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GENETIC DIVERSITY IN DRAGON FRUIT (*HYLOCEREUS* SP) GERMPLASMS REVEALED BY RAPD MARKER

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ABSTRACT

Dragon fruit (*Hylocereus* sp) is considered an important economic fruit species worldwide due to its rich nutrient contents and strong tolerance to drought stress. In order to investigate genetic diversity in dragon fruits in Bangladesh, 15 germplasms were subjected to randomly amplified polymorphic DNA (RAPD) analysis. A total of 43 loci of which 37 (86.05%) were polymorphic amplified from the genomic DNA of germplasms using 5 decamer random primers. Overall gene diversity or heterozygosity and Shannon's information index detected were 0.327 and 0.482, respectively indicating that a substantial level of genetic diversity existed in the studied germplasms. Pair wise band-sharing based similarity indices (ranged from 0.41 to 0.97), Jaccard similarity coefficient (ranged from 0.21 to 0.94) and Nei's genetic distance (ranged from 0.05 to 1.12) indicated that a wide range of genetic variation and relatedness retained between dragon fruit germplasms. The dendrogram constructed on the basis of Jaccard similarity coefficient showed that 15 dragon fruit germplasms grouped into different clusters and that individuals within one cluster possessed similar morphological traits, though there were some discrepancies. RAPD analysis effectively disclosed genetic variation and relatedness between dragon fruit germplasms.

Keywords: RAPD marker, Polymorphism, Genetic distance, Gene diversity, *Hylocereus* sp.

INTRODUCTION

Dragon fruit (*Hylocereus sp.*) is an excellent tropical fruit with exotic aesthetic characteristics belonging to the family Cactaceae. The species, known as pitahaya or dragon fruit, is of great interest to researchers due to its attractive color (Hua *et al.,* 2016), pleasant taste (Garcia-Cruz *et al*., 2017), high content of nutrients (Tze *et al*., 2012) and exceptional drought-tolerance (Nobel and De La Barrera, 2004). Moreover, it has senescence-retarding (Lim *et al.,* 2012; Zhuang *et al.,* 2012), cancer-preventing effects (Yusof *et al.,* 2012), positive effects on metabolism, digestion, immune system, clear vision, oxidative stress, diabetes and cardiovascular diseases (Nurmahani *et al.,* 2012).

There are several species of the dragon fruit commercially grown namely *H. Undatus* (white dragon fruit), *Hylocereus sp.* (pink dragon fruit), *H. Polyrhizus* (red dragon fruit), *H. costaricensis* (purplish-red dragon fruit), and *Selenicereus megalanthus* (yellow dragon fruit) (Hunt, 2006; Hamida *et al.,* 2017). The origin of *Hylocereus spp.* is the tropical and sub-tropical forest regions of Mexico and Central and South America (Ortiz-Hernández and Carrillo-Salazar, 2012). From there, dragon fruits spread to tropical and sub-tropical America, Asia, Australia and the Middle East. It is cultivated in at least 22 countries of the tropics (Mizrahi and Nerd, 1999).

Dragon fruit in Bangladesh is an exotic species. The germplasm of the species (*Hylocereus spp.*) was introduced from Thailand and many other countries (Patwary *et al.,* 2013). Some germplasms collected from Vietnam were conserved at Bangladesh Agricultural University Germplasm Centre (BAU-GPC) in 2007 (personal communication with Professor Dr. Md. Abdur Rahim, Director, BAU-GPC). Since then some research on field performance of the species, its nutritional composition and diseases were performed. Research findings showed that there are tremendous prospects of growing dragon fruit commercially across Bangladesh as the topography and environment of the country favours its commercial farming (Patwary *et al.,* 2013). A number of farmers from different districts of Bangladesh including Dinajpur, Thakurgaon, Panchagarh, Nilphamari, Jhenidah, Dhaka, Natore, and Chattogram have started commercial cultivation of the species (Karmaker, 2015). The fruit attracts the people by its taste and it gets higher market price (400-500 Tk per kg fruit). BAU-GPC has contributed significantly in disseminating cultivation technology of dragon fruits and in some cases initial supply of plant materials to the farmers (Benarjee, 2015; personal communication with Professor Dr. Md. Abdur Rahim, Director, BAU-GPC).

Although some morphological (Patwary *et al.,* 2013) and biochemical (Islam *et al.,* 2012) researches have been conducted, there has been no report on genetic diversity assessment of dragon fruit germplasms using either morphological traits or molecular markers. This is the very first study conducted in Bangladesh to estimate genetic diversity and relationship in the germplasms of *Hylocereus sp.* with the aim to develop a baseline

document on genetic diversity of this important exotic fruit species in Bangladesh. Information on genetic variation of any organism facilitates its genetic improvement, conservation and management.

