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RESEARCH ARTICLE

Assessment of Spine Gourd (*Momordica dioica* Roxb.) with Cross-species Transferable Microsatellite and ISSR Markers

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Abstract

Spine gourd (*Momordica dioica* Roxb.) is a highly nutritious and underutilized potential vegetable containing high amount of protein as compared to other cucurbitaceous vegetables. The present study was under maker to identify and develop the molecular markers for genetic diversity study in spine gourd. In this regard, total 94 (24 *M. dioica*, 50 *M. charatia*, 10 *Cucumis sativus,* 10 *C. melo*) nucleotide sequences were obtained from national center for biotechnology information (NCBI) platform followed by extraction of desirable sequences by Batch primer 3. Out of 94 simple sequence repeats (SSR) sequences, 52 were amplified with spine gourd genotype. However, only seven SSRs were polymorphic and useful for diversity study from the 52 markers. In addition, 10 ISSR markers were also evaluated for genotyping and genetic diversity study. SSR markers were produced 2.42 average alleles per primer, whereas inter simple sequence repeats (ISSR) markers were produced 4.6 average alleles per primer. The average polymorphism information content (PIC) value for SSR markers was 0.32 and for ISSR was 0.73. SSR markers based cluster analysis revealed two major cluster of 41 genotypes in which highest dissimilarity was obtained between genotypes PK-49 and NDM-3 at 60% Jaccard's similarity coefficient. Similarly, ISSR markers based diversity analysis also grouped the 41 genotypes into two clusters in which highest dissimilarity was recorded between PK-35 and PMD-5-1 at 66% dissimilarity level. Diverse genotypes will be very useful in cross-breeding programmes. Molecular markers identified through this study can be used for further crop improvement activities.

Keywords: Genetic diversity, Molecular markers, Polymorphism information content, Spine gourd, Transferability.

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Introduction

Spine gourd (*M. dioica* Roxb.) is an important potential vegetable crop and one of the most consumed during rainy season in India and East Asia. It belongs to the Cucurbitaceae family having chromosome no. 2n=2x-28. This commercial vegetable crop is gaining popularity because it is rich in taste and has high nutritional value. Per 100 g of edible fruit was found to contain carbohydrate 47.92 g, crude protein 19.38 g, crude fiber 21.30 g, crude lipid 4.70 g and ash 6.70 g. It also contained little quantities of essential elements like iron 4.60 mg, phosphorus 42 mg, calcium 33 mg, vitamins like thiamin 0.05 mg, riboflavin 0.18 mg, niacin 0.06 mg, ascorbic acid 29.94 mg and carotene 162 mg (Bharathi *et al.,* 2007 and Aberoumand, 2011).

Spine gourd's medicinal and curative properties are known for ages and are passed on from generation after generation as traditional knowledge. Decoction of leaves reduces fever; tuberous roots help in relieving headaches, excess sweating, stone formation and migraine, while the fruit is quite helpful in controlling diabetes and blood pressure (Ram *et al*., 2001, Talukdar and Hossain, 2014). Tuberous roots are used for curing diarrhea, fever and rheumatism by the tribal's of Chhattisgarh, seeds are used against chest problems and stimulate urinary discharge (Bharathi*et al*., 2007).

Area under spine gourd cultivation has increased day by day because of so many advantages like high demand in the market good nutritional and medicinal value, high keeping quality, ability to withstand long-distance transportation, high market price and good export potential (Rasul, 2003). Thus, this minor vegetable crop has gained popularity among farmers and may be a useful supplement crop in developing nations, especially in overpopulation and undernourished areas. The initiation of any plant breeding programme requires the morphological characterization of germplasm but through morphological characterization, we limited for only few characters and genes.

In the recent era of Omics, all types of information viz., morphological, biochemical, physiological and molecular, were generated in huge quantities and in a very short period, leading to rapid selection and development of new genotypes with diverse characters. The development of molecular markers could complement the morphological markers. This enabled the plant breeders to overcome the limitations faced in the use of morphological markers (Rana and Das, 2016). The knowledge of morphological and molecular diversity in a crop species is essential for their improvement. The genetic diversity among the landraces might contribute to intra-specific crosses among this spine gourd collection landraces having high potential for genetic improvement of this vegetable crop. Spine gourd had great medicinal and nutritional value, but due to some problems like dioecious nature, low seed germination, availability of plant materials and early-stage identification of sex type restrict the domestication process. Recently, the transferability of SSR markers has also been reported in related species of spine gourd (Shukla *et al*. 2017) for understanding the genetic variations between species and genera. To accelerate the domestication of the spine gourd two major objectives were studied in this investigation, which are (1) Morphological characterization of diverse genotypes of spine gourd, and (2) Identification of molecular markers and their utilization for generation of molecular data of spine gourd germplasm.

