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ORIGINAL ARTICLE

A comparative assessment of morphological and molecular diversity among *Sechium edule* (Jacq.) Sw. accessions in India

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Abstract Genetic variation of 36 *Sechium edule* accessions collected across 12 states in India was assessed using morphological traits and DAMD markers. Eighteen fruit morphological traits (both qualitative and quantitative) were evaluated to confirm the variations in the present collection. Quantitative traits showed major variations with respect to fruit weight (7.85–498.33 g/fruit), fruit length (5.8–15 cm/fruit), fruit diameter (6–28 cm/fruit) and length of the spine (0–5 cm). Qualitative traits were also diverse in fruit colour, shape, spine density, reticulation, flexibility of spine and furrow depth. The first six principle components showed 82.88% variation in the principal component analysis. The principal component analysis revealed that fruit weight, fruit width, fruit diameter, fruit shape, length of spine, spine density and furrow depth had a significant contribution to the total variation. The DNA analysis performed using DAMD primers were used for deducing the diversity at DNA level. The collection produced 102 bands out of which 97 were polymorphic and the percentage polymorphism ranged between 66.66 and 100 per primer. Discrete pattern of clustering was obtained using UPGMA method of complete linkage percent disagreement revealing high diversity among the collected accessions. Thus, the present study indicates that molecular and morphological marker map would improve our knowledge of *S. edule* and would facilitate efforts to breed improved *S. edule* cultivars.

Keywords *Sechium edule* · Morphological variation · Principal component analysis · DAMD · Genetic diversity

Introduction

Sechium edule (Jacq.) Swartz (Chayote or air potato) is one of the neglected vegetable crops and belongs to the family Cucurbitaceae. It is an herbaceous, perennial, monoecious climber and is now considered as an important food in tropical and subtropical regions of the world (Morton 1981). The wild species of *S. edule* and the related species are abundantly found in Central America and Mexico, and is the center of origin. The close relatives, *S. compositum*, *S. hintonii* and *S. tacaco* are either endemic or restricted to their original area. *Sechium edule* was introduced in India where it grows widely in the South and North-Eastern parts of India (Newstrom 1991). The shoots, stems, leaves and tuberous roots are edible and the fruits are now being consumed in many countries as it has been reported to have anti-diabetic (Maity et al. 2013), antimicrobial (Ordonez et al. 2003), anti ulcer (Sateesh et al. 2012) and antihypertensive activities (Earl et al. 2014).

Sechium edule fruit displays a great diversity of size, shape, fruit wall features and texture (Lira-Saade 1996). Such collection of germplasm and characterization forms an essential stage of crop improvement and breeding programs. Morphological traits are essential for preliminary evaluation and for assessment of genetic diversity as *S. edule* fruits are the sign of its diversity. Without determining the diversity, it would be difficult to determine the associated qualitative traits (Lansari et al. 1994).

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The precocious germination of the *S. edule* hinders the efforts to conserve and study these resources and the conservation using simple orthodox methods cannot be carried out (Esquivel and Engelmann 2002). Therefore, it has to be subjected to field gene banks, which turns out to be an expensive procedure. The wider population of *S. edule* varieties was not studied or classified systematically until the beginning of the 1980s (Engels 1983). The factors like frost, drought and root diseases led to the decline of the collections gradually. A total number of 375 accessions grown in Costa Rica, Honduras and Guatemala were reduced to almost half by 1981 due to such environmental factors (Newstrom 1986). Similar decline in the number of accessions were observed among the Mexican collections. As a wide diversity is seen among *S. edule* populations in India, both morphological and molecular data are essential and these form a backbone for the conservation of genetic resources for present and future use (Sanwal et al. 2010).

Advancement in field of molecular biology has led to the concise classification of plant genetic resources. Molecular markers can be used for identifying collections, screen its germplasm or genetic diversity and also resolving the taxonomic relationships (Kameswara 2004). DAMD markers are advantageous as they are highly polymorphic. Cucumber (Hu et al. 2010), *Capsicum* spp. (Ince et al. 2009), grapevine cultivars (Seyedimoradi et al. 2012) and mulberry species (Bhattacharya and Ranade 2001) have been evaluated successfully for its diversity studies, but no information is available to compare the morphological and genetic studies of *S. edule* species in India. Therefore, the aim of the present study is to analyze the morphological and genetic variations of *S. edule* collections from different growing locations across India.