The development of DNA marker technology has provided an efficient and more reliable tool to estimate genetic variation of any organism. Although DNA markers like AFLPs and microsatellites are preferred for genetic diversity study due to their informativeness, Randomly Amplified Polymorphic DNA (RAPD) analysis has also been broadly employed to analyze the genetic diversity because it has a universal set of primers, no prior work such as probe isolation, or nucleotide sequencing is necessary (Hadrys *et al.,* 1992). This marker is being widely used to estimate genetic variation and relatedness in different organisms (Mollah *et al.,* 2009; Moghaieb *et al.,* 2014; Dhakshanamoorthy *et al.,* 2015; Saclain *et al.,* 2016; Bala *et al.,* 2017). In the present study, RAPD marker was used to estimate genetic variation and relatedness in fifteen dragon fruit germplasms in Bangladesh.

MATERIALS AND METHODS

Plant materials: For genomic DNA isolation and subsequent RAPD analysis, stem of 15 dragon fruit plants were collected from BAU-GPC (Longitude: 24.7175º N, latitude: 90.4310º E). Dragon fruit germplasms at BAU-GPC were collected from Thailand and Vietnam in 2007. Some important morphological characteristics of the collected dragon fruits that were noted during collections of plant materials are shown in Table 1.

DNA extraction: Genomic DNA from each individual was extracted from tender stem tissue following CTAB method (Xin and Chen, 2012) with some modifications. The stem tissues were cut into small pieces, taken into eppendorf tube, homogenized and digested with extraction buffer (2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 3% β- mercaptoethanol) at 65ºC for 15 min. Tissue lysate was purified with phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v). DNA was precipitated using absolute ethanol and 3 M sodium acetate (pH 5.2) and pelleted by centrifugation. DNA was reprecipitated by adding 70% ethanol, and pelleted by centrifugation. Pellets were air-dried, suspended in TE buffer (10 mM Tris HCL, 1 mM EDTA, pH 8.0) and stored at -20ºC. Extracted DNA samples were confirmed by using 0.8% agarose gel electrophoresis and quantified by a spectrophotometer (Spectronic^{R} Genesis^{TM}, Spectronic Instruments Inc., USA).

Primer test: Twenty five decamer primers of random sequence were initially screened on DNA samples of 3 dragon fruit germplasms to evaluate their suitability for amplifying DNA sequences, which could be accurately scored. A final subset of five primers (Table 1) was selected on the basis of intensity or resolution of bands, their reproducibility, consistency within individuals and potentiality for discrimination of genotypes. In order to ensure reproducibility of RAPD bands further, amplification of DNA samples of 2 dragon fruit germplasms using the selected 5 primers was repeated twice. Similar banding pattern was observed in each case.

PCR analysis: The selected 5 RAPD primers were used to amplify whole sample set of genomic DNA from 15 dragon fruit germplams. The amplification was based on Williams *et al.* (1990) with some modifications. PCR reactions were carried out in 10 µl reaction mix containing 2 µl (100 ng) of genomic DNA, 1 µl of 10x *Taq* buffer, 1 µl of dNTPs (250 µM each) (Takara, Japan), 2 µl (10 µM) of primer (Invitrogen), 0.2 (1 unit) of *Taq* DNA polymerase (Takara, Japan) and required amount of sterile water. PCR was performed in a TProfessional Standard Gradient Thermocycler (Biometra, Germany) as follows: initial denaturation at 94ºC for 3 min; 40 cycles of denaturation at 94ºC for 1 min, annealing at 36ºC for 1 min and extension at 72ºC for 2 min. A final extension step at 72ºC for 7 min was employed to ensure complete amplification of all DNA fragments followed by holding at -4ºC.

Agarose gel electrophoresis: PCR products were separated on 1.4% agarose gel. Molecular weight markers (1kb and 100 bp DNA markers (BIONEER, Republic of Korea) were also run alongside the gel. Gels were stained with ethidium bromide (10 mg/ml), visualized and photographed using a gel documentation system (Biodoc-It™ Gel Imaging System, Cambridge, UK).

