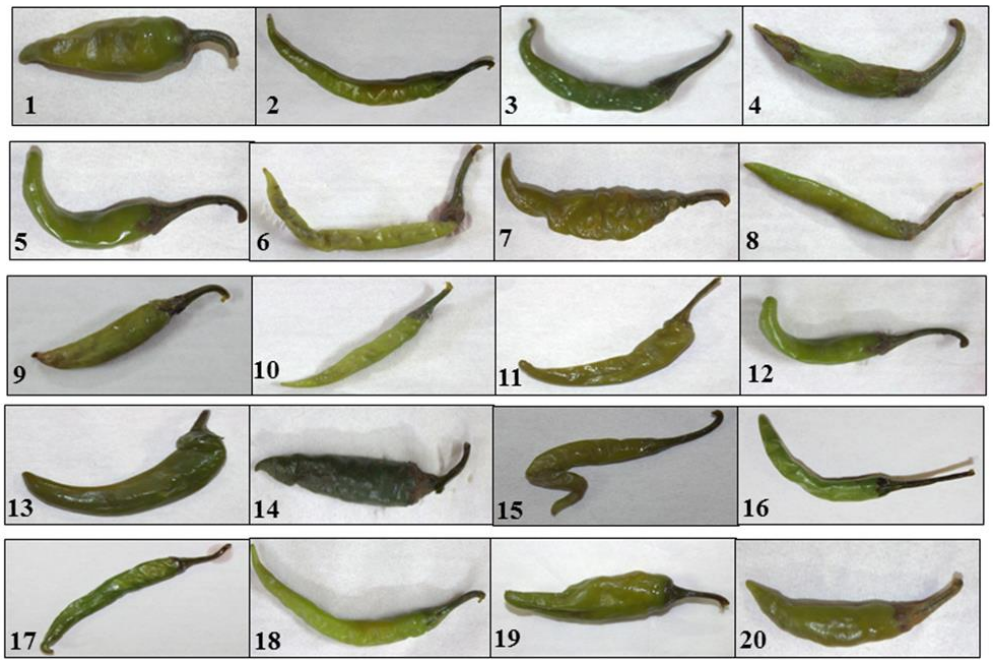


Fig 1: Fruits color (mature) of 20 *Capsicum* genotypes, numbers (1 to 20) corresponds to serial numbers and names in Table 1