Materials and Methods

Experimental Site

Morphological characterization of spine gourd genotypes were done at the research cum instructional farm and molecular markers-based characterization was accomplished at Plant Molecular Biology and Biotechnology laboratory of Rajmohini Devi College of Agriculture and Research Station, IGKV, Ambikapur (Chhattisgarh).

Plant Materials, Planting Geometry and Morphological Characters Recorded

The experimental plant material comprised of 41 genotypes of spine gourd, including two checks. These accessions were originated from different agroecological regions of the country (Table 1). Plant materials were grown at a field in 13 blocks to evaluate morphological characters during the crop season of 2018-19.

The planting geometry between plants was 2 x2 m for each line. The ratio of female: male (F:M) was maintained at 8:1 ratio to obtain sufficient fruit yield. Standard agronomic practices were adopted during the entire crop period. Special mechanical supports were provided to plants through the bamboo sticks for better growth, development and fruit yield.

Total 18 characters, including plant morphology and fruit yield parameters, were recorded according to the standard method during different crop growth stages. Details of the morphological characters and their stage of observation are given in Supplementary Table 1.

Identification and Synthesis of SSR Marker based on Cucurbitaceous Crop Genome Sequence for Spine Gourd

Nucleotide sequences of cucurbitaceous crops *viz*., cucumber, bitter gourd, and watermelon were searched from NCBI platform to identify and synthesize SSR Markers for spine gourd. From such nucleotide sequences, FASTA sequences have been extracted for designing the SSR primers by using primer designing program *i.e*. Batch primer 3. As suggested by Castoe *et al*. (2012) for selecting SSR primer from FASTA sequence, standard criteria was applied during the designing of SSR primers by Batch primer 3. Following this procedure, total 94 (24 *M. dioica*, 50 *M. charatia*, 10 *C. sativus*,10 *C. melo*) SSR primer sequences were obtained synthesized.

All 94 SSR primers were initially screened in a single genotype IK-1 of *M. dioica* to check their quality, annealing temperature, amplification and compatibility with the *M. dioica*genome. Based on this experiment, total 52 SSRs were amplified and showed genome compatibility with *M. dioica*. When these 52 markers were used for finger-printing in 41 genotypes, only seven showed polymorphism. Therefore,only seven SSR markers were identified for fingerprinting and molecular diversity analysis in *M. dioica*. A list of seven SSR markers and name of their source genome and other details are given in Table 2.

ISSR Marker Identification

Total 10 ISSR markers were procured from R. H. Richharia Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur for the current study. Primers details are given in Table 3. All the ISSRs were screened in a genotype of *M. dioica* IK-1 to check their quality, annealing temperature, amplification and compatibility with *M. dioica* genome. All the 10 ISSR markers were amplified however, only seven ISSRs were polymorphic out of the 10 markers. Therefore, seven ISSR markers were identified and used for genetic diversity analysis.

Table 1: List of the genotypes of spine gourd used for the investigation along with their source of collection

S. No.	*Genotypes*	*Sources*
1	IK-1 (C)	Ambikapur, Chhattisgarh
2	RMDSG-3 (C)	Ambikapur, Chhattisgarh
3	PK-5	Surguja, Chhattisgarh
4	PK-9	Surguja, Chhattisgarh
5	PK-26	Surguja, Chhattisgarh
6	PK-34	Surguja, Chhattisgarh
7	PK-35	Surguja Chhattisgarh
8	PK-46	Surguja, Chhattisgarh
9	ASG-18-5	Ambikapur, Chhattisgarh
10	NDM-5	Faizabad, Uttar Pradesh
11	RMF-7-P-1	Rahuri, Maharashtra
12	RMF-P-4	Rahuri, Maharashtra
13	RMF-27	Rahuri, Maharashra
14	RMF-17	Rahuri, Maharashtra
15	RMF-1	Rahuri, Maharashtra
16	PK-49	Surguja, Chhattisgarh
17	PK-33	Surguja, Chhattisgarh
18	PHULLE MD-5-2	Rahuri, Maharashtra
19	DHARAMJAYGARH	Dharmjaygarh, Raigarh, Chhattisgarh
20	PHULLE MD-5-1	Rahuri, Maharashtra
21	RMDSG-1	Surguja, Chhattisgarh
22	KRISHNAPUR	Surguja, Chhattisgarh
23	AMBIKA K-12-1	Pratappur, Balrampur
24	AMBIKA-13-5	Pratappur Mani, Balrampur, Chhattisgarh
25	AMBIKA-13-6	Pratappur, Balrampur
26	AJSG-3	Jashpur, Chhattisgarh
28	RMF-G-49	Rahuri, Maharashta
29	NDM-1	Faizabad, Uttar Pradesh
30	NDM-4	Faizabad, Uttar Pradesh
31	NDM-3	Faizabad, Uttar Pradesh
32	NDM-2	Faizabad, Uttar Pradesh
33	RAIGARH	Raigarh, Chhattisgarh
34	AJSG-4	Jashpur, Chhattisgarh
35	AJSG-1	Jashpur, Chhattisgarh
36	AJSG-2	Jashpur, Chhattisgarh
37	AJSG-5	Jashpur, Chhattisgarh
38	ASG-18-1	Ambikapur, Chhattisgarh
39	ASG-18-2	Ambikapur, Chhattisgarh
40	ASG-18-3	Ambikapur, Chhattisgarh
41	ASG-18-4	Ambikapur, Chhattisgarh

