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Genetic diversity in snake gourd genotypes revealed by RAPD markers

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A genetic analysis of 21 diverse Bangladeshi snake gourd (*Trichosanthes anguina* L) genotypes was performed using Random Amplified Polymorphic DNA (RAPD) markers. Out of 14 random primers screened three were selected, which produced 34 amplicons, among them 33 (95.83%) was considered polymorphic providing an average of 11 amplicons per primer. RAPD amplicons per primer ranged from 8 (OPA-05) to 15 (OPC-02) and varied in size from 150 bp to 1450 bp. The Nei's index of diversity for each primer ranged from 0.0907 to 0.4898 with an average of 0.3356 whereas the Shannon's index of diversity for each primer ranged from 0.1914 to 0.6920 with an average of 0.5051. The UPGMA constructed based on RAPD analysis in 21 snake gourd genotypes in where two major groups were found. Cluster II had 18 genotypes whereas cluster one only had three. The highest genetic distance 0.9614 was estimated between the genotypes TA0021 and TA0002, while the lowest genetic distance 0.1252 was estimated between the genotypes TA0005 and TA0003. RAPD analysis showed an effective tool in estimating genetic variation in snake gourd genotypes. Examined *T. anguina* genotypes were genetically diverse and this variation stressed the need of genetic study and crop improvement of the species.

Key word: Genetic variation, RAPD marker, Snake gourd, UPGMA

Introduction

Commonly known as cucurbits or gourds, under the family Cucurbitaceae includes a large number of cultivated species of global or local economic importance (Robinson and Decker-Walters, 1997). Snake gourd (*Trichosanthes anguina* L) is one of the cucurbitaceous vine crop growing mostly in tropical and sub-tropical regions. The diverse morphological characters of *T. anguina* in Bangladesh provide relatively broad phenotypic species variation (Ahsan *et al*., 2014; Rahman, 2002), indicating a great scope for genetic improvement of the crop. Estimation of

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genetic diversity can increase the efficiency of a breeding program (Fan *et al*., 2006; Paterson *et al*., 1991) since it has been considered as an important tool to quantify genetic variability in both self and cross-pollinated crops (Griffing and Lindstrom, 1954; Murty and Arunachalam, 1966; Gaur *et al.* 1978). As the genotypes are not well characterized yet it is important to differentiate existing genotypes with the level of genetic diversity at the beginning of a breeding program. Morphological and/or molecular markers can be a research tool to determine genetic diversity among individuals. Identification of genotypes based on molecular markers is a quick-reliable method for estimating genetic relationships (Thormann *et al*., 1994). Among different types of molecular marker techniques available, Random Amplified Polymorphic DNA (RAPD) technique (Williams *et al*., 1990) has been most popular because of their simplicity, cost effective, fast and easy to perform (Dos Santos *et al*., 1994; Williams *et al*., 1990).

Although DNA marker exploration helping in such analyses (Staub *et al*., 1996), but limited polymorphic markers have been identified in snake gourd (Alam *et al*., 2012). However, RAPD markers have been efficiently used in genetic diversity analysis of different cucurbits such as cucumber (Staub *et al*., 2005; Ping *et al*., 2002; Meglic *et al*., 1996), Melon (Levi *et al*., 2004; Woo and Hyeon, 2003; Levi *et al*., 2001; Garcia *et al*., 1998; Lee *et al*., 1996), bottle gourd (Srivastava *et al*., 2014; Ram *et al*., 2006; Decker-Walters *et al*., 2001;), ash gourd (Resmi and Sreelathakumary, 2011; Verma *et al*., 2007; Sureja *et al*., 2005), teasle gourd (Rasul *et al*., 2007), sweet gourd (Rahman *et al*., 2007; Gwanama *et al*., 2000), pointed gourd (Khan *et al*., 2009) and bitter gourd (Behera *et al*., 2012; Dalamu *et al*., 2012; Behera *et al*., 2008; Dey *et al*., 2006; Rahman *et al*., 2007; Singh *et al*., 2007), and may have utility for genetic analysis of *T. anguina* genotypes. Genetic diversity analysis by the molecular markers has not been performed in *T. anguina*. Therefore, the study was conducted using RAPD markers to identify potential diverse genotypes for varietal improvement.

Materials and Methods

Plant materials: Twenty-one genotypes of snake gourd were collected by Plant Genetic Resources Center (PGRC), Bangladesh Agricultural Research Institute (BARI) during 2010 from different locations of Bangladesh as mentioned in **Table S1**. Passport information about the genotypes is also listed in **Table S2**. The seedlings of each genotype were raised at the facilities of PGRC. In order to carry out RAPD analysis, fresh young leaves from five randomly chosen seedlings of each genotype of 14-days old were bulked into a single sample in airtight poly bag and preserved in ice box.