Data analysis: Size of each RAPD bands was measured using AlphaEaseFC^{TM} version 4.0 (Alpha Innotech) software program. Then RAPD bands were scored as '1' if present and '0' if absent of bands of same molecular weight. Scores in respect of all primers were then pooled for constructing a single data matrix, which was used to estimate polymorphic loci, overall gene frequencies, gene diversity (Nei, 1973), genetic distance (Nei, 1978) and constructing an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram using POPGENE, version 1.31 (Yeh *et al.,* 1999) and 'Tools for Population Genetic Analyses (TFPGA, Miller, 1997) software packages. NTSYSpc software program (Rohlf, 1997) was also used to measure Jaccard similarity coefficient (Jaccard, 1912) and perform cluster analysis. The band-sharing based similarity indices (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula: Similarity index (SI) = 2NXY/NX+NY, Where, NXY represents total number of RAPD markers shared by individuals X and Y, and NX

and NY are the numbers of markers scored for each individual, respectively (Lynch, 1990).

RESULTS

Five primers (e.g., AO1, AO9, OPB8, GO3, and M16) amplified a total of 43 loci with a size range of 262-2473 bp from genomic DNA of 15 dragon fruit germplasms (Table 2). Primer AO1 generated the highest number of bands (13) whereas primer M16 produced the least number of bands (5). Out of 43 bands, 37 were polymorphic (86.05%) and 6 bands (13.95%) were monomorphic. The average number of scorable bands was 8.6 where the average number of polymorphic fragments was 7.4. Overall gene or allele frequencies varied between 0.133 to 1.000 (Table 3). Allele $OPB8_{1429}$ with a frequency of 0.133 was found in Df-11 and Df-13 genotypes whilst it was absent in other 13 genotypes. Likewise, allele $OPB8_{395}$ was found in genotypes Df-4 and Df-9 at a frequency of 0.133 whereas it was absent in other genotypes.

Mean expected number of alleles was found to be lower (1.570) than that of observed number of alleles (1.861) in the present study. Overall gene diversity (h) and Shannon's information index (i) values across all primers and germplasms were 0.327 and 0.482, respectively (Table 3). The h and i values across primers A01, AO9, OPB8, GO3 and M16 were 0.394 & 0.529, 0.313 & 0.468, 0.367 & 0.549, 0.332 & 0.489 and 0.164 & 0.238, respectively.

Estimates of band-sharing based similarity indices produced from the RAPD marker data for all pair-wise combinations of the 15 dragon fruit germplasms are presented in Table 4. The similarity indices varied from 0.41 to 0.97.The highest SI value (0.97) was found between Df-13 and Df-11 germplasm pair whereas the lowest SI value (0.41) was obtained from Df-4 vs Df-2 germplasms. The overall SI value considering all loci and germplasm was 0.69. To estimate relative contribution of each primer in detecting genetic similarity in fifteen germplasms, we found that the M16 primer resulted in the highest level of similarity (0.87) and the OPB8 caused the lowest level of genetic similarity (0.48). Jaccard similarity coefficient between different dragon fruit genotypes was also determined (Table 4). Like the band-sharing based similarity indices, Jaccard similarity coefficient determined the highest (0.94) and the lowest level of similarities or relatedness (0.21) in Df-11 vs Df-13 and Df-2 VS Df-4 dragon fruit genotypes, respectively.

Nei's unbiased measures of genetic distances (Nei, 1978) between different dragon fruit genotypes computed from combined data for 5 primers were ranged from 0.05 to 1.19 with the least genetic distance between Df-11 and Df-13 and the highest genetic distance between Df-2 and Df-4 (Table 4). Lower level of genetic distances were also found for Df-2 vs Df-3 (GD=0.07), Df-6 vs Df-10 (GD=0.10) and Df-7 vs Df-8 (GD=0.10). On the other hand, higher level of genetic distance was detected in Df-3 vs Df-4 (GD= 1.12), Df-2 vs Df-9 (GD= 0.99) and Df-3 vs Df-9 (GD=0.93) genotype pairs.

Cluster analysis or a UPGMA dendrogram based on the Jaccard similarity coefficient segregated 15 dragon fruit genotypes into 2 major clusters. Cluster 1 consisted of Df-04, Df-09 and Df-15 whereas cluster 2 contained the rest of 12 genotypes. Cluster 2 was further divided into 2 sub clusters: Df-01 and Df-05 in sub cluster1 and other 10 genotypes into sub cluster 2. Sub cluster 2 was further separated to 3 groups: Df-02 and Df-03 in group 1, Df-06, Df-07, Df-08 and Df-10 in group 2 and Df-11, Df-12, Df-13 and Df-14 in group 3.

Table 1. Morphological characteristics of dragon fruit (Df) germplasms.