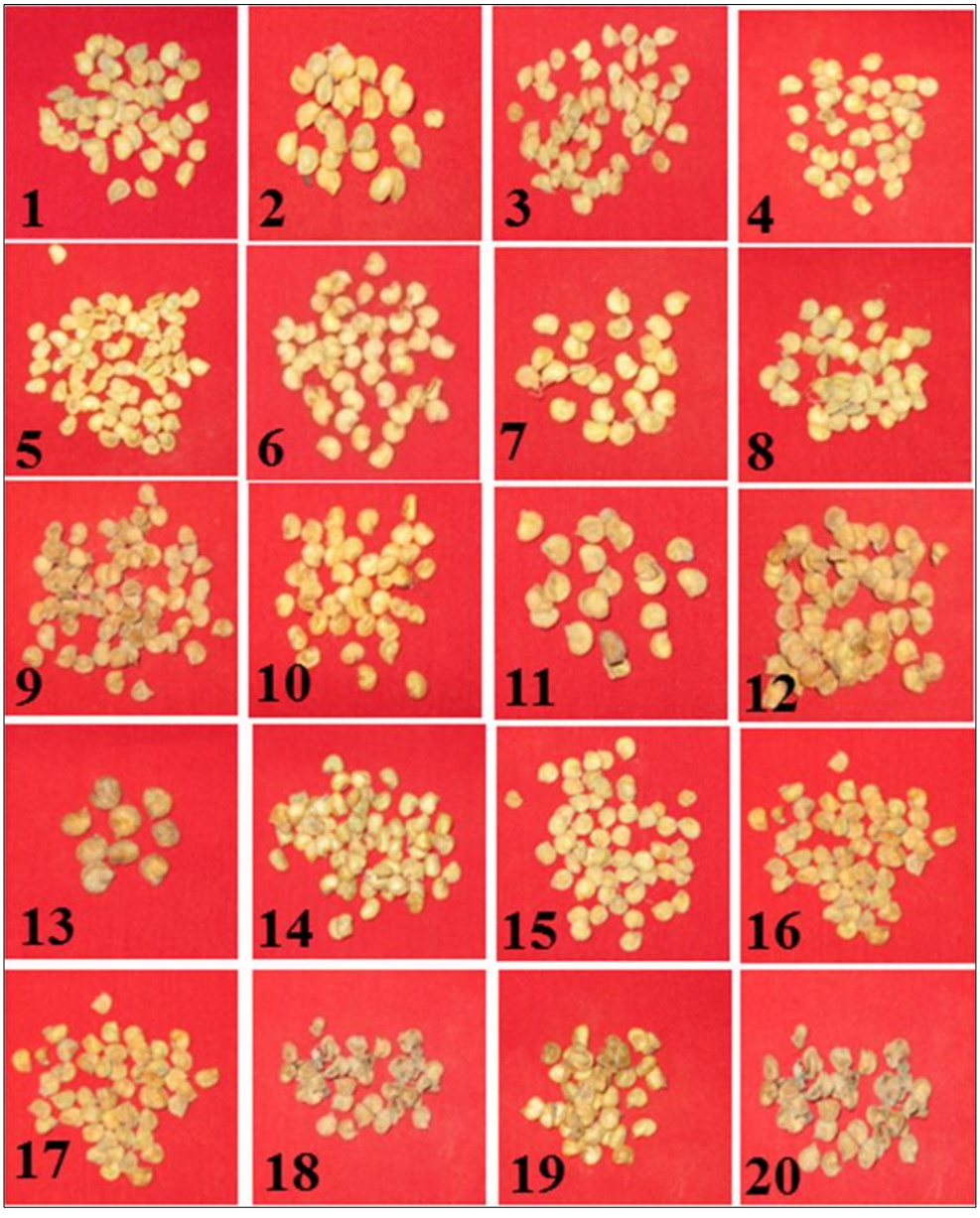


Fig 2. Seed color of 20 *Capsicum* genotypes, numbers (1 to 20) corresponds to serial numbers and names in Table 1