Table S1 Sources of snake gourd genotypes used in the study

Accessions	Collected from	Accessions	Collected from
TA0001	Comilla	TA0012	Jamalpur
TA0002	Comilla	TA0013	Jamalpur
TA0003	Comilla	TA0014	Faridpur
TA0004	Chandpur	TA0015	Faridpur
TA0005	Chandpur	TA0016	Tangail
TA0006	Chandpur	TA0017	Tangail
TA0007	B. Baria	TA0018	Tangail
TA0008	Comilla	TA0019	Laxmipur
TA0009	Mymensingh	TA0020	Feni
TA0010	Mymensingh	TA0021	Chittagong
TA0011	Mymensingh		

Table S2 Morphological description of snake gourd genotypes

Accessions	Vine length after 1st flowering (m)	Node with male flower	Node with female flower	Days to male flowering	Days to female flowering	Fruit length (cm)	Fruit width (cm)	Single fruit weight (g)	Number of fruits/plant	Weight of fruits/plant (kg)	Yield (t/ha)	Color	Smell
TA0001	2.17	22	28	64	76	41.80	6.66	280.00	16	4.48	22.40	Deep green	Absent
TA0002	1.40	23	26	64	75	31.33	6.63	175.00	16	2.80	14.00	Green	Medium
TA0003	1.60	15	32	61	69	35.80	7.17	242.00	15	3.63	18.15	Light green	Absent
TA0004	2.87	21	30	76	81	30.50	6.66	187.50	12	2.25	11.25	Deep green	Absent
TA0005	1.73	16	25	66	72	27.33	8.55	216.67	15	3.25	16.25	Deep green	Absent
TA0006	1.64	16	25	66	71	26.67	7.34	136.67	24	3.28	16.40	Deep green	Absent
TA0007	2.60	14	22	76	83	36.33	6.84	283.33	12	3.40	17.00	Green	Medium
TA0008	2.00	21	20	74	77	30.33	6.34	166.67	21	3.50	17.50	Blue green	Absent
TA0009	0.83	20	28	68	78	47.60	6.40	264.00	13	3.43	17.15	Deep green	Medium
TA0010	2.50	22	34	74	78	37.20	5.33	130.00	17	2.21	11.05	Green	Absent
TA0011	1.62	15	17	60	78	30.25	8.44	137.50	10	1.38	6.90	Light Green	Absent
TA0012	2.56	23	42	78	90	37.00	8.25	233.33	15	3.50	17.50	Deep green	Absent
TA0013	2.30	20	23	78	78	26.50	6.25	122.50	9	1.10	5.50	Deep green	Absent
TA0014	1.47	14	19	67	75	19.50	3.33	87.50	29	2.54	12.70	Deep Green	Absent
TA0015	2.83	20	23	74	78	29.20	6.15	190.00	44	8.36	41.80	Light green	Medium
TA0016	3.82	31	33	89	91	30.25	6.63	172.50	32	5.52	27.60	Deep green	Medium
TA0017	2.70	25	34	72	76	33.25	6.46	200.00	24	4.80	24.00	Deep green	Absent
TA0018	2.40	22	24	73	73	37.67	7.66	283.33	29	8.22	41.10	Green	Absent
TA0019	2.95	21	29	87	87	60.08	6.32	325.00	12	3.90	19.50	Deep green	Absent
TA0020	2.63	20	25	78	75	18.84	9.07	180.00	16	2.88	14.40	Green	Medium
TA0021	1.75	16	28	67	69	46.58	6.11	163.33	18	2.94	14.70	Green	Absent

Extraction of genomic DNA: Genomic DNA was extracted from bulked leaf sample using a CTAB-based method (Stewart and Via, 1993) with minor modification. Concentration of gnomic DNA was confirmed by calculating the ratio of the optical density measured at 260 nm using a spectrophotometer and finally, samples were stored at-20^0C.

Primer selection: Fourteen primers of random sequence (Operon Technologies, Inc, Alameda, California, USA) were used in the study. Two randomly chosen individuals from two different genotypes were used to evaluate their suitability for amplification of DNA sequences, which scored accurately. Primers were evaluated based on intensity of bands consistency within individual, presence of smearing, and potential for population discrimination. Finally, three primers exhibited good quality banding patterns and sufficient variability, those were selected for further analysis showing asterisk in **Table 1**.

