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Research Article

Assessment of genetic diversity in *Solanum torvum* Swartz. from Tripura using SSR and RAPD markers

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Abstract

Wild *Solanum torvum* shows widespread distribution throughout the state of Tripura. In this investigation, diversity analysis of five populations of *S. torvum*, each represented by single accession, from different geographical locations of Tripura has been carried out for the first time using two types of molecular markers, Simple Sequence Repeats (SSR) and Rapid Amplified Polymorphic DNA (RAPD). SSR markers revealed a high degree of polymorphism (86.38%) in comparison to RAPD primers (79.16%). Polymorphic information content ranged from 0.28 to 0.54 for SSR and 0.16 to 0.41 for RAPD, respectively. UPGMA cluster analyses based on molecular data grouped the genotypes into two different clusters. Our findings suggests that SSR has a high discriminatory power wherein it was observed that the genotype from Jampui hills (STP5) with greater genetic diversity is stabilized in the high altitude of Sub- Himalayan region of Tripura in the due course of evolution.

Keywords: Genetic diversity; Polymerase Chain Reaction; RAPD; SSR; *Solanum torvum*

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Introduction

The State of Tripura (20°56′ - 24°34′ North latitude and 91°10′ - 92°21′ East longitude) belongs to Indo-Burma biodiversity hotspot of the world (1, 2) and is situated in the Sub-Himalayan region of North East India. The vegetation of Tripura is very rich and as many as 11 *Solanum* spp. belonging to the family Solanaceae is found in wild condition throughout the state (3). *Solanum torvum* Swartz., commonly known as turkey berry, is widely distributed in India, Pakistan, Malaya, China and Philippines (4). It is found in the forest margins, roadsides and open fields of Tripura and is consumed as raw vegetable by the local people (3, 5-7). From ages, ethnic people had been using different parts of this plant for the treatment of various diseases for its antiviral and antibacterial properties (8-13). It has been reported that extract of this plant has essential minerals like iron, magnesium, zinc, sodium and potassium (14). In addition, this taxon is a good source of vitamins and proteins (14). Fruits of *S. torvum* contain alkaloids, saponins, sugars, phenols, flavonoids, fiber along with steroidal glycosides - torvoside-A, torvoside-H and torvanol-A (13, 15-17). So far investigations on this species were focussed mainly on morphological, chromosomal and phytochemical aspects and little attention had been paid on the assessment of genetic diversity at intra-

species level (18). Now a days, PCR based DNA fingerprinting with arbitrary primers and genomic DNA is effectively used as a powerful tool for detection of genetic variation. DNA based markers (like RAPD, ISSR, SSR, AFLP, etc.) having high polymorphic character allow amplification of particular sequence within the plant genome for evaluation of genetic variability (19-22) to understand the phylogenetic and genetic relationship (23-26). Literature survey revealed that these genetic markers have advantages as well as disadvantages in assessing the diversity index (20, 27). Random amplified polymorphic DNA (RAPD) is a multilocus marker which amplifies the discrete regions of genome using short 10-mer primers (28, 29). Simple sequence repeats (SSRs), or Microsatellites are stretches of DNA, consisting of tandem repeats of mono-, di-, tri-, tetra- or penta-nucleotide units and are interspersed throughout the eukaryotic genome. Though some researchers used different DNA based molecular markers to study the genetic relationship of *Solanum* spp. (30-33) till date, no information is available on the haplotypes of this ethnomedicinaly important species from Tripura. The present investigation has, therefore, been undertaken on the 'Assessment of genetic diversity in *Solanum torvum* Swartz. from Tripura using SSR and RAPD markers' to find out the genetic variation, if any, which will help in future germplasm conservation strategy of *S. torvum*.

Materials and Methods

Plant materials and DNA extraction

Five populations of wild *Solanum torvum* (hereafter described as STP1, STP2, STP3, STP4 and STP5) were collected from different geographical locations (Fig. 1) of Tripura (Table 1). Thus, each population/genotype is represented by single accession. Total genomic DNA of each population was isolated from young tender leaves using the DNeasy ® Plant Mini Kit-Qiagen (part no.69104) according to manufacturer's protocol. The concentration of total DNA was checked by the Nanodrop 2000C spectrophotometer (Thermo Scientific-USA) and finally, visualized in 0.8% agarose gel for qualitative study.