DNA Extraction from Tender Leaves

DNA was isolated with the help of the modified CTAB method. Tender leaves free from diseases, insects, and viruses and developmental deformities, were collected from each genotype. Collected leaves were kept in ice box and brought to the laboratory where they were washed with 70% ethanol to remove traces of dirt and used for DNA extraction. Theleaves were cut into small pieces and kept in mortar which have 400 µL CTAB and ground thoroughly with the help of pestles. Samples were transferred on 1-mL

Table 2: List of seven SSR markers, sequence, annealing temperature, PIC value and name of their source genome

Markers name	*Forward sequence*	*Reverse sequence*	*No. of alleles produced*	*Alleles size (bp)*	*Alleles frequency*	*PIC value*	*Source genome*
SgSSR1	CAGGGGCGATTAGATTATTC	GTGATGTGGGTCGAGCAGTA	2	100 180	0.95 0.05	0.095	*M. dioica*
SgSSR17	AGAGATAACCGCAGTTCATAA	TTCTCTCTTTTCTCTCATCCA	3	100 200 800	0.64 0.2 0.15	0.521	*M. dioica*
SgSSR15	AGTTCTACTGAGATGGAAGTGG	TGTTAAAACCAGAACCTATGC	2	150 500	0.94 0.051	0.097	*M. dioica*
SgSSR13	ATTGGTCATCTCGAAAGGTAT	GTTGGAAAAATGTGGTAACAG	3	150 600 800	0.76 0.078 0.15	0.384	*M. dioica*
SgSSR11	CACATGATTTATGGGTTTCAT	TGAGTAAGAGAGAGAACGAAAA	3	50 100 400	0.57 0.24 0.18	0.58	*M.dioica*
McN12	CAGAGGGGTGGTTCCTCTTT	CCACATGGATGATCGAGAGA	2	100 250	0.71 0.28	0.404	*M. charantia*
McN24	CTCCAACTTGAGGAAAGAAAAC	AGAGCCAATTGGGGCTTTAT	2	50 100	0.91 0.088	0.162	*M. charantia*

Table 3: Details of ISSR markers used for genetic diversity study in spine gourd

S. No.	*Primers*	*Sequence (5′ – 3′)*	*No. of alleles*	*Alleles size (bp)*	*Alleles frequency*	*Annealing temp. (°C)*	*PIC value*
1	UBC-808	AGAGAGAGAGAGAGAGC	4	150 200 250 350	0.26 0.26 0.25 0.21	50	0.748
2	UBC-812	GAGGAGGAGGAGGAGAA	4	100 200 280 450	0.28 0.28 0.28 0.14	52	0.734
3	UBC-824	TCTCTCTCTCTCTCTCG	5	150 220 300 400 500	0.32 0.32 0.025 0.24 0.10	52	0.725
4	UBC-840	GAGAGAGAGAGAGAGAYT	4	120 210 310 450	0.36 0.16 0.14 0.32	52	0.712
5	UBC-841	GAGAGAGAGAGAGAGAYC	6	120 200 250 300 350 400	0.26 0.25 0.20 0.17 0.08 0.01	52	0.786
6	UBC-856	ACACACACACACACACYA	4	180 280 250 300	0.35 0.34 0.17 0.28	51	0.675
7	UBC-873	GACAGACAGACAGACA	5	150 250 400 450 600	0.32 0,30 0.22 0.11 0.02	51	0.734