Table 1 Random primer sequences used for molecular characterization of snake gourd genotypes

Primer code	Sequence (5´- 3´)	GC Content (%)
OPA-02	TGCCGAGCTG	70
OPA-03*	AGTCAGCCAC	60
OPA-05*	AGGGGTCTTG	60
OPA-09	GGGTAACGCC	70
OPA-11	CAATCGCCGT	60
OPA16	AGCCAGCGAA	60
OPB-01	GTTTCGCTCC	60
OPB-05	TGCGCCCTTC	70
OPB-06	TGCTCTGCCC	70
OPB-11	GTAGACCCGT	60
OPC-01	TTCGAGCCAG	60
OPC-02*	GTGAGGCGTC	70
OPC-03	GGGGGTCTTT	60
OPC-07	GTCCCGACGA	70

RAPD assay: PCR mixture was prepared for each DNA sample containing 1.0µl 10X reaction buffer, 1.5µl $MgCl_2$, 1.0µl 250 µM dNTPs, 2.0µl of 10 µM primer, 1 unit of Taq polymerase enzymes and 50 ng genomic DNA made up to final volume 10µl with sterile distilled water. The tubes were then sealed and placed in a thermocyle and the cycling was started immediately. Protocol was used as described by Khan *et al*. (2009) based on Williams *et al.* (1990). PCR products from each sample were confirmed by running 1.5% agarose gel containing TAE buffer at 100V for 1:25 hour. 2µl loading dye was added to the PCR products and loaded in the wells. Molecular weight marker (100bp DNA ladder) was also loaded on either side of the gel. RAPD bands were observed under Ultra Violet (UV)

light on a transilluminator and documented by taking photograph using Gel Cam Polaroid camera.

Data analysis: All RAPD product amplified by given primers were measured as a single locus and data were scored visually on the basis of their presence (1 or more) and absence (0), separately for each genotype and each primer. Two independent persons performed band scoring for more accuracy. DNA fragments those continually amplified were scored and weak bands were not scored for analysis. Besides, the bands intensity was not taken into consideration and the bands with the same mobility were considered to be the same bands. The scores obtained from RAPD analysis for all primers were then pooled for constructing a single data matrix.

The software program POPGENE 32 (Sun *et al*., 2009; Yeh *et al*., 1997) was applied to figure out the genetic diversity parameters. The pooled data matrix was used to estimate polymorphic loci based on Nei's (1973) gene diversity statistics. The Shannon index of diversity was also calculated as described by Lewontin (1972). Gene frequency from the polymorphic loci was estimated as described by Slatkin and Barton (1989). In order to determine genetic relationship among the genotypes, a dendrogram was generated based on Nei's (1972) genetic distance following a cluster analysis by means of UPGMA (unweighted pair group method with arithmetic average) procedure (Rohlf, 2000).

Results and Discussions

RAPD polymorphism

Initially 14 decamer primers were tested two randomly chosen genotypes. Among them three primers OPA-03, OPA-05 and OPC-02 those produced maximum number of high intensity bands with minimal smearing were selected for the study (**Table 1**). DNA polymorphism as revealed by three of the highly polymorphic primers was illustrated in **Fig. 1** in where each band representing a locus. Selected three primers were used in RAPD analysis of 21 snake gourd genotypes which amplified 34 different reproducible loci, among them 33 were found to be polymorphic with an average of 95.85% at the genotypic level (**Table 2**). The number of bands per primer ranged from 8 (OPA-05) to 15 (OPC-02) while average bands per primer was 11.33. The size of the amplified products varied from 150 to 1450 bp. The primers OPA-03 and OPC-02 were 100% polymorphic whereas primer OPA-05 was 87.50% polymorphic. Though RAPD analysis in snake gourd is not yet reported, some other cucurbits showed different level of polymorphism; 86.98% in cucumber (Ping *et al*., 2002), 23.2% in sweet gourd (Gwanama *et al*., 2000), 41.34% in bitter gourd (Behera *et al*., 2012), 60.29% in bottle gourd (Srivastava *et al*., 2014). *Cucumis melo* also showed diverse polymorphism in different studies measuring 25.6% (López-Sesé *et al*., 2002), 61% (Silberstein *et al*., 1999) and 20.32% (Mo *et al*., 1999).

Most of the previous studies showed polymorphism lower than the values obtained in the present study. Present studies showed higher level of polymorphism, it could be due to either high GC contents (60-70%) of the primers and/or origin of genotypes since collected from distant geographical regions of Bangladesh (**Table S1**). There was a high level of genetic variation among the studied genotypes of snake gourd from the proportion of polymorphic point of view indicating studied genotypes are rich in genetic variation. This result supported by Dhillon *et al*. (2007) also where they analyzed diversity among 36 Indian snap melons and concluded that India genotypes were rich in genetic variation.