Table 1. *Solanum torvum* collected from different geographical locations of Tripura

Population Number	Place of collections	Latitude	Longitude	Altitude / MSL (m)
STP1	Dharmanagar	N24°19'31.43	E92°09'25.54	38.0
STP2	Suryamaninagar	N23°46'13.70	E91°15'40.35	28.0
STP3	Sabroom	N22°59'22.20	E91°43'13.70	22.0
STP4	Amarpur	N23°33'30.30	E91°38'48.30	36.0
STP5	Jumpui Hills	N23°48'42.60	E92°15'35.90	918.0

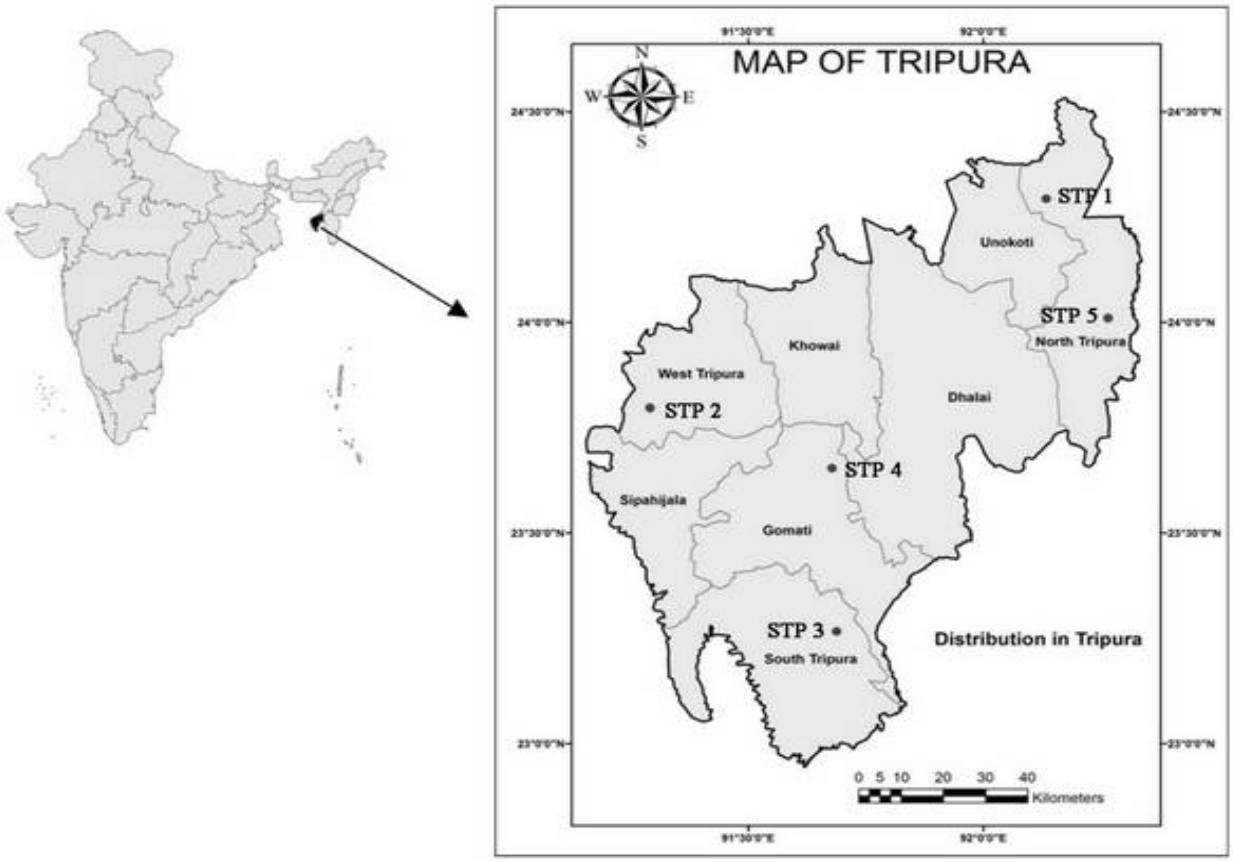

Fig. 1. Map locations of sample collection sites

Molecular fingerprinting using SSR markers

Eight arbitrary SSR markers were selected for assessing the genetic diversity (Table 2). PCR amplification of each sample was done with 25 µL of reaction mixture containing 10 mM dNTPs (Qiagen), 10X Taq buffer (Sigma Aldrich), 10 µM of each primer pair, 25 mM $MgCl_2$ (Sigma Aldrich), ~30 ng/µL of genomic DNA and 2.5 unit of Taq polymerase (Sigma Aldrich). Amplification was performed with initial denaturation at 94°C for 5 minutes followed by subsequent 35 PCR cycles operated at 94°C for 1 minute followed by 1 minute in varying temperature depending on the melting temperature (Tm) of SSR primers. This was followed by primer extension at 72°C for 1 minute and then, final extension was run at 72°C for 20 minutes. The confirmation was done using two replicates of each individual. The amplified products were visualized on 2% agarose gel, stained with Ethidium bromide and photographed by gel documentation system (XR+, Bio-Rad).

Molecular fingerprinting using RAPD markers

For RAPD genotyping, ten primers were selected (Table 2) for the present study. For PCR amplification 25 µL reaction mixture was prepared with 10 mM dNTPs (Qiagen), 25 mM $MgCl_2$ (Sigma aldrich), 10X *Taq* buffer (Sigma aldrich), 10 µM of each primer, 2.5 Unit of *Taq* polymerase (Sigma aldrich) and 30 ng/µL of genomic DNA. All PCR reactions were performed initially at 94°C for 5 minutes followed by 40 cycles having three ranges of temperature: 94°C for 1 minute, 40°C for 1 minute for primer annealing and 74°C for primer extension and finally at 74°C for 15 minutes. The amplified products were visualized with the help of gel documentation system (XR+, Bio-Rad) using 2% agarose gel.