Materials and methods

Collection of plant material and data collection

The fruits for the investigation comprised of 36 *S. edule* accessions collected from 12 *S. edule* growing states across India. An initial attempt to grow the plants in uniform field collections was not successful as the collections from the North-Eastern regions of India failed to germinate and we had to continue our studies only with the fruit-related traits. These fruit collections were maintained at −80 °C at Jain University—CPGS campus, Bangalore, India. Eighteen qualitative and quantitative characteristics were recorded for the fruit-related trait as mentioned by Newstrom (1986) and Engels (1983). The traits include shape of the fruits (FS), color (FC), length (FL), width (FW), diameter (FD), weight (W), density of the spines present over the fruits (SD), longitudinal furrow depth (LFD), reticulation (R), cross section profile (CSP), spine length (LS) and spine distribution (SPD) (Table 1) were used in the present investigation.

Table 1 List of the morphological traits used as descriptors for *S. edule* in the present study

Sl. no.	Character	Character code	Character and descriptive value
1	Fruit shape	FS	1 (pyriform), 2 (subpyriform), 3 (ovoid), 4 (round), 5 (flattened)
2	Fruit length	FL	In centimeters
3	Fruit width	FW	In centimeters
4	Fruit ratio	R	Fruit length/fruit width
5	Fruit diameter	FD	In centimeters
6	Fruit weight	W	In grams
7	Spine density	SD	1 (None), 2 (very low), 4 (intermediate), 6 (very high)
8	Fruit color	FC	1 (Whitish), 2 (light green), 3 (green), 4 (dark green)
9	Longitudinal furrow depth	LFD	1 (None), 2 (very superficial), 4 (intermediate), 6 (very deep)
10	Reticulation	R	1 (None), 2 (very little), 4 (intermediate), 6 (very intense)
11	Cross section profile	CSP	1 (Round), 3 (oval), 5 (flattened)
12	Spine distribution	SPD	0 (None), 1 (distally), 3 (proximally), 5 (lines), 7 (covering entire fruit)
13	Length of spine	LS	In millimeters
14	Size of base spine	SBP	0 (None), 1 (small), 5 (medium), 9 (wide)
15	Flexibility of spine	FS	0 (None), 1 (flexible), 2 (hard)
16	Furrows	F	0 (None), 1 (short distal), 3 (short proximal), 5 (short distal and proximal), 7 (medium, not total length), 9 (long, total length)
17	Depth of furrows	DF	0 (None), 1 (superficial), 5 (medium), 9 (deep)
18	Ridges	RD	0 (None), 1 (superficial), 5 (medium), 9 (deep)

Molecular characterization

DNA extraction

Total genomic DNA was extracted from the frozen fruit rind of all the 36 accessions by CTAB method (Jain et al. 2015). The total DNA pellet after purification was subjected to RNase treatment at 37 °C. DNA quantification, as well as quality assessment, was carried out spectrophotometrically (A260/280) and similarly the purity of DNA was calculated (Asish et al. 2010). The samples were then stored at −20 °C and aliquots were maintained at 4 °C. A total of 12 DAMD primers were used to screen and amplify the genomic DNA of 36 accessions of *S. edule* (Hu et al. 2010). PCR amplification was performed in 25 µl of the reaction mixture containing 25 ng sample DNA, 1 mM dNTP, 10 pM primer and 2 units *Taq* polymerase with 2.5 µl of PCR buffer containing 15 mM $MgCl_2$ per reaction. The temperature profile was as follows: 94 °C for 4 min, followed by 40 cycles at 94 °C for 1 min, annealing temperature (50–61 °C) for 1 min, 72 °C for 1 min and 72 °C for 5 min final extension. The PCR products were separated by electrophoresis through 1.2% agarose gel and the profiles were analyzed (Hu et al. 2010). The presence and absence of bands were recorded; the binary data generated was used to estimate the level of polymorphism using UPGMA.

