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GENETIC VARIATION AND MOLECULAR RELATIONSHIPS AMONG EIGHT TAXA OF *DESMODIUM* DESV. BASED ON RAPD MARKERS

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Abstract

Genetic variation and molecular relationships among eight taxa of *Desmodium* Desv. were assessed on the basis of random amplified polymorphic DNA (RAPD) markers. The banding patterns of eight taxa namely, *Desmodium gangeticum* (L.) DC., *D. heterocarpon* (L.) DC., *D. heterophyllum* (Willd.) DC., *D. motorium* (Houtt.) Merr., *D. pulchellum* (L.) Benth., *D. triflorum* (L.) DC., *D. triquetrum* (L.) DC. and *D. triquetrum* subsp. *alatum* (DC.) Prain were compared. A total of 81 DNA fragments were detected by 11 primers. Among the taxa studied *D. triquetrum* and *D. triquetrum* subsp. *alatum* were found to be most closely related followed by close proximity between *D. gangeticum* and *D. motorium*. The highest genetic distance was observed between *D. triflorum* and *D. heterophyllum* followed by *D. heterocarpon* and *D. heterophyllum*. UPGMA dendrogram was constructed to show the genetic relatedness among the taxa employed and the tree revealed a close proximity among *D. pulchellum*, *D. gangeticum* and *D. motorium*. In contrast, *D. heterophyllum* was found distantly related with rest of the taxa.

Introduction

Desmodium Desv. belongs to the family Fabaceae comprises about 280 species widespread in the tropical and subtropical regions (Puhua and Ohashi, 2010). In Bangladesh *Desmodium* is represented by 19 taxa (Ahmed *et al.*, 2009). They are annual to perennial herbs, undershrubs or shrubs, and characterized by possessing uni- or tri-foliolate leaves, simple raceme or panicle inflorescence and distinctly jointed pods. The systematics of the genus *Desmodium* is confusing and not yet resolved completely (Ohashi and Mill, 2000). Several taxonomic studies on *Desmodium* were carried out based on morphology and anatomy (Pedley and Rudd, 1996; Shaheeen, 2008; Puhua and Ohashi, 2010). Recently, Rahman and Rahman (2012) conducted a morphometric study of *Desmodium* and showed interspecific relationships among 14 species of the genus. However, molecular studies employing different DNA markers on this genus are very scanty (Yue *et al*., 2010; Ahmad Haji *et al*., 2016).

Recent progress in DNA marker technology have augmented the marker resources for genetic analyses of a wide variety of genomes. The development of random amplified polymorphic DNA (RAPD) markers generated by polymerase chain reaction (PCR) using arbitrary primers has resulted in alternative molecular markers for the detection of nuclear DNA polymorphism (Williams *et al*., 1990). RAPD markers have application in many fields including DNA fingerprinting (Elavazhagan *et al*., 2009), assessment of genetic diversity (Bodo Slotta and Porter, 2006), cultivar identification (Sipahi *et al.,* 2010), estimation of population genetics (Sales *et al.*,

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2001), hybridization (Caraway *et al.*, 2001), systematics (Vilatersana *et al.*, 2005), phylogeny reconstruction (Ahmed *et al.,* 2005), and genome mapping (Krutovaskii *et al*., 1998).

In legume species, RAPD markers have proven to be a useful tool in studies analyzing genetic variation (Yamaguchi and Jabadi, 2004; Bisoyi *et al*., 2010). Previous studies on *Desmodium* using isozymes were conducted mainly with species which are important as forage (Smith and Schaal, 1979; Imrie and Blogg, 1983). Application of RAPD markers for detecting genetic variation and interspecific relationships of *Desmodium* is very limited. Bedolla-Garcia and Lara-Cabrera (2006) applied RAPD markers to detect genetic variation within and among five population of *Desmodium sumichrastii* from Mexico. Very recently, Singh *et al.* (2016) employed RAPD analysis for DNA fingerprinting of only two species of *Desmodium*, *viz*., *D. gangeticum* and *D. laxiflorum*. However, no detailed study based on RAPD markers for detecting genetic diversity and interspecific relationships in *Desmodium* was carried out so far. Therefore, the aims of the present study are two-fold: i) to detect the genetic diversity among eight *Desmodium* taxa, and ii) to infer the relationship among these taxa of *Desmodium* based on RAPD analysis.

Materials and Methods

Plant materials

Eight taxa of *Desmodium* were collected from different places of Bangladesh, *viz*., *Desmodium gangeticum* (L.) DC., *D. heterocarpon* (L.) DC., *D. heterophyllum* (Willd.) DC., *D. motorium* (Houtt.) Merr., *D. pulchellum* (L.) Benth., *D. triflorum* (L.) DC., *D. triquetrum* (L.) DC. and *D. triquetrum* subsp. *alatum* (DC.) Prain (Table 1). Leaf samples were used for DNA isolation and were preserved at -80°C until further use. The voucher specimens are deposited at Dhaka University Salar Khan Herbarium (DUSH).

Table 1. List of *Desmodium* Desv. taxa used for RAPD analysis.