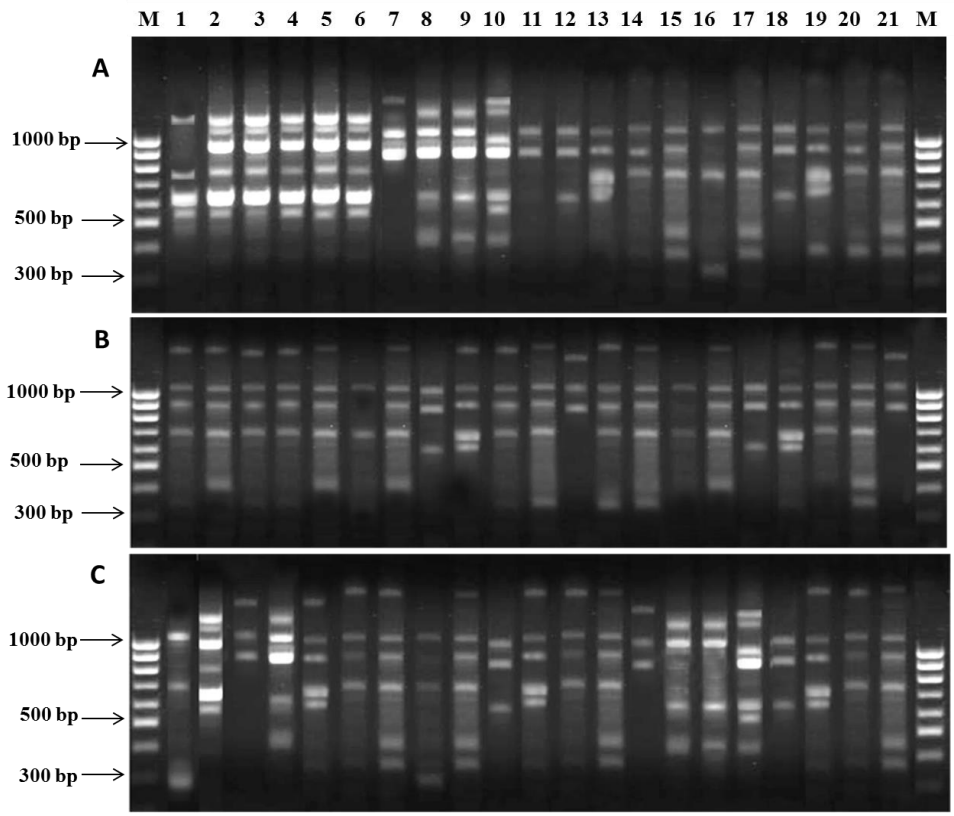

Fig. 1. RAPD profiles of 21 snake gourd genotypes using primer OPA-03 (A), OPA-05 (B) and OPC-02 (C). M: Molecular weight marker (100bp)

Table 2 RAPD polymorphism observed in 21 snake gourd genotypes

Primer code	Number of loci analyzed	Number of polymorphic loci	Proportion of polymorphic loci (%)	Size ranges (bp)
OPA-03	11	11	100.00	200-1220
OPA-05	8	7	87.50	250-1450
OPC-02	15	15	100	150-1450
Total	**34**	**33**	**287.50**	**-**
Average	**11.33**	**11.00**	**95.83**	**-**

Frequency of polymorphic loci

Frequency of RAPD loci was estimated based on the assumption of a two alleles system where only one was capable of amplification and other was the "null" incapable of amplification. So, each RAPD band treated as a locus with two alleles, M and m, respectively in where "1" represents genotype MM or Mm and "0" represents genotype mm. It is assumed that the gene frequency within a genotype was under Hardy-Weinberg equilibrium. Bands with $q^2<3/N$ (q was the frequency for recessive gene and N was the sample size) were discarded according to Lynch-Milligan (Lynch and Milligan, 1994) method for calibrating RAPD data from a small sample size.

The frequency of polymorphic loci varied from primer to primer. A total of 33 polymorphic loci from 3 primers were found in the study (**Table 2**). The maximum gene frequencies found from the polymorphic loci OPA05-3 (1.0000) followed by OPA03-3 & OPA05-4 (0.9048), OPA05-5 (0.8095), and OPC02-5 (0.7143) and rest of the loci showed moderate to low gene flow (Fig. 2). Similar pattern of gene frequency was observed by Khan *et al.* (2009) in pointed gourd. Though no genotypes-specific marker has been scored in the present study, the high level of polymorphism (95.83%) revealed by the study indicating potentiality of RAPD markers for estimating genetic diversity in snake gourd genotypes. However, genotypes could be differentiated by a combination of fragments and differences between clusters reflected variation in frequencies rather than presence or absence of genotypes specific fragments.