Statistical analysis

The phenotypic data were used for the assessment of morphological characteristics by principal component analysis (PCA) to define the Eigen values using a multivariate analysis program (Unscrambler X, CAMO, Bangalore, Karnataka, India). Similarly, a statistical software package (Statistica) was used to evaluate the correlations between the characteristics and a dendrogram was created by complete linkage percent disagreement using UPGMA method (Manohar and Murthy 2012).

Results

Morphological variation

Thirty six accessions were evaluated for fruit morphological variations using external fruit characters of *S. edule*. Eighteen qualitative and quantitative characteristics were used as descriptors as per Engels (1983) and Newstrom (1986) listed in the Table 1. A large variation among the collected accessions was observed in case of fruit color, its shape, size and spine density. Among all, 17 accessions were found to be ovoid in shape followed by 13 accessions of subpyriform shape. The length of the fruit varied from 5.8 to 15 cm and the maximum weight recorded was 498.33 g with a minimum of 7.85 g. The color of the fruit varied from dark green to white. Variations were also observed in case of furrows and ridges. Cumulative variations of the quantitative characters were calculated as shown in the Table 2. The maximum cumulative variation is shown in case of length of spines, i.e., 100%. Principal component analysis was used to access the variability in *S. edule* accessions. The percentage of variation explained by the first six components was as follows: 30, 20, 11, 9, 8 and 6. Eigen vectors that delineated the accessions into separate groups in the first six components are represented in the Table 3.

The fruit shape, length, weight, spine density and longitudinal furrow depth were the important characters for the formation of different clusters. Figure 1 depicts the formation of five clusters with one un-clustered *S. edule* accession. By these components, first cluster had majority of the accessions from diverse locations (Sikkim, Assam, Meghalaya, Manipur, West Bengal, Chhattisgarh, Andhra Pradesh, Karnataka and Tamil Nadu) having fruit length of 6.5–14 cm and no or very low spine density and majorly having intermediate furrow depth.

Two accessions from Manipur and West Bengal were grouped into another cluster (2). These two accessions were green in color, had no spines and bearing very deep longitudinal furrow depth. Similarly, cluster (3) had collections from Sikkim, Meghalaya, Manipur, Mizoram, Andhra Pradesh and Tamil Nadu. The fruit length among these collections ranged from 11.5 to 13.5 cm having low to intermediate spine density. The spines spreads across the entire surface of the fruit except for the collection from Meghalaya, with the fruits bearing long/total length furrows for most of them.

Cluster (4) had a similar number of collections from different locations, i.e., Assam, Manipur, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. These collections were

Table 2 Variability in some quantitative characters of *S. edule* accessions

Traits	Maximum values	Minimum values	Mean ± SD	Cumulative variations (%)
Fruit length (cm)	15	5.8	11.70 ± 2.09	17.86
Fruit width (cm)	9	2	6.60 ± 1.27	19.24
Fruit diameter (cm)	28	6	20.55 ± 3.99	19.41
Fruit weight (g)	498.33	7.85	247.96 ± 108.81	43.88
Length of spine (cm)	5	0	1.40 ± 1.4	100

Table 3 Contribution percentages and quantitative traits associated with the first six principal components of 36 *S. edule* accessions and their Eigen vectors