Eppendorf tube and again 400 µL CTAB was added then kept in the water bath for 20 minutes at 65°C. After incubation, 700 µL of 24:1 chloroform: Iso-amyl alcohol was added and mixed well by using vertex. Later tubes were centrifuged at 14,000 rpm for 5 minutes to settle down the debris. Supernatant was taken out into new 1.5 mL centrifuge tube with the help of micro pipettes. Steps 5 and 6 were repeated one more time. Then 900 µl of chilled ethanol was added and kept for incubation at -20°C for 2 hours. After incubation, tubes were centrifuged at 14,000 rpm for 20 minutes and the supernatant was discarded. DNA pellet was washed with 50 µL of 70% ethanol. Tubes were centrifuged for 3 minute at 14,000 rpm and upper layer of ethanol was discarded. Pellet was dried through air at room temperature. 100 µL of TE buffer was added to dissolve the pellet.

Table 4: PCR mix of 20 µL volume was prepared in 1.5 mL centrifuge tube by using the following components

Stock	*Final Concentration*	*Volume (µL)*
Sterile ddH_2O	-	11.5
PCR buffer	10 X	2.5
dNTPs (mix)	1.0 mM	1.5
Primer (forward)	5 ρmol.	1
Primer (reverse)	5 ρmol.	1
DNA Template	100 ηg/µL	2
Taq polymerase	5 U/µL	0.5
Total	-	20

Quantification of DNA

Quality and quantity of DNA were checked by electrophoresis on 1% agarose gel. Total 6 µL of solution was prepared by adding 3 µL of extracted DNA and 3 µL of 6X loading dye (bromo phenol blue). The solution was mixed well and vortexed to settle down into the bottom and loaded into the wells of agarose gel. Current of 50V was applied till the initial movement of DNA from the wells and later increased to 70V. Once the tracking dye reached to 90% length of gel, electrophoresis was stopped and the visualized in gel documentation system E-BOX CX5.TS (20M). A single intact band of genotype indicated the good quality and quantity of DNA. Based on the band intensity, extracted DNA was diluted and further used for PCR amplification by using SSR and ISSR markers.

PCR Amplification

PCR mix of 20 µL volume was prepared in 1.5 mL centrifuge tube by using several components as shown in Table 4. PCR cycle consisted of initial denaturation at 94°C for 5 minutes; followed by 40 cycles of amplification, at 94°C for 30 seconds (denaturation), 50 to 60°C for 1-minute (annealing) and 72°C for 1-minute (extension). A final extension step at 72°C for 7 minutes was followed by termination of the cycle and stored the PCR product at 4°C.

Agarose Gel Electrophoresis and Visualization

A total of 1% agarose gel (1-gm agarose + 100 mL 1X TBE + 2 µL ethidium bromide) was used for separation and

Table 5: Descriptive statistics of various morphological characters in spine gourd genotypes

S. No.	*Characters*	*Mean*	*Range*		*Standard Deviation*	*Coefficient of Variation (%)*
			Minimum	*Maximum*		
1	Days to 1st flowers	41.20	32.00	54.00	5.82	14.14
2	No. of first flowering node	14.85	6.00	24.00	5.08	34.21
3	No. of stem per plant	10.32	5.00	18.40	3.57	34.61
4	Leaf Length (cm)	7.35	5.34	9.60	0.97	13.17
5	Stem color	1.15	1.00	2.00	0.36	31.22
6	Leaf width (cm)	7.20	5.58	9.10	0.83	11.51
7	Pedicel length (cm)	2.91	1.52	4.08	0.52	17.79
8	Ovary length (mm)	1.70	1.40	2.44	0.24	14.27
9	Ovary diameter (mm)	0.71	0.52	0.86	0.07	10.24
10	Style length (mm)	0.77	0.62	1.02	0.09	12.04
11	Pistil length(mm)	0.60	0.40	0.76	0.10	16.48
12	Fruit length (cm)	4.19	2.84	5.96	0.69	16.53
13	Fruit diameter (cm)	3.31	2.36	4.56	0.64	19.40
14	No. of fruit per plant	112.28	103.20	121.20	4.49	4.00
15	Single fruit weight (g)	14.78	9.60	19.00	2.52	17.05
16	Fruit yield per plant (kg)	1.67	1.07	2.52	0.30	18.14
17	No. of seed per fruit	9.77	7.80	11.20	0.80	8.19
18	100 Seed weight (g)	26.52	14.50	41.00	7.03	26.49
19	Fruit yield (q/ha)	33.37	21.35	50.42	6.05	18.14

Table 6: List of top performing three genotypes for fruit yield and related traits