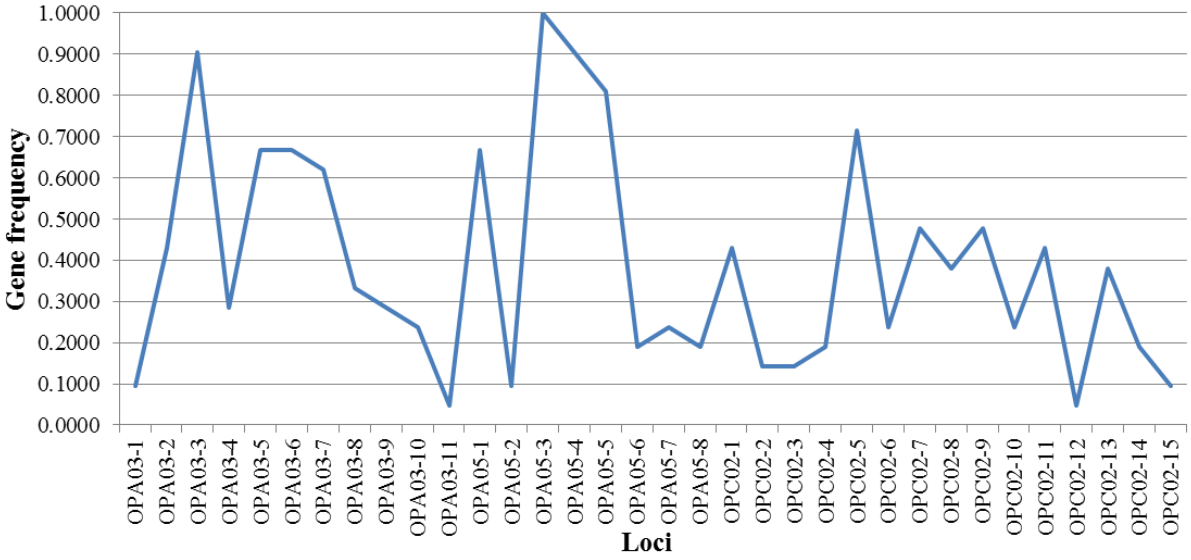

Fig. 2 Gene frequency for 34 loci based on RAPD marker analysis

Gene diversity

Gene diversity is a measure of the expected heterozygosity in a sample of gene copies collected at a single locus. It is a summary statistic used to represent patterns of molecular diversity within a sample of gene copies. The expected heterozygosity is calculated under the assumption that the sample of gene copies was drawn from a population at Hardy-Weinberg equilibrium. Various indices are used for analyses and comparisons of gene diversity within plant species. The Nei and Shannon index of diversity are the two most commonly used nonparametric measures of species diversity. The Nei's index accounts for partitioning the total genetic diversity to within and between subpopulation components, while the Shannon's index accounts for both richness and evenness of the species present. Here we used both indices together just to enhance the significance of conclusions. The Nei's index of diversity for each primer ranged from 0.0907 to 0.4898 with an average of 0.3356 whereas the Shannon's index of diversity for each primer ranged from 0.1914 to 0.6920 with an average of 0.5051 (**Table S3, Fig. 3**). The Shannon's index of diversity showed higher than the Nei's index of diversity, though genes distribution trends for both of the diversity indices were very similar for 34 loci. This type of diversity was also found by Khan *et al.* (2009) and Barroso *et al.* (2004) in different cucurbits. Estimation of average Nei's gene diversity and Shannon's index of diversity across 34 loci strongly support the existence of high level of genetic variation in 21 genotypes of snake gourd. High genetic diversity is attributable to a number of factors (Zawko *et al*., 2001); reduction of population size, insufficient time for isolation or extensive recurrent gene flow (Chiang *et al*., 2006). Field observation showed that snake gourd is biannual highly cross pollinated crop, which largely promotes outcrossing

indicating naturally maintained genetic polymorphisms across the population. Our findings supported by Baba *et al*. (2002) in where reported molecular diversity of Hungarian melon varieties. Wider genetic variability in studied snake gourd genotypes indicates a valuable gene pool for future breeding programs (Behera *et al*., 2012; Dalamu *et al*., 2012).

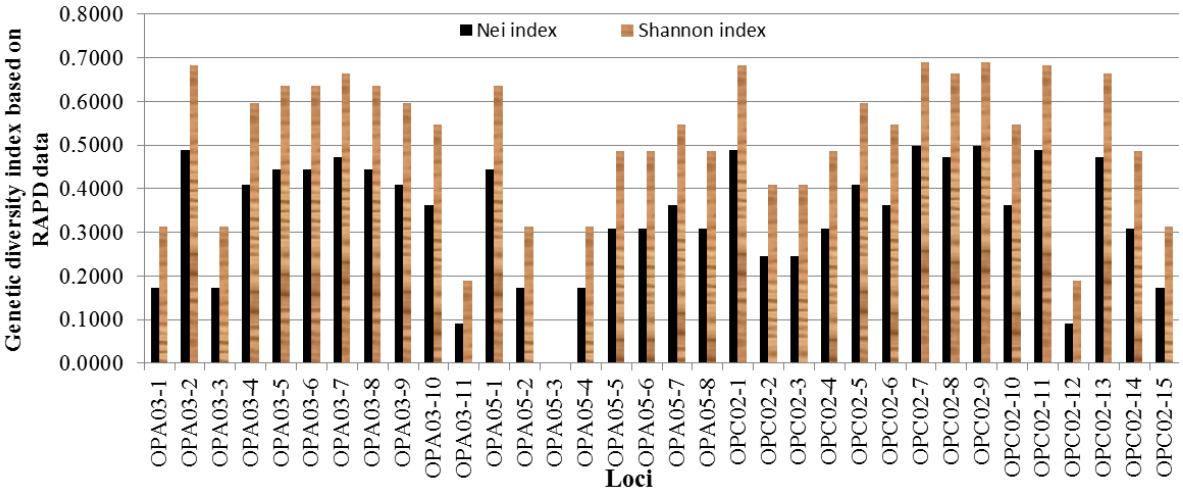

Fig. 3 Histogram of genetic diversity indices for 34 loci based on RAPD analysis.