Principal component	PC-1	PC-2	PC-3	PC-4	PC-5	PC-6
Explained proportion of variation (%)	30.11	19.57	11.27	8.62	7.57	5.74
Cumulative proportion of variation (%)	30.11	49.68	60.95	69.57	77.14	82.88
Traits	Eigen vector					
Fruit shape	0.0983	−0.3126	−0.4831	−0.6006	0.0809	0.3510
Fruit length	0.5764	−0.3757	0.2053	0.5169	0.2740	−0.2024
Fruit width	0.5943	−0.5997	−0.3520	0.2036	0.1242	−0.0364
Fruit diameter	0.6271	−0.6725	−0.2972	0.1413	0.0332	−0.0638
Fruit ratio	−0.1940	−0.5433	0.6213	0.2811	0.2237	−0.0137
Fruit weight	0.6907	−0.5010	−0.0947	0.2834	0.2420	0.0821
Spine density	0.7491	0.1101	0.2014	−0.5029	0.0632	−0.0146
Mature fruit skin color	0.3146	−0.1332	0.1186	−0.0027	−0.6908	−0.2126
Longitudinal furrow depth	−0.5515	−0.6732	0.4098	−0.1475	0.1809	0.0011
Reticulation	0.0575	−0.1115	0.2247	0.4219	−0.4470	0.6693
Cross section profile	−0.0311	0.3224	−0.1342	−0.0184	0.4718	0.4612
Spine distribution	0.8224	0.1462	0.2888	−0.0755	0.0373	0.1164
Length of the spine	0.7422	−0.0559	0.2557	−0.3451	−0.0834	−0.1362
Size of base spine	0.6792	0.1168	0.3607	0.0175	0.1123	0.2518
Spine flexibility	0.7895	0.1990	0.4082	−0.2451	−0.0307	0.0114
Furrows	−0.1574	−0.5971	0.1774	−0.0409	−0.3899	0.2355
Depth of furrows	−0.5515	−0.6732	0.4098	−0.1475	0.1809	0.0011
Ridges	−0.4565	−0.6605	0.4582	−0.2056	0.1228	−0.0039

mostly subpyriform or ovoid in nature. These were large in size and the length of the fruit was ranging from 12 to 15 cm. The weight of these fruits ranged from 234.16 to 440.86 g having spines mostly covering entire plant and furrows being long and total length. Cluster (5) had three collections from Sikkim and Manipur. The shape of the fruits was more or less ovoid or round. These three fruits had a very high or an intermediate spine density bearing a very superficial longitudinal furrow depth.

The un-clustered landrace, collected from Sikkim had a length of 5.8 cm and was the tiniest among the collections. It weighs 7.85 g having very low spine density. The minimum, maximum and the mean values to express the range of variability are presented in Table 2.

Genetic diversity and cluster analysis based on DAMD

In the present study, 12 minisatellite core sequences primers were screened among 36 accessions for scoring the amplified DNA sample and to reveal genetic variability of *S. edule* accessions (Table 4). By optimizing annealing temperature for amplification, each of the primers produced distinct banding patterns with good reproducibility and resolutions. All primers were capable to amplify polymorphic bands. A total of 102 bands were obtained out of which 97 were polymorphic and the average percentage polymorphism of the bands was found to be 95.09%. Eight primers gave 100% polymorphism (URP2F, URP25F, URP38F, HVR (−), OGRBO1, M13, 14C2 and HBV5). The DAMD profile obtained with primer 14C2 is given in the Fig. 2. The percentage polymorphism ranged from 66.66 to 100 and the lowest was recorded for URP1F indicating that DAMD primers could be used to access the genetic variations for *S. edule* accessions.

The dendrogram, resulting from the complete linkage percent disagreement using UPGMA method, revealed that 36 accessions were grouped into three major clusters Fig. 3. First cluster had 13 accessions from NE India (Sikkim, Manipur, Meghalaya, Mizoram, West Bengal, Chhattisgarh and Assam) with more or less superficial or intermediate furrow depth. Accession SEC-28 and SEC-2 is distinct from the group belonging from Karnataka and Sikkim being ovoid in shape and having no spines. The second cluster also had 13 accessions from South India (Karnataka, Andhra Pradesh, Tamil Nadu and Kerala) with one accession SEC-9 from Meghalaya being similar to