S. No.	*Name of traits*	*Genotype-1*	*Genotype-2*	*Genotype-3*
1	Days to 1st flowers	IK-1 (32)	PK-34 (33)	ASG-18-4 (34)
2	Leaf length (cm)	PK-9 (9.6)	PK-34 (8.8)	RMF-7-P-1 (8.74)
3	Fruit length (cm)	PK-49 (5.96)	PK-26 (5.88)	RMF-7-P-1 (5.68)
4	Fruit diameter (cm)	PK-33 (4.56)	AMBIKA 13-6 (4.32)	RMF-G-39 (4.22)
5	No. of fruit per plant	ASG-18-5 (121.2)	RMF-1 (121)	IK-1 (120.4)
6	Single fruit weight (g)	AMBIKA 13-5 (19)	KRISHNAPUR (18.6)	IK-1 (18.4)
7	Fruit Yield per plant (kg)	RMDSJ-1 (2.52)	IK-1 (2.22)	AMBIKA 13-5 (2.09)
8	No. of seed per fruit	NDM-1 (11.2).	AMBIKA 13-5 (11.2)	RMF-G-39 (11.2)
8	100 Seed weight (g)	ASG-18-1 (41)	PK-35 (38.54)	ASG-18-2 (38.5)
9	Fruit yield (q/ha)	RMDSG-1 (50.42)	IK-1 (44.31)	AMBIKA 13-5 (41.88)

visualization of PCR products. In 2 µL loading dye (10x) was added to PCR products and mixed well. In 10 µL of PCR mix were loaded into the wells. Total 50 bp DNA ladder was loaded in the first well. Gel was run at 70 volts for SSR and 80 volts for ISSR for 40 minutes till the dye reached to the last end of the gel. Thereafter, gel was visualized by E-BOX CX5.TS (20M) having UV Trans-illuminator and gel pictures were saved for further scoring of bands.

Scoring of Bands

The banding pattern of genotypes, developed by each set of primer was scored separately. If band is present for a specific amplicon size it was scored as '1' (allele is present) and absence of any band for that position was scored as '0' (allele is absent). Allele size of different markers was determined based on the banding pattern of 50 bp DNA ladder.

Bio-statistical Analysis of Morphological and Molecular Data

Morphological data obtained from 5 plants of each genotype were used for descriptive statistics analysis by using the XLSTAT v18.3 software. Mean data were used for genetic diversity study by following and UPGMA (Un-weighted Pair Group Method with Arithmetic averages) algorithm and Euclidean distance by using XLSTAT v18.3 software.

Scoring of banding pattern of SSR and ISSR markers were used for genetic diversity analysisby following the UPGMA algorithm and Jaccard's similarity coefficient by using NTSYS-pc software. A dendrogram showing the distance-based relationship between the genotypes was constructed by following this method.

Results and Discussion

Performance of Genotypes based on Morphological Traits

Descriptive statistical parameters of various morphological traits are given in Table 5 and list of top performing three genotypes for fruit yield and related traits are given in Table 6. Days to 1st flowering ranged from 32 days IK-1 to 54 days RMF-27 from date of sowing with an average 41 days and standard deviation 5.68 days. Coefficient of variation for days to 1st flowering was 14.14%. Genotype IK-1 showed earliest flowering followed by genotype PK-34. Leaf length ranged from 5.34 to 9.60 cm with average 7.35 cm and standard deviation 0.97 cm. Coefficient of variation for leaf length was 13.17%. Genotype PK-9 had longest leaf followed by genotype PK-34. Fruit length was ranged from 2.84 to 5.96 cm with an average of 4.19 cm and standard deviation 0.69cm. Coefficient of variation for the trait is 16.53%. Genotype PK-49 had longest fruit followed by genotype PK-26. Fruit diameter was ranged from 2.36 to 4.56 cm with an average of 3.31 cm and standard deviation 0.64 cm. Coefficient of variation for the trait is 19.40%. Genotype PK-33 had highest fruit diameter followed by genotype Ambika 13-6. Number of fruits per plant ranged from 103 to 121 with an average of 112 and standard deviation 4.49. Coefficient of variation for the trait is 4%. Genotype ASG-18-5had the highest number of fruits followed by genotype RMF-1 and genotype IK-1. Weight of single fruit ranged from 9.60 g to 19 g with an average of 14.78 g and standard deviation 2.52 g. Coefficient of variation for the trait is 17.05%. Genotype Ambika13-5 had highest single fruit weight followed by genotype Krishnapur. Fruit yield per plant ranged from 1.07 to 2.52 kg with an average of 1.67 kg and standard deviation 0.30 kg. Coefficient of variation for the trait is 18.14%. Genotype RMDSG-1 had highest fruit yield per plant followed by genotype IK-1. Number of seeds per fruit ranged from 7.80 to 11.20 with an average of 9.77 and standard deviation 0.80. Coefficient of variation for the trait is 8.19%. Genotype NDM-1 had the highest number of seeds per fruit followed by genotype AMBIKA 13-5. Hundred seed weight ranged from 14.50 to 41 g with an average of 26.52 g and standard deviation 7.03 g. Coefficient of variation for the trait is 26.49 %. Genotype ASG-18-1 had highest single fruit weight followed by genotype PK-5. Ample amount of variability is present among the genotypes that can be utilized for improvement of this crop.