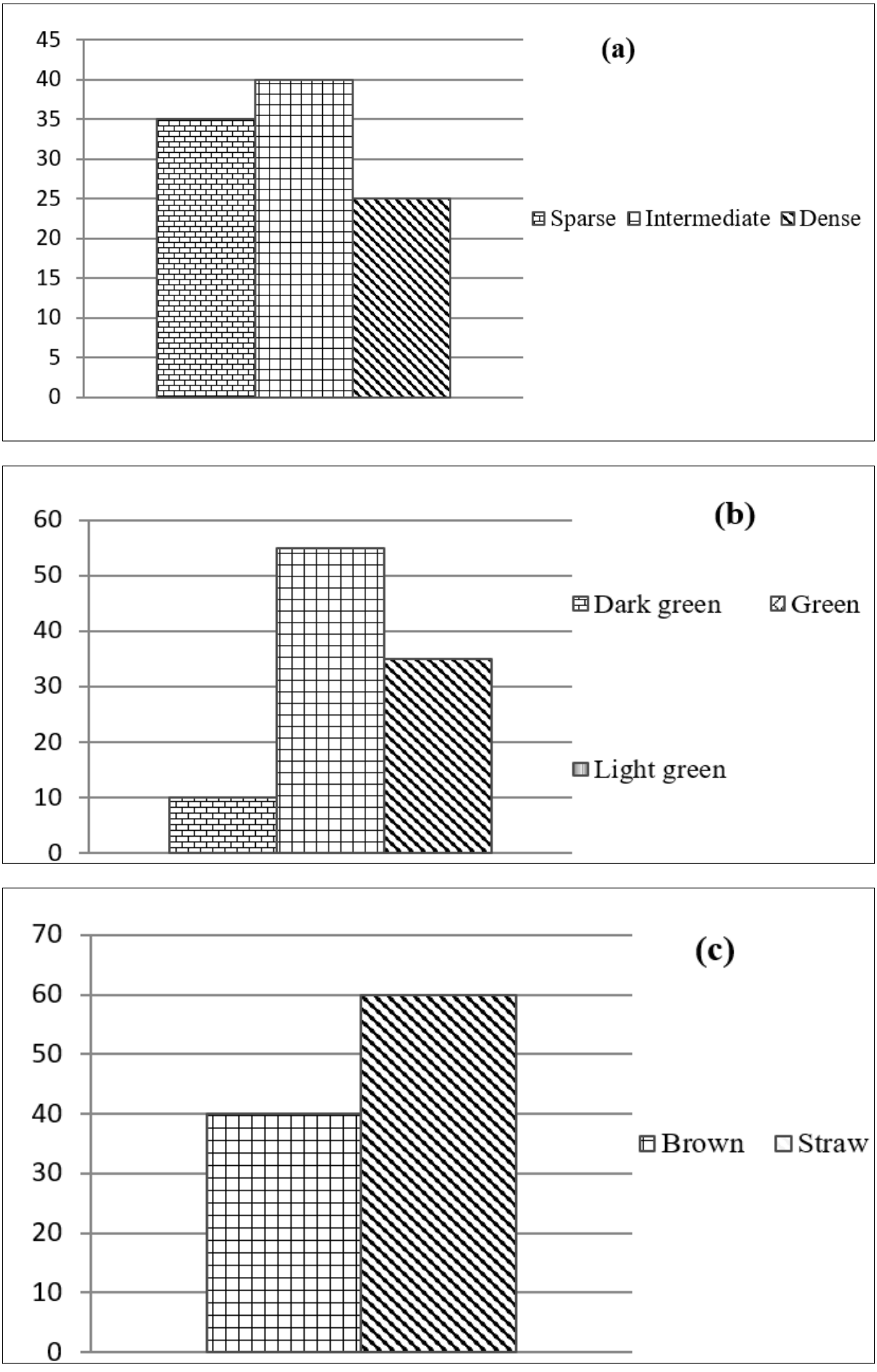


Fig 3: Graph showing frequency percent for qualitative characters of Chilli genotypes (a) Plant branching type, (b) Fruit color and (c) Seed color