Data Analysis

The amplified fragments (Fig. 2) were scored as '1' for presence and '0' for absence of each fragment. The average number of polymorphic and monomorphic bands was calculated for each primer in all the populations studied. The mean value for Polymorphic Information Content (PIC) was determined using the formula PIC = $1-\sum pi^2$, where *pi* is the frequency of i^{th} allele at a given locus (34) and Marker Index (MI) was calculated (35). The number of observed alleles (n_a), mean number of effective alleles (*ne*) (36), and Nei's (37) gene diversity index (*h*) were calculated with the help of POPGENE software (38). Shannon index (*I*) (39) and DICE's coefficient (40) to determine the level of similarity between the populations were also calcualted. The dendrogram was constructed using the SAHN subroutine through the NTSYS pc (Numerical Taxonomy System, 2.21q version) (41). Further, Principal Coordinate Analysis (PCA) was carried out with modules of STAND, CORR and EIGEN of NTSYS pc-2.21q software.

Results and Discussion

In the present investigation, eight SSR primer pairs produced 151 amplified fragments with an average of 18.88 (Table 3). There were 131 polymorphic bands and 20 monomorphic bands, which were amplified in the range of 200 - 5000 bp. The percentage of SSR polymorphic bands were found to range from 76.47 – 96.42 and the maximum number of bands were recorded in At5 (28). The lowest and highest PIC values for SSR primers were recorded in At5 (0.28) and CBT08 (0.54) with an average of 0.36. The MI value ranged from 23.74 to 51.68 with an average of 32.24 per locus (Table 3). The RAPD data showed that 10 loci yielded 70 bands (Table 3) of which 56 bands were found to be polymorphic (79.16%) and the highest number of polymorphic bands (9) was recorded in OPA09. The average number of polymorphic bands obtained per primer 5.6 and the range of PIC value was 0.16 to 0.41 with an average of 0.30 (Table 3). The average MI value was found to be 24.19. The mean values of effective number of alleles (n_e), observed number of alleles (n_a), Nei's gene diversity index (*h*) and