Table S3 Genetic diversity for all loci of snake gourd genotypes

	Loci	Nei's gene diversity	Shannon's index of diversity	Gene frequency
OPA03	OPA03-1	0.1723	0.3145	0.0952
	OPA03-2	0.4898	0.6829	0.4286
	OPA03-3	0.1723	0.3145	0.9048
	OPA03-4	0.4082	0.5983	0.2857
	OPA03-5	0.4444	0.6365	0.6667
	OPA03-6	0.4444	0.6365	0.6667
	OPA03-7	0.4717	0.6645	0.6190
	OPA03-8	0.4444	0.6365	0.3333
	OPA03-9	0.4082	0.5983	0.2857
	OPA03-10	0.3628	0.5489	0.2381
	OPA03-11	0.0907	0.1914	0.0476
OPA05	OPA05-1	0.4444	0.6365	0.6667
	OPA05-2	0.1723	0.3145	0.0952
	OPA05-3	0.0000	0.0000	1.0000
	OPA05-4	0.1723	0.3145	0.9048
	OPA05-5	0.3084	0.4869	0.8095
	OPA05-6	0.3084	0.4869	0.1905
	OPA05-7	0.3628	0.5489	0.2381
	OPA05-8	0.3084	0.4869	0.1905
OPC02	OPC02-1	0.4898	0.6829	0.4286
	OPC02-2	0.2449	0.4101	0.1429
	OPC02-3	0.2449	0.4101	0.1429
	OPC02-4	0.3084	0.4869	0.1905
	OPC02-5	0.4082	0.5983	0.7143
	OPC02-6	0.3628	0.5489	0.2381
	OPC02-7	0.4989	0.6920	0.4762
	OPC02-8	0.4717	0.6645	0.3810
	OPC02-9	0.4989	0.6920	0.4762
	OPC02-10	0.3628	0.5489	0.2381
	OPC02-11	0.4898	0.6829	0.4286
	OPC02-12	0.0907	0.1914	0.0476
	OPC02-13	0.4717	0.6645	0.3810
	OPC02-14	0.3084	0.4869	0.1905
	OPC02-15	0.1723	0.3145	0.0952
	Mean	**0.3356**	**0.5051**	**0.3890**
	STDEV	**0.1384**	**0.1738**	**0.2675**

Genetic divergence among genotypes

Genetic distance is a measure of the genetic divergence between species or between populations within a species. It is also used for understanding the origin of genotypes. In order to determine the genetic relationship among the genotypes, the Nei's genetic distance was calculated using a distance matrix based on UPGMA algorithm. UPGMA dendrogram based on pairwise genetic distance identified two major cluster among 21 snake gourd genotypes, i.e. (TA0015, TA0016 and TA0017) *vs.* all others, of which [(TA0001, TA0003, TA0004, TA0005, TA0006), (TA0002, TA0010)], [(TA0007, TA0009, TA0013, TA0021), (TA0008, TA0012, TA0018, TA0019)] and [TA0014, TA0011, (TA0019, TA0020)] were further clustered (**Fig. 4**). Genetic analysis showed that the highest genetic distance 0.9614 was observed between the genotypes TA0021 and TA0002, whereas the lowest genetic distance 0.0924 was observed between the genotypes TA0005 and TA0003 (**Table 4**). This estimation was completely coincided to the estimate of genetic identity in where genotypes TA0003 and TA0005 found very similar, while the genotypes TA0002 and TA0021 very dissimilar (Table 4). Based on RAPD analysis genetic distances were estimated in different cucurbits; ranging 0.03-0.28 in bitter gourd (Behera *et al*., 2012), 0.13-0.41 in sweet gourd (Gwanama *et al*., 2000), 0.52 in two group of bottle gourd (Srivastava et al., 2014) and 0.4328 ± 0.078 in *Cucurbita maxima* (Ferriol *et al*., 2001). Present study estimated higher genetic distance compared to previous studies that indicating the presence of wider variability among the genotypes. Moreover, the resulted dendrogram showed two main groupings which could be associated with the most commercial cultivars and the geographic origin of the genotypes (**Fig. 2**), supported by Youn and Chung (1998). Besides, intragroup genetic relationships based on marker differences were closer than the intergroup genetic relationship that is also observed by Grisales *et al*. (2015) in *Cucurbita moschata*.