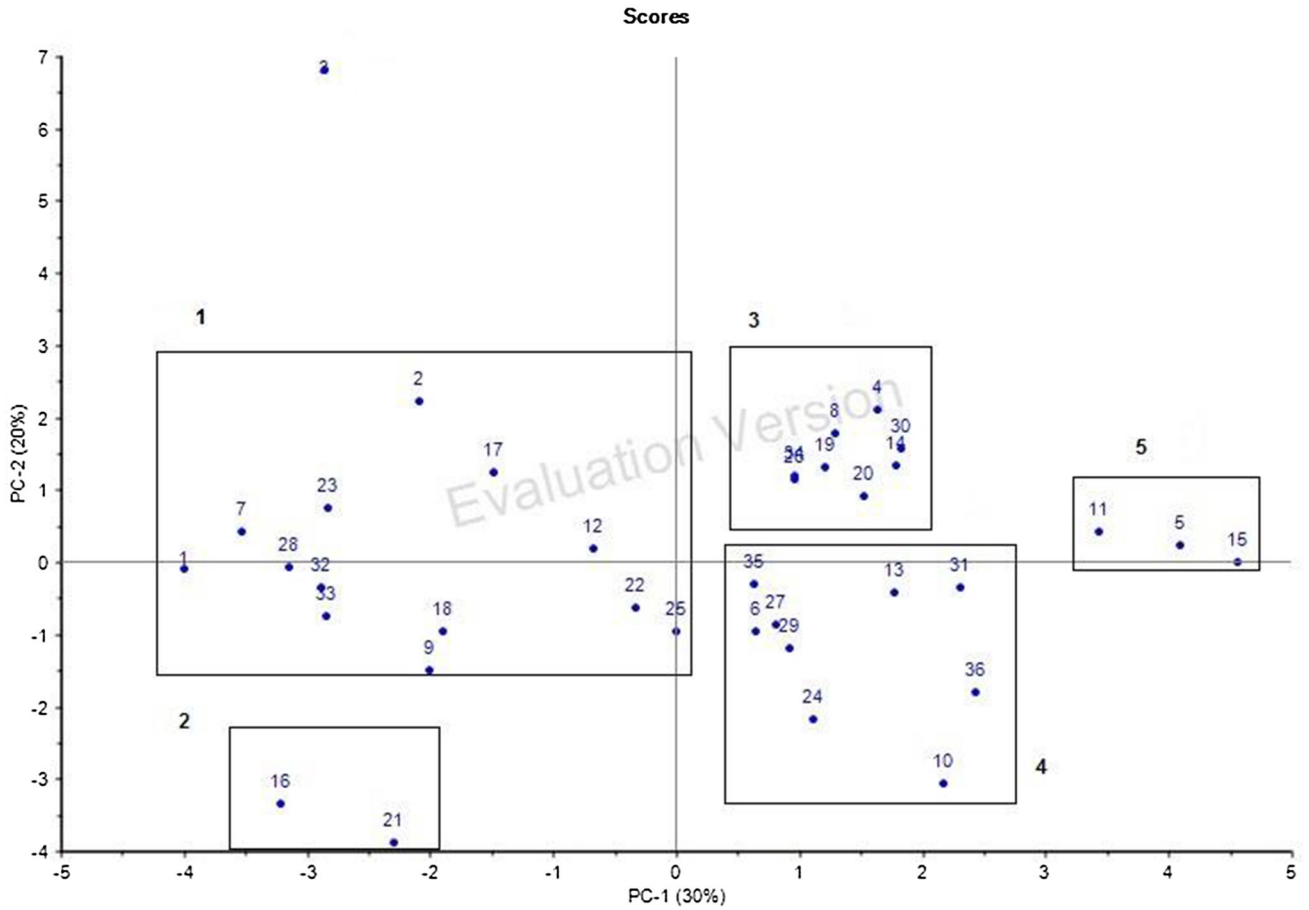

Fig. 1 The first and second principal component scores (PC-1 and PC-2) for the identification of *S. edule* accessions performance based on 18 qualitative and quantitative traits