Genetic Diversity based on Morphological Traits

Morphological character based genetic diversity analysis was performed to construct a dendrogram by following Euclidean distance and UPGMA algorithm through Sim Qual program NTSYSpc software version 2.02 (Rohlf, 1998) (Figure 1). All the 41 genotypes were grouped into two major clusters in which cluster-I have 40 genotypes whereas cluster-II have 1 genotype (AJSG-1). The Euclidean distance ranged from 0 to 15. In pairwise comparison, the maximum distance was obtained between AGSG-1 and PK-46 with the Euclidean distance of 15, whereas Raigarh and RMF-G-39 showed least distance (ED-5). Cluster-I further partitioned into two sub clusters in which first sub cluster consisted 30 genotypes whereas Sub cluster-II has consisted 10 genotypes.

Although, the dendrogram generated from similarity or genetic distance matrix based on morphological traits has provided an overall pattern of variation as well as the degree of relatedness among genotypes. Genotypes showing maximum dissimilarity could be used in hybrid breeding programs for developing good hybrids or for developing better transgressive segregates. Results obtained through current study have been corroborated by the outcomes of by Bhagat *et al*. (2017) on spine gourd. Similar morphological based cluster results were obtained by Singh *et al.* (2016) in cucumber.

Informativeness of SSR Markers and their Utilization in Genetic Diversity Study

A total of 52 SSR primers (9 Cucumber, 6 Muskmelons, 19 Spine gourd and 18 Bitter gourd) were amplified from which only seven SSR primers were found to be polymorphic. A total of 17 alleles were produced by 7 SSR primers with an average of 2.42 alleles per primer. Number of alleles amplified for each primer ranged from 2 to 3. The polymorphic information content (PIC) for these primers were ranged from 0.097 (SgSSR15) to 0.580 (SgSSR11) with average PIC 0.32 (Table 2).

The microsatellite primer SgSSR11, SgSSR13, SgSSR17 generated three alleles while SgSSR1, SgSSR15, McN12 and McN24 exhibited two polymorphic alleles. The PIC provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. Seven primers produced 361 bands across the 41 varieties and of which 123 were polymorphic (34%). Similar type result was obtained by Jacob *et al*. (2016) in bottle gourd landraces; Cui *et al*. (2017) in bitter gourd.

SSR markers based cluster analysis was performed by following theJaccard's similarity coefficient and UPGMA algorithm to generate a dendrogramfor 41 varieties which is being presented in Figure 2. Total genotypes were grouped into two major clusters *viz.,* cluster-I and cluster-II. Highest dissimilarity was obtained between PK-49 and NDM-3. The similarity coefficient ranged from 0.60 to 0.95.

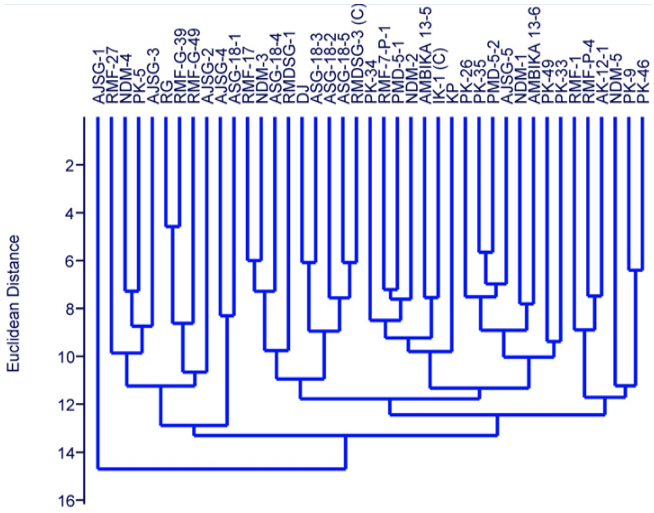

Figure 1: Morphological character based dendrogram depicting the relatedness among the 41 genotypes of spine gourd