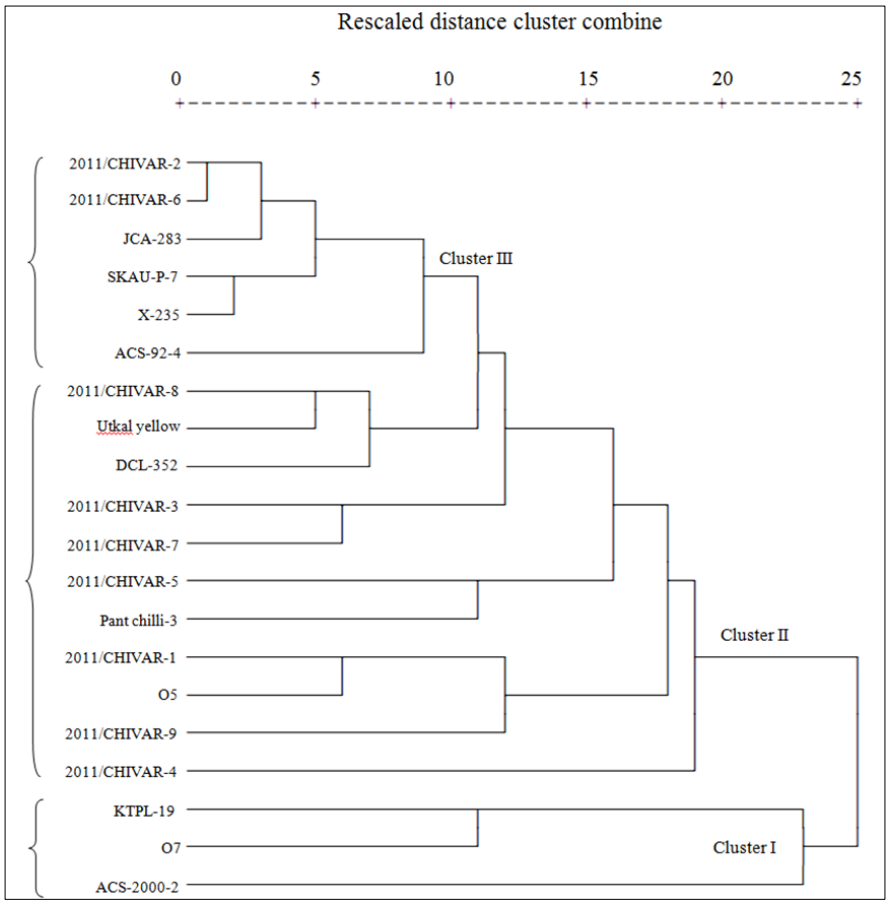
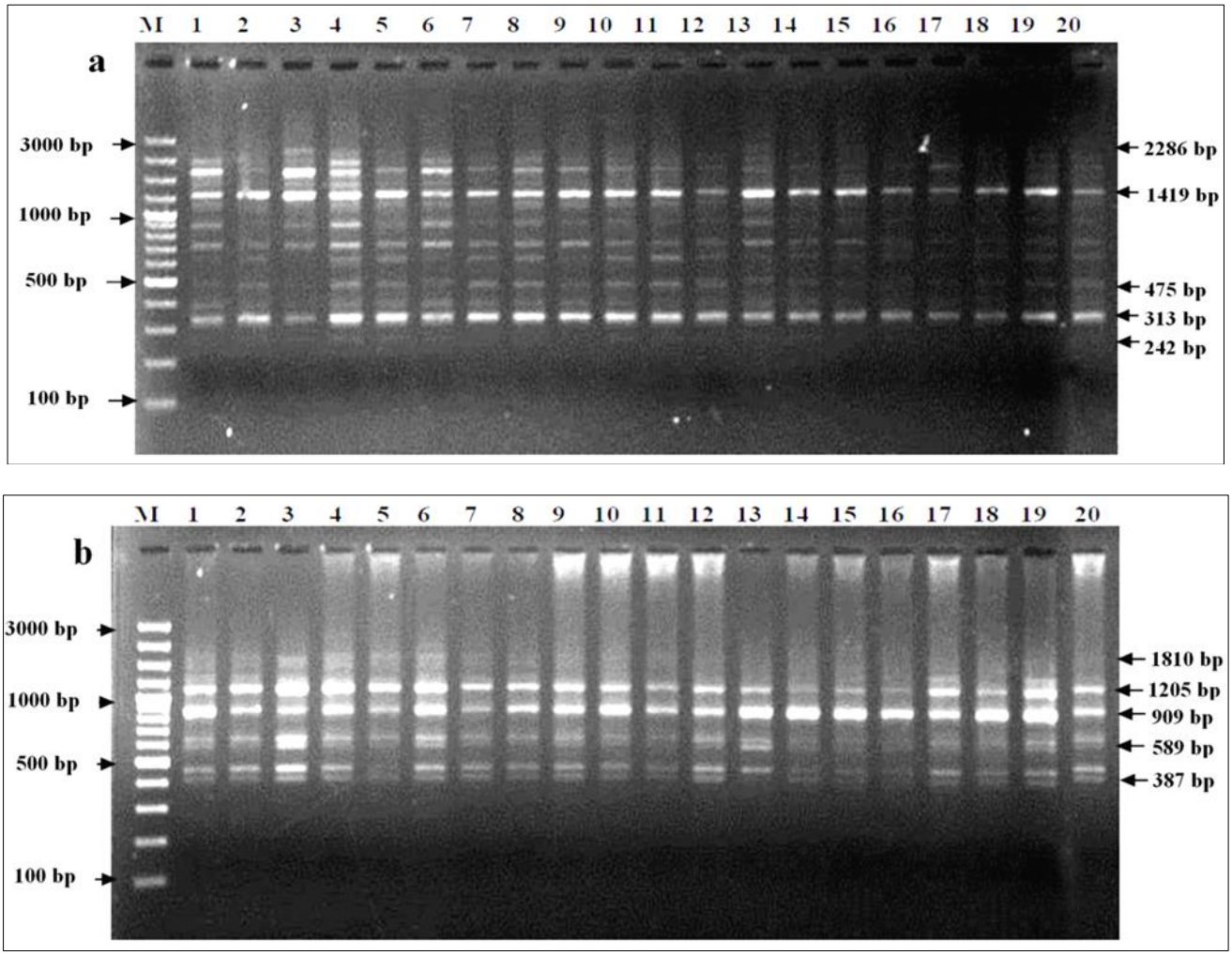