Table 4 Polymorphism revealed by DAMD primers in *S. edule* accessions

Sl no.	Primers	Primer sequence 5′–3′	Ta (°C)	No. of bands	Polymorphic bands	% Polymorphism
1	URP1F	ATCCAAGGTCCGAGACAACC	55	6	4	66.66
2	URP2F	GTGTGCGATCAGTTGCTGGG	61	5	5	100
3	URP4F	AGGACTCGATAACAGGCTCC	58	6	5	83.33
4	URP25F	GATGTGTTCTTGGAGCCTGT	58	8	8	100
5	URP38F	AAGAGGCATTCTACCACCAC	60	9	9	100
6	HVR (−)	CCTCCTCCCTCCT	50	8	8	100
7	OGRBO1	AGGGCTGGAGGAGGGC	55	6	6	100
8	6.2H (+)	AGGAGGAGGGGAAGG	56	9	8	88.88
9	M13	GAGGGTGGCGGCTCT	57	9	9	100
10	14C2	GGCAGGATTGAAGC	53	10	10	100
11	YNZ^{22}	CTCTGGGTGTGGTGC	56	10	9	90
12	HBV5	GGTGAAGCACAGGTG	53	11	11	100

these accessions. All accessions had long total length furrows except the accession SEC-30 having short distal. Cluster three forms a group of accessions belonging from Manipur having a linkage distance of 0.3. The fruit length was above 8.5 and fruit weight was above 175 g. These accessions had very low or intermediate or no spines bearing fruits. The maximum linkage distance obtained was 0.4. Thus, the present study indicates that the DAMD makers can be efficiently used to characterize *S. edule* accessions.

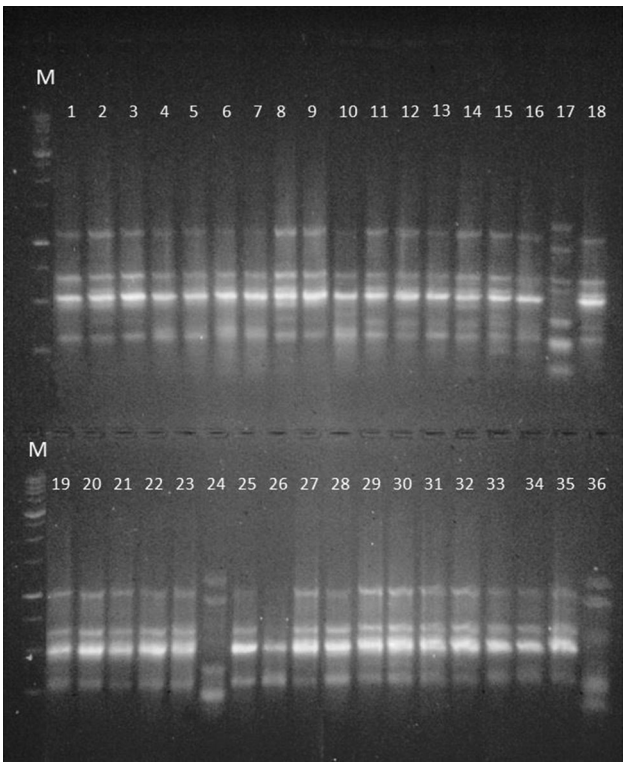

Fig. 2 Gel profile obtained with DAMD primer (14C2). The profiles were resolved in 1.2% agarose gels in 1 X TBE. The *lanes* marked as marker (*M*) contain DNA fragment size marker

Discussion

India is rich in *S. edule* genetic resources and the identification or utilization of these diverse germplasm is the central criteria in plant breeding. Thorough knowledge and understanding can provide a platform for accurate assessment of plant cultivars. Analysis of genetic diversity can assist in reliable classification of collections required for crop improvement (Mohammadi and Prasanna 2003; Manohar and Murthy 2012). Morphological analysis has been used for characterizing large data sets and such description has become a valuable source of information for agronomic and breeding programs (Boczkowska et al. 2014). In the present study, the morphological classification of 36 accessions revealed a great diversity in fruit morphological traits across India. The first six components give a total variation of 84%. The characters with such high variability will be expected to provide high level of gene availability for transfer during the breeding programs (Aliyu et al. 2000). The maximum variation was seen in case of length of spines followed by weight of the fruit, with prominent variations in color, size, shape and presence of furrows. The presence of spines was attributed to the lower amounts of gibberellic acid as demonstrated by Cadena-Iniguez (2005) in an experiment where the application of gibberellic acid promoted spine development to the fruits showing certain level of dependency over hormones.