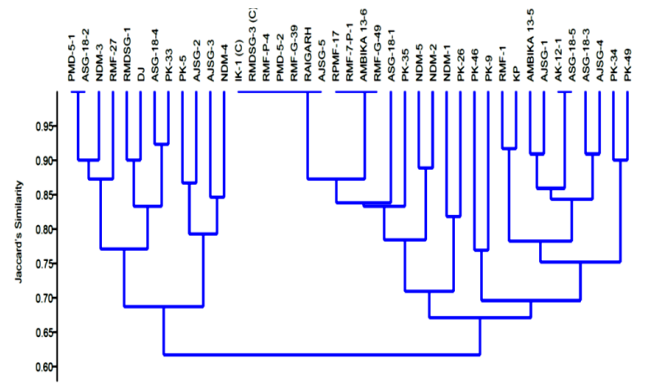

Figure 2: SSR marker based dendrogram depicting the relatedness among the 41 genotypes of spine gourd

According to pairwise comparison, the maximum similarity (100%) was obtained among the genotypes namely, IK-1, RMDSG-3, RMF-P-4, PHULLE MD-5-1, RMF-G-39, RAIGARH & AJSG-5; RPMF-17 & RMF-7-P-1, AMBIKA 13-6, RMF-G-49; AMBIKA-K-12-1 & ASG-18-5 in cluster-I whereas PHULLE MD-5-1 and ASG-18-2 in cluster-II. Cluster-I consisted of 29 varieties whereas cluster-II consisted of 12 genotypes. Highest dissimilarity in clustered-I was recorded between ASG 18-1 and PK-49 whereas highest dissimilarity in cluster-II was recorded between NMD-4 and NMD-3. Similar kind of results were also found by Yang *et al*. (2015) while analyzing the 42 Chinese accessions (mean similarity coefficient= 0.76) of cucumber. Dissimilar genotypes obtained through SSR marker based diversity analysis will be useful in recombination breeding to obtain the maximum heterosis for fruit yield per plant.

Informativeness of ISSR Markers and their Utilization in Genetic Diversity Study

Seven ISSR markers were used for discriminating 41 genotypes and all ISSR primer were found to be polymorphic.

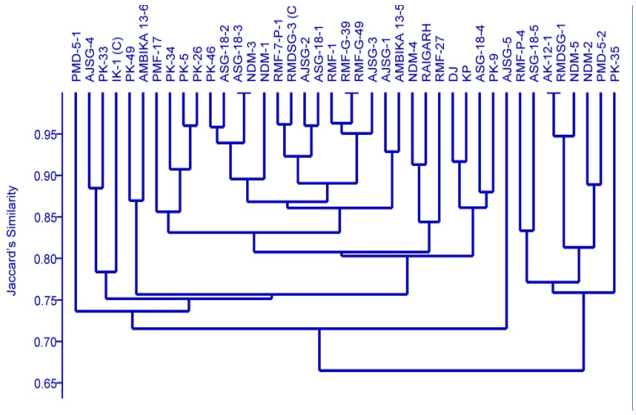

Figure 3: ISSR marker based dendrogram depicting the relatedness among the 41 genotypes of spine gourd.

Total 32 alleles were produced by the seven ISSR primers with an average 4.6 alleles per primer. The number of alleles amplified for each primer pair ranged from 4 (UCB-808, UCB-812, UCB-840, UBC-856) to 6 (UBC-841) and the polymorphic information content (PIC) for these primers ranged from 0.675 to 0.786 with an average PIC value of 0.73 (Table 3). PIC is a parameter that refers to the value of a marker for detecting the degree of polymorphism within a population. To determine PIC values of each ISSR primer we analyzed the mean of PIC values for all loci of each ISSR primer. It is assumed that a PIC > 0.5 accounts for a highly informative marker, 0.5 > PIC > 0.25 for an informative marker, and PIC < 0.25 for a slightly informative marker (Botstein *et al*. 1980). A total of 932 bands amplified, of which 645 were polymorphic bands (69.20%). Similar results obtained by Singh *et al*. (2016) through genetic diversity by using ISSR markers in 11 cucumber genotypes.