Germplasms	Fruit characteristics				
	Fruit shape	Fruit color	Flesh color	Taste of fruit flesh	Thorn
Df-1	Oval	Red	Pinkish red/Red	Sweet	Dense
Df-2	Oval	Red	Pinkish red/Red	Sweet	Dense
Df-3	Oval	Red	Pinkish red/Red	Sweet	Dense
Df-4	Oval	Red	Pinkish red/Red	Sweet	Dense
Df-5	Oval	Red	Pinkish red/Red	Sweet	Dense
Df-6	Round	Dark red	Dark red	Sweet	Sparse
Df-7	Round	Dark red	Dark red	Sweet	Sparse
Df-8	Round	Dark red	Dark red	Sweet	Sparse
Df-9	Round	Dark red	Dark red	Sweet	Sparse
Df-10	Round	Dark red	Dark red	Sweet	Sparse
Df-11	Oval	Red	White	Salty sweet	Sparse
Df-12	Oval	Red	White	Salty sweet	Sparse
Df-13	Oval	Red	White	Salty sweet	Sparse
Df-14	Oval	Red	White	Salty sweet	Sparse
Df-15	Oval	Red	White	Salty sweet	Sparse

Table 2. RAPD primers with corresponding loci with their size range, number and proportion of polymorphic loci observed in dragon fruit germplasm.

Primer codes	Sequence (5'-3')	GC Content (%)	Total number of loci scored	Size range (bp)	Number of polymorphic loci scored	Proportion of polymorphic loci (%)
AO1	CAGGCCCTTC	70	13	262-1894	12	92.30
AO9	GGGTAACGCC	70	11	301-1536	10	90.90
OPB8	GTCCACACGG	70	8	280-2473	8	100.00
GO3	GAGCCCTCCA	70	6	337-1124	5	83.33
M16	GTAACCAGCC	60	5	352-967	2	40.00
Overall			43	262-2473	37	86.05

Table 3. Estimation of genetic variation in dragon fruit germplasm: gene frequencies (gf), observed number of alleles (na) and effective number of alleles (ne), Nei's (1973) gene diversity (h) and Shannon's information index (I).

Locus	Gf	na	Ne	h	I
$AO1_{1894}$	0.667	2.000	1.800	0.444	0.637
$AO1_{1647}$	0.333	2.000	1.800	0.444	0.637
$AO1_{1083}$	0.800	2.000	1.471	0.320	0.500
$AO1_{942}$	0.733	2.000	1.642	0.391	0.580
$AO1_{782}$	0.467	2.000	1.991	0.498	0.691
$AO1_{713}$	0.933	2.000	1.142	0.124	0.245
$AO1_{591}$	0.933	2.000	1.142	0.124	0.245
$AO1_{503}$	0.600	2.000	1.923	0.480	0.673
$AO1_{437}$	0.600	2.000	1.923	0.480	0.673
$AO1_{389}$	0.467	2.000	1.991	0.498	0.691
$AO1_{354}$	0.400	2.000	1.923	0.480	0.673
$AO1_{331}$	0.333	2.000	1.800	0.444	0.636
$AO1_{262}$	1.000	1.000	1.000	0.000	0.000
$AO9_{1536}$	0.667	2.000	1.800	0.444	0.637
$AO9_{1305}$	0.667	2.000	1.800	0.444	0.637
$AO9_{1109}$	0.667	2.000	1.800	0.444	0.637
$AO9_{899}$	0.733	2.000	1.642	0.391	0.580
$AO9_{764}$	0.400	2.000	1.923	0.480	0.673
$AO9_{713}$	0.267	2.000	1.642	0.391	0.580
$AO9_{620}$	0.867	2.000	1.301	0.231	0.393
$AO9_{469}$	0.933	2.000	1.142	0.124	0.245
$AO9_{408}$	0.933	2.000	1.142	0.124	0.245
$AO9_{346}$	0.467	2.000	1.991	0.498	0.691
$AO9_{301}$	1.000	1.000	1.000	0.000	0.000
$OPB8_{2473}$	0.400	2.000	1.923	0.480	0.673
$OPB8_{1429}$	0.133	2.000	1.301	0.231	0.393
$OPB8_{1539}$	0.467	2.000	1.991	0.498	0.691
$OPB8_{957}$	0.800	2.000	1.471	0.320	0.500
$OPB8_{693}$	0.267	2.000	1.642	0.391	0.580
$OPB8_{523}$	0.733	2.000	1.642	0.391	0.580
$OPB8_{395}$	0.133	2.000	1.301	0.231	0.393
$OPB8_{280}$	0.267	2.000	1.642	0.391	0.580
$GO3_{1124}$	0.733	2.000	1.642	0.391	0.580
$GO3_{956}$	0.733	2.000	1.642	0.391	0.580
$GO3_{660}$	1.000	1.000	1.000	0.000	0.000
$GO3_{466}$	0.533	2.000	1.991	0.498	0.691
$GO3_{405}$	0.733	2.000	1.642	0.391	0.580
$GO3_{337}$	0.800	2.000	1.471	0.320	0.500
$M16_{967}$	0.800	2.000	1.471	0.320	0.500
$M16_{648}$	0.533	2.000	1.991	0.498	0.691
$M16_{550}$	1.000	1.000	1.000	0.000	0.000
$M16_{455}$	1.000	1.000	1.000	0.000	0.000
$M16_{352}$	1.000	1.000	1.000	0.000	0.000
Mean		1.861	1.570	0.327	0.482
St. Dev		0.351	0.345	0.172	0.235