No.	Taxa	Habit	Voucher specimens
1.	*Desmodium gangeticum* (L.) DC.	Undershrub	Dhaka: 27.9.2011, Zahid 85 (DUSH); Munshigonj: 1.1.2011, Zahid 7 (DUSH).
2.	*D. heterocarpon* (L.) DC.	Undershrub	Cox's Bazar: Teknaf, 24.4.2011, Zahid 28 (DUSH); Gazipur: Rajendrapur, 5.11.2011, Zahid 91(DUSH).
3.	*D. heterophyllum* (Willd.) DC.	Herb	Gazipur: Rajendrapur, 15.7.2011, Zahid 50 (DUSH); Cox's Bazar: Pekua, 21.8.2011, Zahid 74 (DUSH).
4.	*D. motorium* (Houtt.) Merr.	Undershrub	Dhaka: 23.12.2010, Zahid 02 (DUSH).
5.	*D. pulchellum* (L.) Benth.	Shrub	Gazipur: Rajendrapur, 15.7.2011, Zahid 48 (DUSH).
6.	*D. triflorum* (L.) DC.	Herb	Cox's Bazar: Kutubdia island, 17.7.2011, Zahid 63 (DUSH); Narsingdi: Wari Boteshwar, 10.11.2011, Zahid 96 (DUSH).
7.	*D. triquetrum* (L.) DC.	Shrub	Cox's Bazar: Teknaf, 24.4.2011, Zahid 31(DUSH).
8.	*D. triquetrum* subsp. *alatum* (DC.) Prain	Shrub	Cox's Bazar: Teknaf, 19.7.2011, Zahid 73 (DUSH).

Genomic DNA isolation

DNA was isolated from leaves using the CTAB (Cetyl trimethyl ammonium bromide) method following Doyle and Doyle (1987). The isolated DNA was preserved in TE buffer and stored at –20°C.

RAPD amplification

The oligonucleotide primers tested for RAPD analysis were presented in Table 2. These primers were chosen by their number and consistency of amplified fragments for analyzing *Desmodium* taxa. The amplification reaction contained 50 ng of genomic DNA, 0.5 unit of Taq DNA polymerase, 0.5 µl of each dNTPs, 10 mM $MgCl_2$, 1µl decamer random primers (Operon Biotechnology, Germany) and 2.5 µl 10X amplification buffer in a total volume of 25 µl. The amplifications were performed in triplicate using PCR thermal cycler (Biometra UNOII, Germany) with initial denaturation of 5 min at 94°C, followed by 42 cycles at 94°C for 5 sec, 33°C for 1 min and 72°C for 2 min with final extension of 5 min at 72°C. The amplified products were separated on 1% agarose gel containing ethidium bromide, and photographed under UV light.

Table 2. List of primers used in RAPD analysis.

Primer Code	Sequence (5´-3´)	G + C content (%)
OPA-1	TGCCGAGCTC	70
OPA-2	TGCCGAGCTG	70
OPA-3	AGTCAGCCAC	60
OPA-6	GGTCCCTGAC	70
OPA-7	GAAACGGGTG	60
OPA-8	GTGACGTAGG	60
OPA-9	GTGATCGCAG	60
OPA-10	GTGATCGCAG	60
A15	TTCCGAACCC	60
B14	TCCGCTCTGG	70
BO6	TGCTCTGCCC	70

Data analysis

RAPD bands were recorded in a binary data matrix scored as presence (1) or absence (0). The score obtained using all primers in the RAPD analysis were then combined to create a single data matrix. The size of amplification products were estimated by comparing the migration of each amplified fragments with that of a known size fragments of 1 kb molecular weight marker. Genetic linkage distance was determined using the data matrix. UPGMA (Unweighted pair group method with arithmetic means) dendrogram was constructed to show the genetic relationships among the species (Sneath and Sokal, 1973). All analyses were performed using the Statistica program.

Results and Discussion

A total of 81 RAPD bands were scored with eleven RAPD primers in eight *Desmodium* taxa. The highest number of fragments was detected in *Desmodium heterophyllum* (31) followed by *D. triflorum* (20) and *D. heterocarpon* (7), while the lowest band observed in *D. motorium* (1). The highest number of fingerprints were generated by the primer OPA-8 and least number in OPA-1.

The RAPD markers have been found efficient to detect genetic variation in *Desmodium.* The highest dissimilarity (41.0) was observed between *D. triflorum* and *D. heterophyllum* followed by *D. pulchellum* and *D. heterophyllum* (35.5) and *D. heterophyllum* and *D. heterocarpon* (35.0) (Table 3). The lowest genetic distance (1.0) was found between *D. gangeticum* and *D. motorium* indicating that these species are very closely related (Table 3).