The fruits collected in the present study had a maximum weight of 498.33 g and a minimum of 7.85 g and the fruit length varied from 5.8 to 15.0 cm when compared to the collections from Sikkim as observed by Kapoor et al. (2014) (maximum fruit weight of 461 g and a minimum of 74.1 g; maximum fruit length of 8.43–16.76 cm). Similar diversity was observed with that of fruit colour, shape and spine density. Maximum diversity was observed in the present study than that of the Kapoor et al. (2014) as they

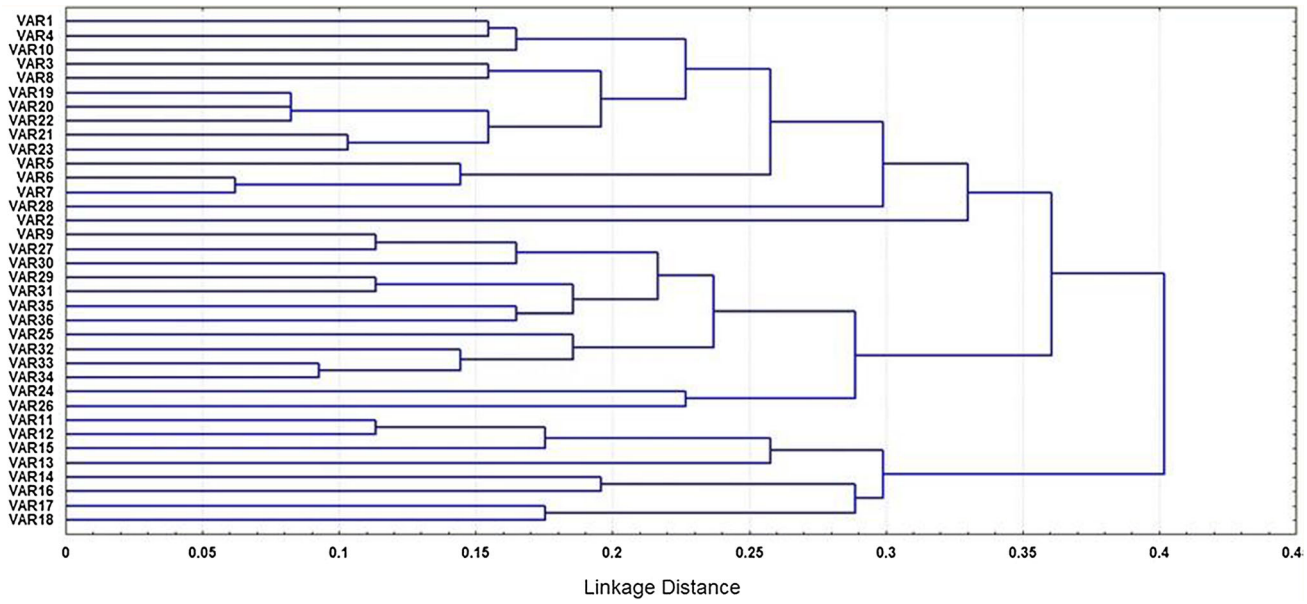

Fig. 3 UPGMA dendrogram for molecular data relating 36 *S. edule* accessions

had concentrated their collection only to Sikkim state of India. Sanwal et al. (2010) observed a similar morphological diversity in the North-Eastern germplasm of India as that of Kapoor et al. (2014) with the length of the fruit (5.70–15.80 cm), diameter (5.2–10.90) and weight (134–406 g) showing maximum variations. In a study conducted by Cadena-Iniguez et al. (2008) fruits of *Sechium edule* were collected in the central region of Veracruz, Mexico, and classified them under eight groups according to their characteristics. The fruit length varied from 3 to 15 cm which is similar as obtained in the present study. It projected the quantitative, pseudo-qualitative and qualitative characters of leaf flower and fruit depicting morphological and anatomical variations of *S. edule* and the greatest variation was observed among the cultivated varieties. The variations observed may be due to the environmental and soil conditions (pH 7.3–7.9) and the presence of Ca and Mg can cause physiological stress leading to low levels of chlorophylls and thus evoking color loss of the fruits of the plant (Cadena-Iniguez et al. 2007).