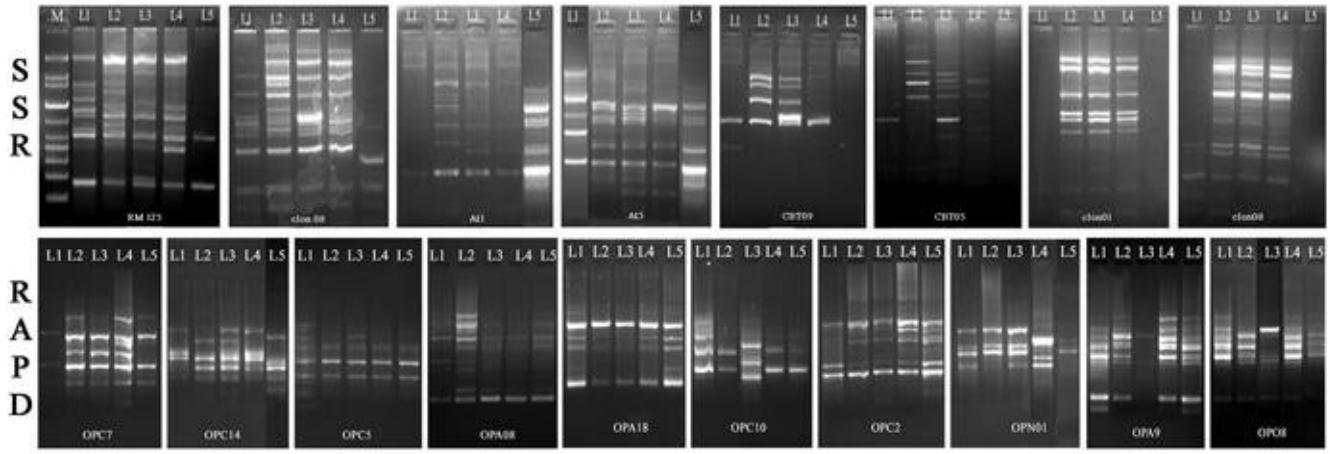

Fig. 2. SSR and RAPD fingerprints of five populations of *Solanum torvum*, M- 1kb plus DNA ladder (Qiagen), L1-L5 represents population 1, 2, 3, 4 and 5 respectively.

Table 2. Total number of amplified fragments generated by PCR using SSR and RAPD Primers

Primer Code (SSR)	Sequence (5′-3′)	Total Bands	Primer Code (RAPD)	Sequence (5′-3′)	Total Bands
At1	F:GCTTCACCTCCAGTTTCC R:GTCCTCCTTGTCCACCC	18	OPC07	GTCCCGACGA	7
At5	F:AATGCAAATGGTGGACG R:GGATAAATCAAAGAGGAGGA	28	OPC14	TGCGTGCTTG	7
CBT05	F:CTCTGTCTCCTCCCCCGCGTCG R:TCAGCTTCTGGCCGGCCTCCTC	20	OPC05	GATGACCGCC	8
CBT08	F:CAGCAGATTTTTGCTCCG R:GTCGCGTTCGTGGAAAT	22	OPA08	GTGACGTAGG	8
CBT09	F:AGGGGGCAGTGGAGAG R:ACGTTCCTGCACTTGACG	11	OPA18	AGGTGACCGT	5
RM125	F:ATCAGCAGCCATGGCAGCGACC R:AGGGGATCATGTGCCGAAGGCC	17	OPC10	TGTCTGGGTG	7
Clon08	F:CCGGTGAGGGTGATATCTTG R:AAGCTCAAGCTCAAGCCAAT	22	OPC02	GTGAGGCGTC	7
Clon01	F:ACTGGACTGTCCGAGAGCAT R:TCGTTTAGCGACAACGGATT	15	OPN01	CTCACGTTGG	6
			OPA09	GGGTAACGCC	9
	Total bands	151	OPO08	CCTCCAGTGT	6
			Total Bands		70