ISSR marker based cluster analysis was performed by following theJaccard's similarity coefficient and UPGMA algorithm to generate a dendrogram for 41 varieties which is being presented in Figure 3. All the 41 genotypes were grouped into two major clusters *viz.,* Cluster-I and Cluster-II. The similarity coefficient ranges from 0.66 to 100. Highest dissimilarity was recorded between PK-35 and PMD-5-1 at 66% dissimilarity level. Cluster-I consisted 33 genotypes whereas cluster-II have 8 genotypes. In cluster-I, highest similarity was obtained between ASG-18-3 & NDM-3; RMF-G-49 & RMF-G-49 (100%) and highest dissimilarity recorded between AJSG-5 and PHULLE MD-5-1. Cluster-II consisted 8 varieties wherethe highest similarity was recorded between AMBIKA-K-12-1 and RMDSG-1 (100%) whereas highest dissimilarity obtained between PK-35 and RMF-P-4. Cluster-I further classified two sub-clusters, whereas sub-cluster-I consisted 32 genotypes whereas sub-cluster-II has one genotype (AJSG-5). Highest dissimilarity recorded between PHULLE MD-5-1 and PK-9 genotypes in sub-cluster-I. Similar results were found by Ehsan *et al*. (2015) in *C. melo* L. and by Singh *et al*. (2016) in cucumber genotypes.

Conclusion

This study reported morphological characterization and novel molecular markers to develop a unique identity of spine gourd germplasm. The cross-species transferable microsatellite were detected from bitter gourd, cucumber and musk melon in spine gourd and it will provide a platform for comparative mapping to understand genomic relationships among these four species. A total of 94 microsatellites and 10 ISSR markers were used, but only 7 microsatellites and 7 ISSR were found polymorphic and useful for diversity study. Though, it is suggested that genotyping by sequencing of spine gourd germplasm will aid in development of new markers and precise molecular characterization. Information generated from GBS could be applied for comparative mapping, map construction, marker- assisted trait selection and provide important marker data, which is limited in this potential vegetable crop. The genetic diversity among the landraces might contribute to intraspecific crosses among landraces of this spine gourd collection having high potential for genetic improvement of this vegetable crop.

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Supplementary Table 1: Details of the morphological characters and their stages of observation.

S.N.	*Characteristics*	*Parameters*	*Codes*	*Stages of observation*
1.	Days to first flowering	Early (<40 days) Medium (41–50 days) Late (>50 days)	1 3 5	I
2.	Number of first flowering node	5–10 nodes 11–15 nodes 16–20 nodes 21–25 nodes	1 2 3 4	I
3.	Stem colour	Light green (L.G) Green (G) Dark green (D.G)	1 3 5	I
4.	No. of stem per plant	Few (<10 stems) Moderate (11–15 stems) Many (>15 stems	1 5 9	I
5.	Leaf length (cm)	Short (<6.5 cm) Medium (6.6–8 cm) Long (>8 cm)	1 5 9	I
6.	Leaf width (cm)	Narrow (1–6.5 cm) Medium (6.6-8 cm) Wide (>8 cm)	1 5 9	I
7.	Pedicel length (cm)	Short (1–2.5 cm) Medium (2.6–3.5 cm) Long (>3.6 cm)	1 5 9	I
8.	Ovary length (cm)	Short (>1–10 mm) Medium (11–20 mm) Long (>20 mm)	1 5 9	I
9.	Ovary diameter (cm)	Small (<7 mm) Large(>7.1 mm)	1 5	I
10.	Style length (cm)	Short (<7 mm) Medium (7.1–9 mm) Long (>9.1 mm)	1 5 9	I
11.	Pistil length (cm)	Short (<4 mm) Medium (4.1–6.0 mm) Long (>6 mm)	1 5 9	I
12.	Fruit length (cm)	Short (<3.0 cm) Medium (3.0–4.0 cm) Long (>4.1 cm) Very long(>51)	1 3 5 7	II
13.	Fruit diameter (cm)	Small (<30 mm) Medium (31–40 mm) Large (>40 mm)	1 5 9	II
14.	No. of fruits per plant	Few (<100) Moderate (100–120) Many (>120)	1 5 9	II
15.	Single fruit weight (g)	Light (1–10 g) Medium (11–15 g) Heavy (>15 g)	1 5 9	II
16.	Fruit yield per plant (kg)	Low(<2 kg) Medium (2–3 kg) Good (>3 kg)	1 3 5	II
17.	No. of seed per fruit	Less (<5) Medium (5–10) Many (>10)	1 5 9	III
18.	100 Seed weight (cm)	Light (<20 g) Medium bold (20–40 g) Bold (>40 g)	1 3 5	III
19.	Fruit yield (q/ha)	Low (<10 q/ha) Medium (11–20 q/ha) Good (21–30 q/ha) Very good (31–40 q/ha) Bumper (>41 q/ha)	1 3 5 7 9	III