Fig 4: Hierarchical cluster analysis for combined morphological and biochemical studies based on squared Euclidean distance



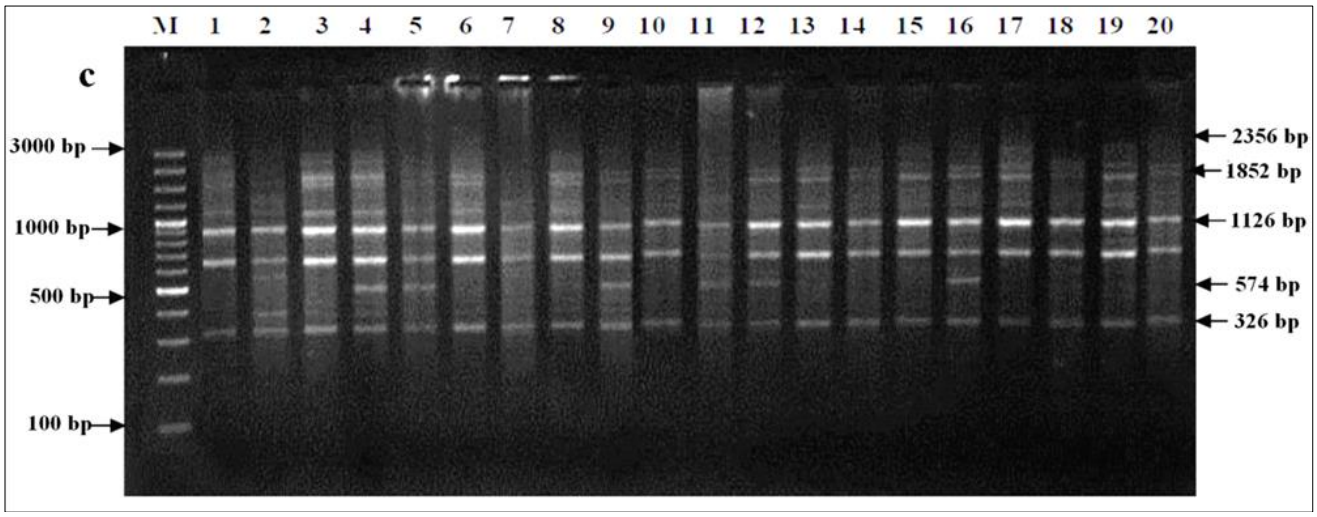


Fig 5: RAPD profile of 20 Chilli genotypes generated by (a) OPE09, (b) OPG09 and (c) OPG12 primers

Where, M: 100bp plus DNA ladder

1. 2011/CHIVAR-1	6. 2011/CHIVAR-6	11. SKAU-P-7	16. X-235
2. 2011/CHIVAR-2	7. 2011/CHIVAR-7	12. DCL-352	17. JCA-283
3. 2011/CHIVAR-3	8. 2011/CHIVAR-8	13. KTPL-19	18. Pant chilli-3
4. 2011/CHIVAR-4	9. 2011/CHIVAR-9	14. ACS-2000-2	19. O5
5. 2011/CHIVAR-5	10. Utkal yellow	15. ACS-92-4	20. O7