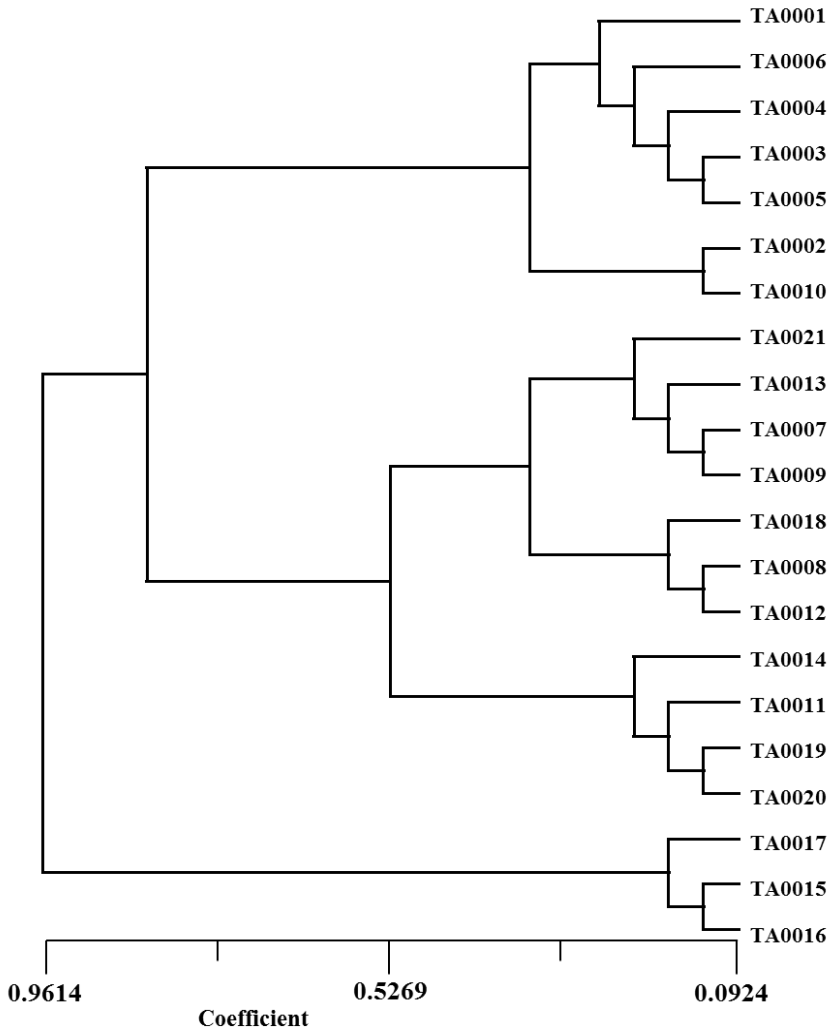

Fig. 4 UPGMA dendrogram showing the relationship and diversity among 21 genotypes of *T. anguina* based on Nei's (1972) genetic distance.

Table 4 Nei's pairwise genetic identity (above diagonal) and genetic distance (below diagonal) among the genotypes based on RAPD analysis.