Table 4. Pair-wise band-sharing based similarity indices (Lynch, 1990) (above diagonal), Jaccard similarity coefficient (above diagonal in parentheses) and Nei`s (1978) genetic distances (below diagonal) estimated according to the RAPD analysis of 15 dragon fruit (Df) germplasms.

Populations	Df-1	Df-2	Df-3	Df-4	Df-5	Df-6	Df-7	Df-8	Df-9	Df-10	Df-11	Df-12	Df-13	Df-14	Df-15
Df-1	***	0.83	0.74	0.45	0.77	0.69	0.76	0.80	0.59	0.78	0.62	0.75	0.59	0.64	0.55
		(0.65)	(0.56)	(0.30)	(0.64)	(0.49)	(0.59)	(0.65)	(0.45)	(0.58)	(0.46)	(0.58)	(0.43)	(0.44)	(0.41)
Df-2	0.30	***	0.91	0.41	0.67	0.71	0.77	0.80	0.50	0.76	0.71	0.78	0.71	0.70	0.51
			(0.90)	(0.21)	(0.61)	(0.68)	(0.65)	(0.70)	(0.29)	(0.78)	(0.65)	(0.69)	(0.66)	(0.64)	(0.33)
Df-3	0.39	0.07	***	0.43	0.69	0.76	0.73	0.73	0.50	0.79	0.78	0.71	0.72	0.72	0.58
				(0.22)	(0.58)	(0.69)	(0.67)	(0.63)	(0.30)	(0.75)	(0.67)	(0.61)	(0.68)	(0.66)	(0.34)
Df-4	0.67	1.19	1.12	***	0.61	0.53	0.57	0.58	0.87	0.55	0.62	0.49	0.64	0.57	0.75
					(0.41)	(0.35)	(0.40)	(0.42)	(0.76)	(0.37)	(0.32)	(0.31)	(0.34)	(0.38)	(0.64)
Df-5	0.26	0.36	0.39	0.50	***	0.64	0.70	0.73	0.74	0.71	0.64	0.68	0.64	0.69	0.76
						(0.46)	(0.56)	(0.61)	(0.52)	(0.55)	(0.51)	(0.54)	(0.49)	(0.54)	(0.59)
Df-6	0.58	0.32	0.30	0.82	0.67	***	0.94	0.90	0.56	0.94	0.79	0.84	0.82	0.81	0.54
							(0.86)	(0.81)	(0.40)	(0.89)	(0.67)	(0.70)	(0.72)	(0.70)	(0.37)
Df-7	0.39	0.36	0.33	0.67	0.47	0.12	***	0.96	0.60	0.86	0.80	0.88	0.82	0.80	0.58
								(0.89)	(0.44)	(0.81)	(0.68)	(0.77)	(0.69)	(0.68)	(0.42)
Df-8	0.32	0.29	0.39	0.67	0.39	0.18	0.10	***	0.64	0.85	0.80	0.86	0.81	0.80	0.62
									(0.50)	(0.87)	(0.69)	(0.78)	(0.70)	(0.68)	(0.43)
Df-9	0.47	0.99	0.93	0.12	0.39	0.77	0.63	0.54	***	0.61	0.65	0.54	0.68	0.60	0.84
										(0.45)	(0.37)	(0.35)	(0.39)	(0.43)	(0.70)
Df-10	0.43	0.21	0.23	0.82	0.50	0.10	0.18	0.12	0.67	***	0.74	0.83	0.77	0.83	0.56
											(0.63)	(0.71)	(0.68)	(0.71)	(0.39)
Df-11	0.63	0.36	0.33	0.87	0.54	0.36	0.33	0.33	0.82	0.43	***	0.88	0.97	0.88	0.68
												(0.77)	(0.94)	(0.82)	(0.50)
Df-12	0.39	0.30	0.39	0.87	0.47	0.30	0.21	0.21	0.82	0.30	0.21	***	0.85	0.87	0.62
													(0.73)	(0.77)	(0.44)
Df-13	0.72	0.36	0.33	0.87	0.63	0.30	0.33	0.33	0.82	0.36	0.05	0.26	***	0.89	0.62
														(0.83)	(0.47)
Df-14	0.63	0.36	0.33	0.67	0.47	0.30	0.33	0.33	0.63	0.30	0.15	0.21	0.15	***	0.67
															(0.53)
Df-15	0.50	0.82	0.77	0.21	0.30	0.82	0.67	0.67	0.18	0.82	0.50	0.58	0.58	0.43	***