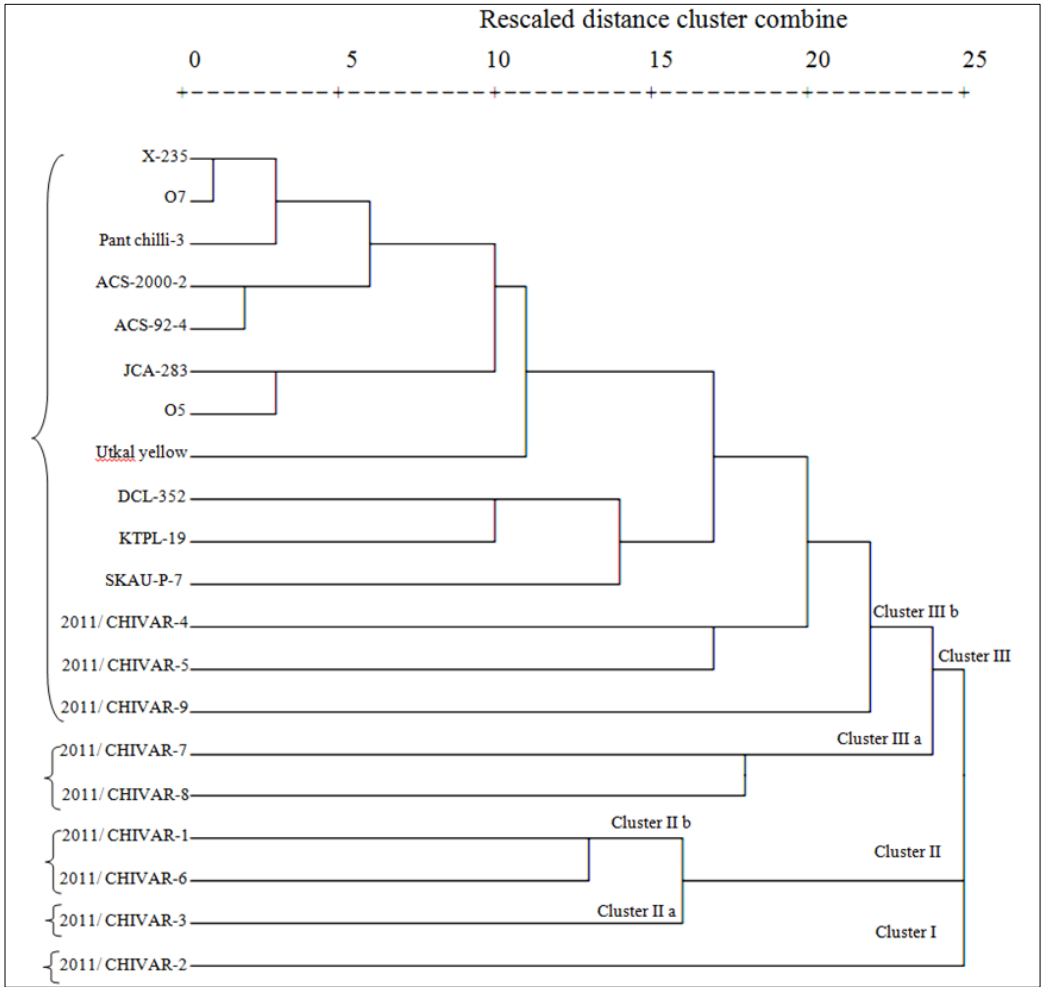


Fig 6: Hierarchical cluster analysis for RAPD based on squared Euclidean distance.

Conclusion

Several types of markers like morphological, biochemical and molecular markers could be used to characterize and study genetic diversity among 20 different chilli genotypes. Although, biochemical and morphological markers could be influence by the environmental factors, whereas molecular markers are stable in these condition. The combined study of morphological and biochemical characters could produce more comprehensive clusters, but as these characters are affected by environmental factors. Molecular

characterization based on RAPD could find out precise genetic diversity among Chilli genotypes. These molecular markers are considered as powerful tools for estimating diversity and are not affected by environmental factors. Results of this experiment contributed to the knowledge of the existing genetic status of *Capsicum* genotypes. Thus, present studies indicated that morphological descriptors and biochemical tests are only useful for grouping and classifying Chilli genotypes. Moreover, the results produced in this study could be further used by a Chilli breeder for their genetic improvement programme.

Acknowledgment
We sincerely acknowledge the Department of Vegetable Science, ASPEE College of Horticulture and Forestry, Navsari (Gujarat), India, for providing seed material for the present study.

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