Table 3. Degree of polymorphism and polymorphic information content in five populations of *Solanum torvum*

SSR						
Locus name	Number of Score Bands	Polymorphic bands	Monomorphic bands	Percentage of polymorphic band (PPB)	Polymorphic information content (PIC)	Marker Index (MI)
At1	18	15	3	83.33	0.32	27.40
At5	28	27	1	96.42	0.28	34.15
Clon01	15	13	2	86.66	0.37	32.34
Clon08	22	17	5	77.27	0.34	26.41
`CBT05	20	17	3	85.00	0.32	27.88
CBT08	20	19	1	95.00	0.54	51.68
CBT09	11	10	1	90.90	0.37	34.34
RM125	17	13	4	76.47	0.31	23.74
Total	151	131	20			
Mean	18.9	16.4	2.5	86.38	0.36	32.24
RAPD						
OPC07	7	6	1	85.71	0.30	25.47
OPC14	7	7	0	100.00	0.41	41.14
OPC05	8	5	3	62.50	0.22	13.75
OPA08	8	7	1	87.50	0.34	29.75
OPA18	5	2	3	40.00	0.16	0.06
OPC10	7	7	0	100.00	0.32	32.00
OPC02	7	5	2	71.43	0.30	21.22
OPN01	6	6	0	100.00	0.40	40.00
OPA09	9	7	2	77.78	0.27	20.74
OPO08	6	4	2	66.67	0.27	17.78
Total	70	56	14			
Mean	7	5.6	1.4	79.16	0.30	24.00

Table 4. Genetic diversity parameters in *S. torvum*

Markers	Observed no. of alleles (n_a)	Effective no. of alleles (n_e)	Nei's gene diversity (h)	Shannon index (I)
SSR	1.87±0.34	1.60±0.32	0.35±0.16	0.51±0.22
RAPD	1.80±0.40	1.52±0.39	0.30±0.19	0.44±0.26
Combined (SSR+RAPD)	1.85±0.36	1.52±0.36	0.30±0.17	0.45±0.24

Shannon index (I) in SSR profile was found to be 1.60, 1.87, 0.35 and 0.51 respectively (Table 4). The RAPD data however, showed that the observed number of alleles (n_a), effective number of alleles (n_e), Shannon index (I) and Nei's gene diversity index (h) was 1.80, 1.52, 0.44 and 0.30

respectively (Table 4). The combined data analysis of SSR and RAPD revealed that the PIC value ranged from 0.16 to 0.54 with an average of 0.33. The average MI value was found to be 28.22. In the combined data the mean values of observed number of alleles (n_a), effective number of alleles (n_e), Nei's gene diversity index (h) and Shannon index (I) were 1.85, 1.52, 0.30 and 0.45 respectively (Table 4).