Cluster analysis of the genetic similarity estimates from RAPD markers was performed to generate the UPGMA dendrogram for showing genetic relationship among the taxa of *Desmodium* (Fig. 1). The dendrogram revealed that *D. pulchellum*, *D. gangeticum*, *D. motorium*, *D. triquetrum*, *D. triquetrum* subsp. *alatum*, and *D. heterocarpon* grouped together and formed a cluster showing a close relationships among them. This cluster further consisted of two sub-clusters, the first one contained *D. pulchellum*, *D. gangeticum* and *D. motorium* showing a close affinity between these three species, while the second sub-cluster comprised *D. triquetrum* subsp. *alatum*, *D. triquetrum*, and *D. heterocarpon*. The highest relatedness was observed between *D. gangeticum* and *D. motorium* among all the taxa employed in this study. The RAPD analysisalso shown that *D. heterophyllum* and *D. triflorum* retained ungrouped and they are distantly related from other taxa of *Desmodium*.

Table 3. Genetic variation among studied taxa of *Desmodium*.

Taxa	*D. pulche-llum*	*D. triflo-rum*	*D. hetero-carpon*	*D. hetero-phyllum*	*D. triquetrum* subsp. *alatum*	*D. trique-trum*	*D. gange-ticum*	*D. moto-rium*
D. pulchellum	0							
D. triflorum	28.3	0						
D. heterocarpon	14.1	32.2	0					
D. heterophyllum	35.5	41.0	35.0	0				
D. triquetrum subsp. *alatum*	8.0	26.3	14.1	33.5	0			
D. triquetrum	8.0	26.3	12.1	33.5	8.0	0		
D. gangeticum	6.0	24.3	12.1	33.6	6.0	6.0	0	
D. motorium	5.0	25.4	11.1	32.6	5.0	5.0	1.0	0

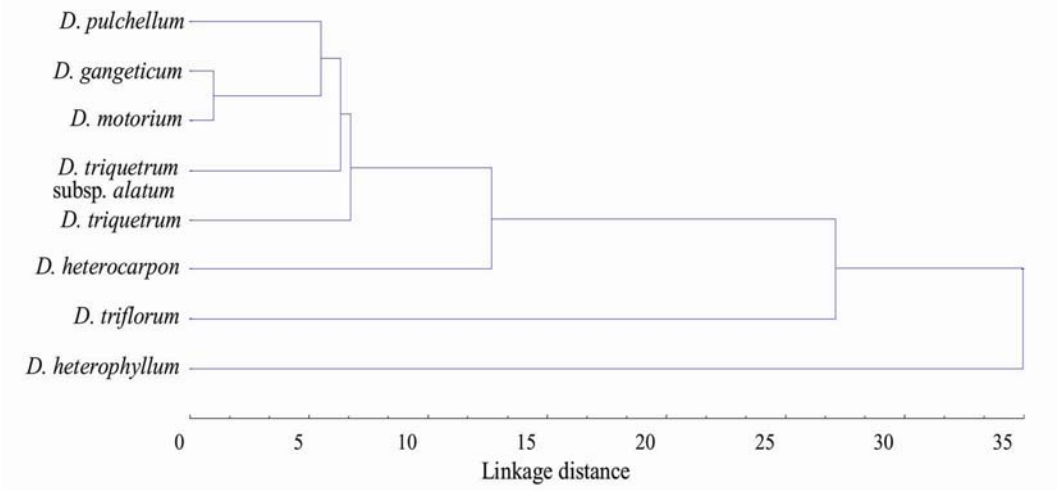

Fig. 1. UPGMA dendrogram showing the genetic relationship among studied Desmodium taxa based on RAPD markers.

The RAPD method is popular because of its technical simplicity and speed. The present study reveals that RAPDs are useful markers in identifying *Desmodium* species. The RAPD data shows that *D. gangeticum* and *D. motorium* are genetically closely related. This result is congruent with the previous study based on morphological characters such as unifoliolate, lanceolate leaves and triangular stipules (Ahmed *et al.,* 2009); and also supported by foliar anatomical investigation (data not shown). Bedolla-Garcia and Lara-Cabrera (2006) studied genetic variation within and among five populations of *Desmodium sumichrastii* from Mexico based on RAPD analysis. Singh *et al.* (2016) employed RAPD approach for the genetic fingerprinting of *Desmodium gangeticum* and *D. laxiflorum* and found 60-65% similarity between these two species. Irshad *et al.* (2009) studied three species of *Desmodium*, viz. *D. gangeticum*, *D. triflorum* and *D. velutinum* (Willd.) DC. and compared with commercial samples of various origin. Among these *D. triflorum* appears closer to *D. gangeticum* reflecting narrow genetic diversity. However, the present study shows that *D. triflorum* and *D. gangeticum* are distantly related. Very recently, Malgaonkar *et al*. (2016) determined the genetic relatedness and diversity among accessions of four *Desmodium* species using RAPD markers, namely *D. dichotomum* (Willd.) DC., *D. laxiflorum* DC., *D. scorpiurus* (SW.) Poir. and *D. triflorum*. A close affinity has been observed between *D. laxiflorum* and *D. scorpiurus*. In the present study *D. pulchellum* has been found close to *D. gangeticum* and *D. motorium* indicating that these three species are closely related. *D. triflorum* and *D. heterophyllum* are closely allied as evidenced by anatomical study (Data not shown), however, this affinity is not supported by RAPD analysis. In order to have better understanding about genetic relatedness and interspecific relationships inclusion of more taxa with additional markers is necessary.

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