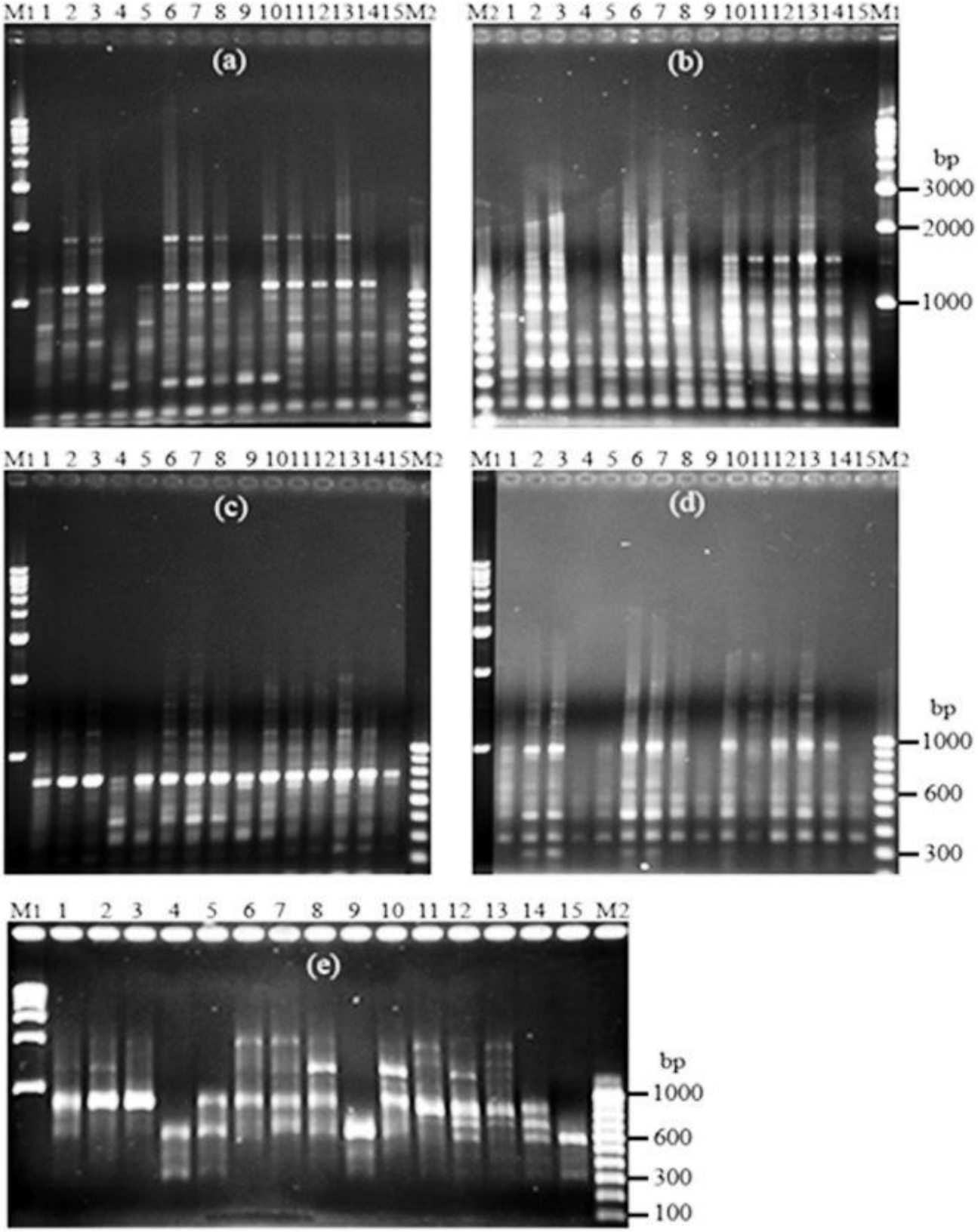

Figure 1. RAPD profiles of 15 dragon fruit germplasms using primers AO1 (a), AO9 (b), OPB8 (c), G03 (d) and M16 (e). Lane 1-15: Dragon fruit germplasms. M1 and M2: 1kb and 100 bp molecular weight markers, respectively.