From the present study, it was observed that the maximum diversity of *S. edule* was concentrated in the North-Eastern India when compared to low diversity in the Southern India. The North-Eastern India has a different set of landraces that does not grow in the planes of Southern India as it grows in high altitude regions of India. This study provides a detailed diversity of the fruit morphology present in India and the same were also observed in the Central American collections (Newstrom 1991). The collections of Costa Rica and Mexico had fruit sized up to 25 cm, which weighed at least 1000 g (Engels 1983). Such huge fruits were not observed in Indian collections.

Even though the morphological characters are generally employed to estimate genetic diversity, such a method has its own limitations as the traits are heavily influenced by the environmental conditions and climate being the main factor influencing the growth and development of the species (Cadena Iniguez and Arevalo Galarza 2011). As a result, the *S. edule* accessions have also been studied using molecular markers. To have a comprehensive idea of the variability among the *S. edule* accessions, DAMD analysis was carried out. The results obtained showed a higher percentage of polymorphism, i.e., ranging from 66.66 to 100. Eight primers showed 100% polymorphism, i.e., URP2F, URP25F, URP38F, HVR (−), OGRBO1, M13, 14C2 and HBV5. Four primers were found to have 100% polymorphism as compared to studies conducted by Hu et al. (2010) using DAMD markers for cucumber collections. Avendano-Arrazate et al. (2012) reported high degree of polymorphism in Mexican accessions of *S. edule* but such studies was limited to the use of isozyme systems. The high level of percentage polymorphism obtained indicates that DAMD primers can be used for *S. edule* species to screen its diversity.

The UPGMA method of clustering using complete linkage percent disagreement revealed three clusters. The maximum linkage distance observed was 0.4. The dendrogram obtained showed that all accessions had a discrete pattern of clustering which have been grouped more or less according to their state or geographical distribution. From the above results, North East and South India collections were easily separated suggesting a practicability of using DAMD markers for germplasm identification.

There was less correlation between the divergence of morphological traits and the data obtained using molecular marker which suggests that the morphological variation may be determined by environmental factors and also by genetic factors as reported for other crops (Seyedimoradi et al. 2012; Ashish et al. 2014; Kumar and Nair 2013). It is frequently observed that the genetic variation determined by molecular markers can produce different results due to analysis in different regions in the genome captured by the respective markers. Morphological traits are associated with a relatively small number of specific gene loci; thus, there could be loss of potential difference in the analysis of large amounts molecular data (Diederichsen 2009) suggesting that DAMD markers varied in terms of comparability with morphological traits of *S. edule* accessions. From a wide variety of colors, shapes and flavors, this species is widely accepted as a regional food across world. Though *S. edule* has economic, cultural and environmental importance, comprehensively it has not been addressed in research process (Cadena Iniguez and Arevalo Galarza 2010).

There are no reports on the genetic diversity of *S. edule* accessions using morphological characters and molecular markers so far. This remains the first study using morphological and genetic diversity characterization of *S. edule* in India and the study reveals a high degree of diversity among the Indian *S. edule* accessions which can be further used for crop improvement. This may provide an opportunity to enhance and boost the breeding strategy.

In conclusion, the accessions used in the present study showed a wide variation in the morphological characters. All the minisatellite core sequences revealed high level of polymorphism in *S. edule* accessions. The PCA analysis and phylogenetic data obtained based on DAMD markers generated a specific clustering patterns which revealed geographical variation due to environmental conditions and the three groups obtained in a dendrogram demonstrated the genetic relationship among the germplasm. Such characterization of genetic resources forms an important factor for crop improvement program. It also confirms the importance of molecular studies besides the morphological data in detecting the genetic variation among the diverse

accessions to carry out further crossing studies successfully.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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