Accession	TA0001	TA0002	TA0003	TA0004	TA0005	TA0006	TA0007	TA0008	TA0009	TA0010	TA0011	TA0012	TA0013	TA0014	TA0015	TA0016	TA0017	TA0018	TA0019	TA0020	TA0021
TA0001	-	0.6471	0.8235	0.7647	0.7353	0.7941	0.6176	0.7647	0.6471	0.7059	0.6176	0.6765	0.7059	0.6471	0.5294	0.6176	0.4118	0.6765	0.7059	0.6765	0.5588
TA0002	0.4353	-	0.7059	0.7647	0.7941	0.6176	0.4412	0.4706	0.4118	0.7647	0.5588	0.4412	0.4706	0.6471	0.5882	0.7353	0.5294	0.6176	0.6471	0.5588	**0.3824**
TA0003	0.1942	0.3483	-	0.8824	**0.9118**	0.8529	0.6176	0.6471	0.6471	0.7059	0.6765	0.6765	0.7059	0.7059	0.5294	0.6176	0.4706	0.6765	0.6471	0.6176	0.5000
TA0004	0.2683	0.2683	0.1252	-	0.8529	0.7941	0.6176	0.5882	0.6471	0.6471	0.6765	0.6176	0.7059	0.5882	0.5294	0.6176	0.5294	0.6176	0.6471	0.5588	0.5000
TA0005	0.3075	0.2305	**0.0924**	0.1591	-	0.7647	0.5882	0.5588	0.5588	0.6765	0.7059	0.5882	0.6176	0.6176	0.5000	0.6471	0.4412	0.6471	0.6765	0.5882	0.4118
TA0006	0.2305	0.4818	0.1591	0.2305	0.2683	-	0.6471	0.6765	0.6765	0.6176	0.6471	0.7647	0.7353	0.5588	0.5588	0.5294	0.4412	0.6471	0.6765	0.6471	0.5882
TA0007	0.4818	0.8183	0.4818	0.4818	0.5306	0.4353	-	0.6176	0.8529	0.5000	0.7647	0.7647	0.8529	0.6176	0.5588	0.6471	0.5000	0.6471	0.6765	0.7647	0.7059
TA0008	0.2683	0.7538	0.4353	0.5306	0.5819	0.3909	0.4818	-	0.7647	0.6471	0.6176	0.7941	0.6471	0.5882	0.5882	0.5000	0.5882	0.7941	0.6471	0.6176	0.6765
TA0009	0.4353	0.8873	0.4353	0.4353	0.5819	0.3909	0.1591	0.2683	-	0.5882	0.6765	0.7353	0.8235	0.5294	0.5294	0.5000	0.5294	0.6765	0.6471	0.6176	0.6765
TA0010	0.3483	0.2683	0.3483	0.4353	0.3909	0.4818	0.6931	0.4353	0.5306	-	0.6176	0.5588	0.5294	0.7059	0.6471	0.6176	0.5294	0.7353	0.6471	0.5588	0.5000
TA0011	0.4818	0.5819	0.3909	0.3909	0.3483	0.4353	0.2683	0.4818	0.3909	0.4818	-	0.7647	0.7941	0.7353	0.6176	0.6471	0.5588	0.7647	0.7941	0.7647	0.5882
TA0012	0.3909	0.8183	0.3909	0.4818	0.5306	0.2683	0.2683	0.2305	0.3075	0.5819	0.2683	-	0.7941	0.6176	0.5588	0.5294	0.5588	0.7647	0.7353	0.7059	0.7647
TA0013	0.3483	0.7538	0.3483	0.3483	0.4818	0.3075	0.1591	0.4353	0.1942	0.6360	0.2305	0.2305	-	0.7059	0.5882	0.6176	0.5294	0.6765	0.7647	0.7941	0.7353
TA0014	0.4353	0.4353	0.3483	0.5306	0.4818	0.5819	0.4818	0.5306	0.6360	0.3483	0.3075	0.4818	0.3483	-	0.7059	0.7353	0.5882	0.7353	0.7059	0.7941	0.6176
TA0015	0.6360	0.5306	0.6360	0.6360	0.6931	0.5819	0.5819	0.5306	0.6360	0.4353	0.4818	0.5819	0.5306	0.3483	-	0.7941	0.7647	0.6765	0.6471	0.6176	0.6765
TA0016	0.4818	0.3075	0.4818	0.4818	0.4353	0.6360	0.4353	0.6931	0.6931	0.4818	0.4353	0.6360	0.4818	0.3075	0.2305	-	0.6176	0.6471	0.6176	0.6471	0.5294
TA0017	0.8873	0.6360	0.7538	0.6360	0.8183	0.8183	0.6931	0.5306	0.6360	0.6360	0.5819	0.5819	0.6360	0.5306	0.2683	0.4818	-	0.6765	0.5882	0.5588	0.6765
TA0018	0.3909	0.4818	0.3909	0.4818	0.4353	0.4353	0.4353	0.2305	0.3909	0.3075	0.2683	0.2683	0.3909	0.3075	0.3909	0.4353	0.3909	-	0.7941	0.7059	0.6471
TA0019	0.3483	0.4353	0.4353	0.4353	0.3909	0.3909	0.3909	0.4353	0.4353	0.4353	0.2305	0.3075	0.2683	0.3483	0.4353	0.4818	0.5306	0.2305	-	0.8529	0.7353
TA0020	0.3909	0.5819	0.4818	0.5819	0.5306	0.4353	0.2683	0.4818	0.4818	0.5819	0.2683	0.3483	0.2305	0.2305	0.4818	0.4353	0.5819	0.3483	0.1591	-	0.7647
TA0021	0.5819	**0.9614**	0.6931	0.6931	0.8873	0.5306	0.3483	0.3909	0.3909	0.6931	0.5306	0.2683	0.3075	0.4818	0.3909	0.6360	0.3909	0.4353	0.3075	0.2683	-

Limitations of this study and future direction

Despite the great nutritive and economic importance of snake gourd, no research finding on genetic analysis of this crop involving DNA markers is available in Bangladesh. This is the first ever approach of molecular characterization using RAPD markers of this species in Bangladesh. The study established a molecular assessment, but constant research on snake gourd genotypes is necessary. The snake gourd accessions presented here will be a worthy of selecting accessions with broader diversity for further evaluation or in future breeding program. Moreover, the phenotypic and molecular marker data can be combined for association mapping aiming development of key molecular markers associated with important traits.

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