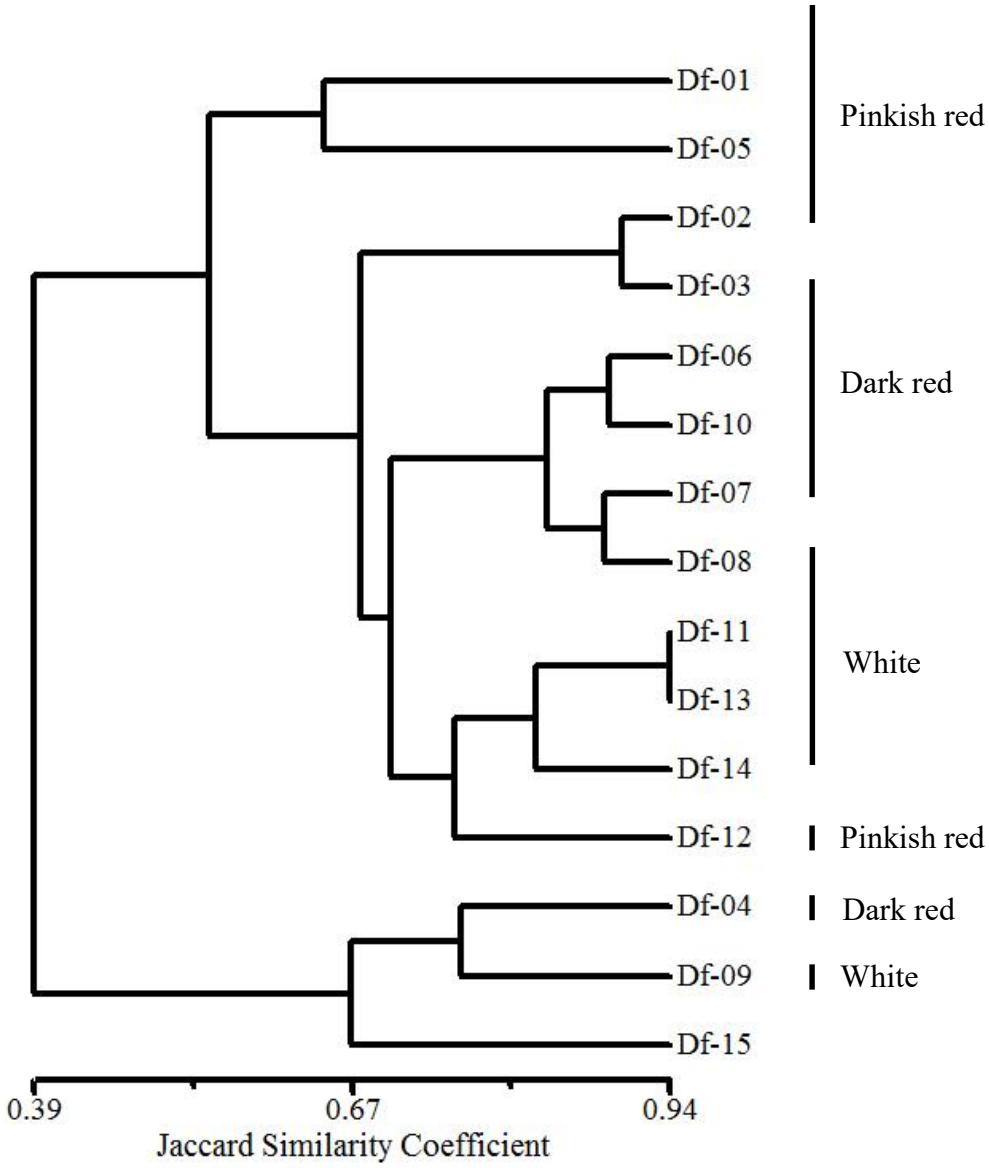

Figure 2. A UPGMA dendogram based on Jaccard similarity coefficient, summarizing data on genetic variation between dragon fruit (Df) germplasms according to RAPD analysis.