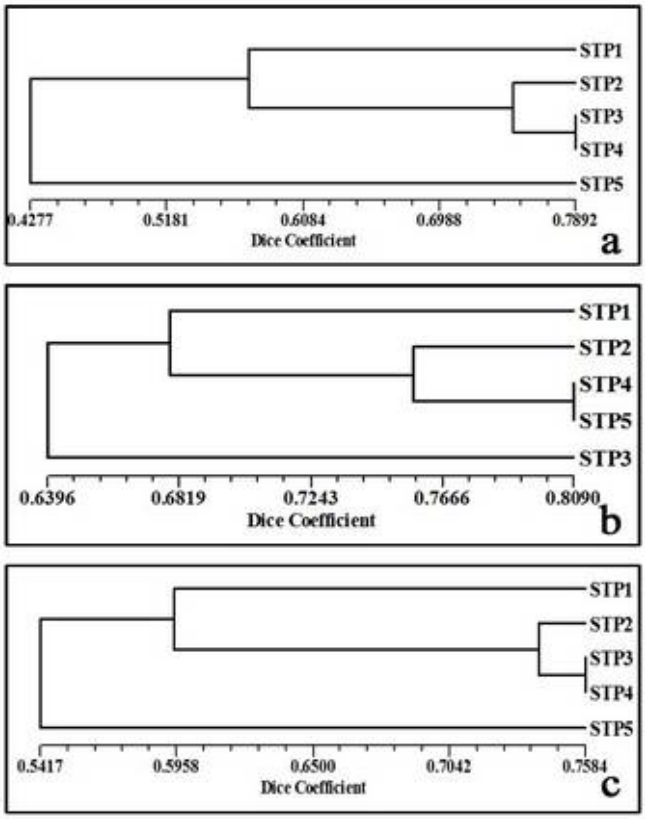

Fig. 3. UPGMA dendrograms based cluster analysis representing the genetic variability of *S. torvum* using (a) SSR, (b) RAPD and (c) SSR+ RAPD markers

The UPGMA dendrogram based on SSR data (Fig. 3a) reveals that STP1, STP2, STP3 and STP4 of *S. torvum* are grouped together in a single cluster whereas STP5 forms a separate cluster. It also reveals that STP3 and STP4 form a subgroup with highest similarity coefficient value (0.789). Interestingly, STP1 and STP5 are distantly placed in two different clusters showing greater diversity (0.444) (Table 5). PCA of SSR data showed that the first three components accounted for 33.99%, 24.32% and 22.98% variations (Fig. 4a). A similar result has also been found in combined analysis of SSR and RAPD data (Fig. 3c), where STP3 and STP4 show highest Dice similarity coefficient value (0.758) (Table 5). The combined PCA plot also reflects similar pattern of relationship like that of SSR data (Fig. 4c). The RAPD dendrogram, however is different from SSR profile wherein, STP1, STP2, STP4 and STP5 form a single cluster and STP3 is placed in another cluster (Fig. 3b). The dendrogram also shows that STP4 and STP5 are close to each other with similarity coefficient value (0.809) (Table 5). PCA plot for RAPD data showed that the first three principle coordinate components accounted for 30.30%, 27.05% and 23.85% variations (Fig. 4b). Our results are in partial agreement with previous findings (18, 42, 43).

Undoubtedly, SSR and RAPD dendrogram obtained by UPGMA analysis shows a location based clustering pattern suggesting the existence of intra-specific diversity (44, 18, 45). Literature survey reveals that RAPD is inconsistent and not reproducible though it amplifies both coding and non-coding regions of the genome. The results obtained suggest that the RAPD profile does not reflect altitudinal based clustering pattern like that of SSR profile (Table 1). The SSR data suggests that though the different individuals of *S. torvum* are inherited from a common ancestor, eventually the accession (STP5) with greater genetic diversity is stabilized in the high altitude of Sub - Himalayan region of Tripura in due course of evolution. High percentage of polymorphic bands suggests that the arbitrary primer sets are appropriate for assessment of genetic diversity in *S. torvum*. The PIC value and MI value obtained indicate that SSR markers are more informative than RAPD. This investigation reveals that a reasonable degree of genetic variation exists among the genotypes of *S. torvum* and it also suggests that effective strategy should be taken to conserve the germplasms.

Table 5. Dice similarity coefficient among the five populations of *S. torvum*

		STP1	STP2	STP3	STP4	STP5
SSR	STP1	1.000				
	STP2	0.565	1.000			
	STP3	0.522	0.749	1.000		
	STP4	0.632	0.747	0.789	1.000	
	STP5	0.444	0.429	0.411	0.426	1.000
RAPD	STP1	1.000				
	STP2	0.667	1.000			
	STP3	0.543	0.707	1.000		
	STP4	0.697	0.733	0.690	1.000	
	STP5	0.674	0.782	0.617	0.809	1.000
Combined (SSR+RAPD)	STP1	1.000				
	STP2	0.599	1.000			
	STP3	0.529	0.737	1.000		
	STP4	0.656	0.743	0.758	1.000	
	STP5	0.546	0.556	0.486	0.578	1.000

Conclusions

The outcome suggests that SSR marker itself has the greater discriminating power to assess the intra-specific genetic diversity due to high degree