DISCUSSION

Detection of genetic differences and elucidation of genetic relationships among genotypes of any plant are of high importance for both proprietary rights protection, conservation, utilization and improvement of its genetic resources (Liu *et al.,* 2011). Up to date, there has been no report on genetic diversity assessment of dragon fruit germplasms in Bangladesh. We performed a systematic baseline assessment of genetic diversity in 15 dragon fruit germplasms using RAPD makers and related this molecular based diversify with their some important phenotypic characteristics.

Our study effectively used RAPD technique in detecting a degree of polymorphism (polymorphic loci, 86.05% and gene diversify, 0.327) among dragon fruit germplasms. Like the present study, Junqueira *et al.* (2010) investigated genetic diversity in 13 dragon fruit accessions using 164 RAPD markers. The percentage of polymorphic markers of their study was 95.06% which is higher than that of our study (86.05%). Tao *et al.* (2014) on the other hand, revealed 66.12% polymorphic loci using 111 Inter-Simple Sequence Repeat (ISSR) markers to discriminate 50 accessions of dragon fruit. The variation in detection of polymorphism in different experiments might be due to differences in dragon fruit

accessions, type of markers, primers, and overall experimental set up followed.

A major objective of our study was to determine genetic relatedness or differences between 15 dragon fruit genotypes. Band-sharing based similarity indices or Jaccard similarity coefficient values suggested that a wider range of genetic relationship existed between dragon fruit genotypes. Estimation of pair-wise Nei's genetic distances (Nei, 1978) further confirmed the diversified genetic relationships between the genotypes. Intriguingly, in most cases genotypes having morphological similarities trended to group into the same cluster. For instance, Df-6, 7, 8 and 10 possessing dark red flesh color and sparse-thorn made one cluster with a average Jaccard similariy coefficient of 0.86. Likewise, Df-11-14 containing white flesh, salty-sweet taste and sparse-thorn grouped to the same cluster. Moreover, Df-1 and 5 or Df-2 and 3 having pinkish red flesh, sweet taste and dense-thorn belonged to the same cluster with a higher level of genetic similarity. This implies that genotypes having morphological similarities might possess similar genetic makeup or more likely they come from the same origin. In other words, genotypes clustered in the same group might belong to the same species or variety. This is consistent with the results from Pan *et al.* (2017) who studied genetic diversity among 46 pitaya accessions using SSR markers and found that accessions having similar identities were grouped into the same cluster. Similarly, species or variety-wise separation of different accessions of dragon fruits in the dendrogram constructed from morphological, ISSR and RAPD data was reported by Tao *et al.* (2014) and Junqueira *et al.* (2010). According to researchers, there are a number of dragon fruit species, which vary from each other dominantly by their fruit flesh colors (pink, red, white, purplish-red, yellow etc) (Hamidah *et al.,* 2017). These species are being widely cultivated in Southeast Asian countries including Indonesia, Malaysia, Vietnam, and Thailand. In Bangladesh, dragon fruits are exotic and a number of germplasms have been collected from Southest Asian Countries particularly from Thailand and Vietnam randomly. Therefore, there is a possibility that more than one species of dragon fruit existed in Bangladesh. Based on the cluster of genotypes in the dendrogram, it can be concluded that 15 dragon fruit genotypes might come from three different species.

In contrast, three genotypes namely Df-4 (pinkish red), 9 (dark red) and 15 (white) that possess different flesh color, taste and types of thorns grouped into one cluster. The reason of clustering these three genotypes is difficult to explain. However, existence of individual differences within one species or variety according to Campbell *et al.* (2003) could lead to discrimination among individuals within a species and place them differently in the dendrogram. Within species differences in dragon fruit was also reported by Hamidah *et al.* (2017) while investigating phenotypic-based genetic diversity in *Hylocereus* sp.

In conclusions, analyses of 43 RAPD markers efficiently estimated genetic diversity among 15 dragon fruit germplasms by revealing 86.05% polymorphic loci and heterozygosity of 0.372. Although there existed discrepancy in genetic relatedness between RAPD loci and morphological data for 3 genotypes, other 12 genotypes grouped into 3 clusters with higher degree of both RAPD and morphological based genetic relatedness. Finally, based on the cluster analysis of the present study and morphological data recorded, it is expected that studied 15 germplasms have come from at least 3 different species or verities. This information could be valuable for the view points of nomenclature, management, conservation and improvement of the dragon fruit in Bangladesh. Moreover, our preliminary attempt regarding genetic diversity study of dragon fruit could be used as a baseline data for the detailed investigation of genetic structure of this important fruit crop in future.

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