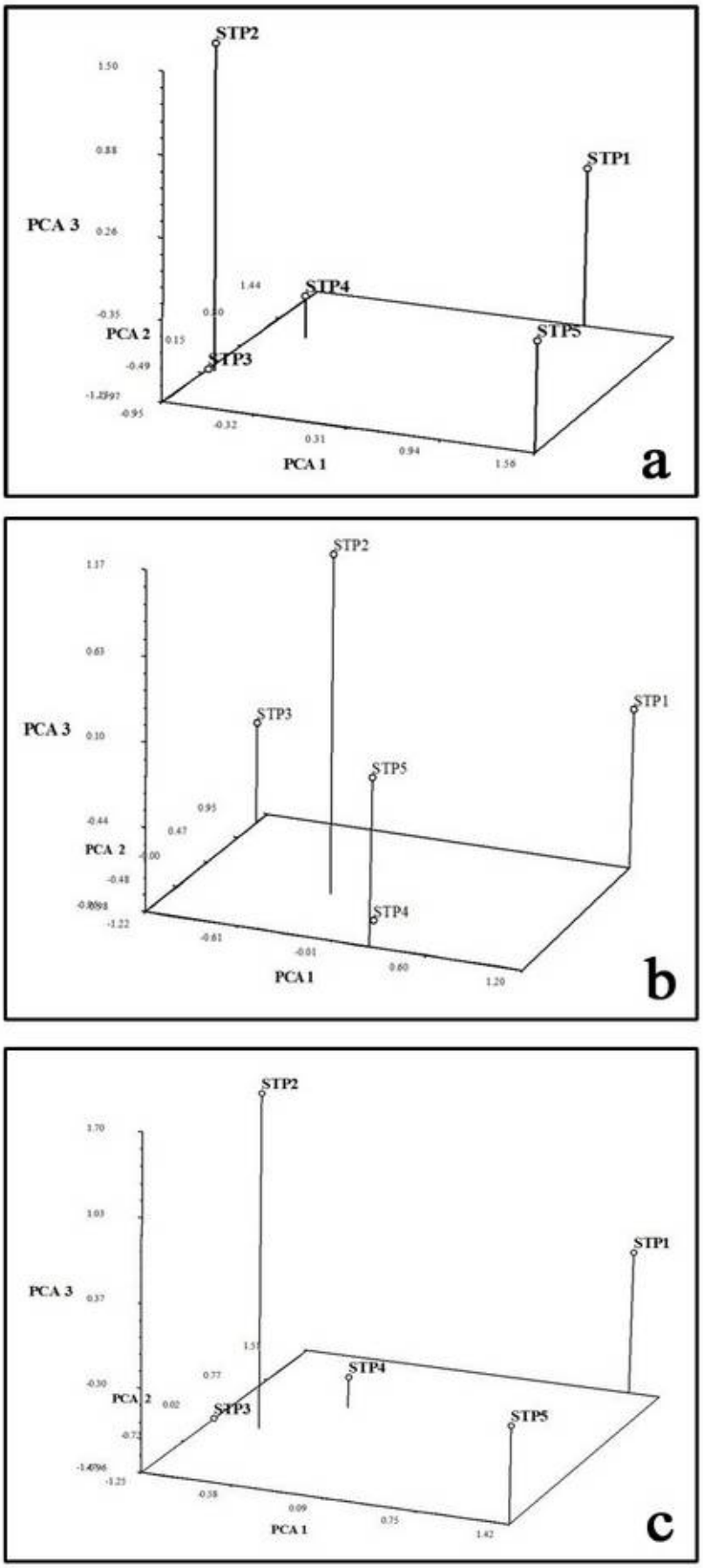

Fig. 4. Principal coordinate analysis (PCA) map for five different populations of *S. torvum* (a) SSR, (b) RAPD and (c) SSR+ RAPD

of polymorphism (86.38%). The SSR dendrogram using UPGMA algorithms also justify the altitude based clustering pattern. The data obtained from this result have a great significance in germplasm conservation strategy.

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Authors' contributions

MD, KS and HRS designed the experiment and wrote the manuscript.

Competing Interests

The authors have no conflict of interests.

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