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Full Length Research Paper

Estimation of genetic variability in *Trigonella* cultivars by RAPD analysis

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Trigonella **is receiving great importance due to its rear medicinal properties. Gene banks harbor scanty germplasm. The neglected use of this important herb had received less attention due to which very little information is available about its genetic diversity. As such, an attempt was made to study the genetic diversity of this important plant. For the present study, five cultivars of** ***Trigonella*** **were analyzed to estimate genetic diversity by using eleven random primers. A total of 80 bands were detected, 66 of which were polymorphic with a polymorphism of 82.50%. The amplified DNA fragments ranged in size from 200-3060 bp. Jaccard's similarity coefficient ranged from 0.266 to 0.615. A dendrogram constructed based on UPGMA revealed two clusters. Cluster 1 consists of three accessions - T3, T1 and T2, while cluster 2 consists of two accessions - T4 and T5. It is evident from the dendrogram that accession T3 and T5 are genetically diverse; hence it is recommended that these should be used for future breeding programs to create higher amount of genetic variability in** ***Trigonella*****.**

Key words: *Trigonella*, genetic diversity, polymorphism, dendrogram, monomorphic.

INTRODUCTION

Genetic diversity is at the lowest hierarchy, without genetic diversity, a population cannot evolve and adapt to environmental changes. The genetic diversity has an impact on the higher levels of biodiversity. Analysis of genetic structure at intra specific level of medicinal plant species is important to the development of conservation strategies, exploration of plant genetic resources and future breeding programs of wild plants. Estimation of genetic relationships among genotypes is helpful in selecting parental combinations for hybrid breeding to gain desirable traits (Becelaere et al., 2005). Genetic diversity refers to any variation in the nucleotides, genes, chromosomes or whole genomes of organisms. Genetic diversity at its most elementary level is represented by differences in the sequences of nucleotides that form the DNA within the cells of the organism (Andayani et al., 2001). Information on genetic diversity and relationships among and between individuals, populations, plant varieties, animal breeds and species are of importance to plant and animal breeders for the improvement of crop plants and animal breeds for conservation biology and for studying the evolutionary ecology of populations. Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification (Duran et al., 2009).

Molecular tools provide valuable data on diversity through their ability to detect variation at the DNA level. In population studies, molecular tools are being used to identify whether two individuals are from the mating of specific parents, estimating the degree of relatedness among individuals, determination of social behavior, reproductive success and mating choice and it allows direct access to the hereditary material (Paterson et al.,

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1991). These have been used to monitor DNA sequence variation in and among the plant species (Wang et al., 2008) and are best suited for detecting genetic diversity within populations (Maria et al., 2005). Large scale characterization of plant species in varying geoclimatic conditions can be performed using various parameters such as seed morphometric traits and isozymes. However, environmental factors as well as the developmental stage of the plant influence such traits. DNA based markers provide more detailed genetic information to either the increased variability of loci or the greater number of the available loci (Cruzan, 1998; Ashley and Dow, 1994). These markers have successfully been used to estimate levels of relatedness among the individuals, studies of mating systems, and successful establishment in natural population. With the advent of plant molecular biology, a number of molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism (AFLP) have been developed and used extensively in studying genetic diversity, genetic relationship and germplasm management. Recent developments in DNA markers have made it possible to uncover a large number of genetic polymorphisms at DNA sequence level, and to use them as markers for the evaluation of genotypes for the observed phenotypic variability. RAPD - PCR reaction is a means of detecting polymorphisms for genetic mapping and strain identification. It is simple, cost-effective and a powerful tool in the analysis of plant genome characterization. Although, RAPD is criticized for its low reproducibility (Virk et al., 2000), it is overcome by optimization of the RAPD reaction and maintenance of stringent conditions. RAPD has, therefore, been extensively used in assessing genetic diversity and relationship measures in various plant species (Bauvet et al., 2004; Upadhyay et al., 2004).

Fenugreek is gaining importance due to its rare medicinal properties (Sharma, 1990). Among *Trigonella* species, *Trigonella foenum graecum* (commonly known as fenugreek) is a flowering annual, with autogamous white flowers occasionally visited by insects indigenous to countries on the eastern shores of Mediterranean, Irano-Turanian and Europe-Siberian phytogeographical regions. Fenugreek is widely cultivated in India, Egypt, Ethiopia, and Morocco and occasionally in England (Polhil and Raven, 1981). Fenugreek is reported to have anti-diabetic, anti-fertility, anticancer, anti-microbial, anti-parasitic and hypocholesterolaemic effects (Al-Habori and Raman, 2002). In India, fenugreek is used as a lactation stimulant (Tiran, 2003). Fenugreek seed in powder or germinated form exhibits anti-diabetic properties (Broca et al., 2004; Devi et al., 2003; Hannan et al., 2003; Tahiliani and Kar, 2003b; Thakaran et al., 2003; Vats et al., 2003). Across the world, only known and well-defined cultivars are grown in specific areas. Gene banks also harbor scanty germplasm collection of *Trigonella* species (Hymowitz, 1990). The neglected and the underuse status of these locally important crops indicates a risk of disappearance of important plant material developed over thousands of years of cultivation. One of the important factors restricting their large-scale production and development of better varieties is that very little information is available about their genetic diversity, inter and intra-specific variability and genetic relationship among these species. Therefore, attempts to analyze possible untapped genetic diversity become extremely essential for breeding and crop improvement. Random Amplified Polymorphic DNA (Williams et al., 1990) technology is a reliable method for characterizing variation among species and among populations (Gustine and Huff, 1999). RAPD profile construction has several advantages, such as rapidity of process, low cost and the use of small amounts of plant material (Jain et al., 1994; He et al., 1995; Lopez-Brana et al., 1996).

MATERIALS AND METHODS

The present study was carried out on *Trigonella foenum graecum* belonging to family fabaceae used both as herb (the leaves) and as a spice (the seed, often called *methi*). The plant is cultivated worldwide as a semi-arid crop and is a common ingredient in many curries. The cuboid yellow to amber colored fenugreek seeds are frequently used in the preparation of pickles, curry powders, and pastes, and the spice is often encountered in the cuisine of the Indian subcontinent. The dried leaves - also called *kasuri methi* (or *kasoori methi* in India), after the region of Kasur in Punjab, Pakistan province, where it grows abundantly - have a bitter taste and a characteristically strong smell. When harvested as microgreens, it is also known as Samudra Methi, in Maharashtra, especially in and around Mumbai, where it is often grown near the sea in the sandy tracts, hence the name (Samudra means “ocean” in Sanskrit). Fenugreek is used in Eritrean and Ethiopian cuisine. The word for fenugreek in Amharic is *abesh* (or *abish*), and the seed is used in Ethiopia as a natural herbal medicine in the treatment of diabetes.

The seeds of five accessions (IC 332236; IC 144225; IC 143851; IC 371755; IC 433589) were collected from National Beareu of Plant Genetic Resource, New Delhi to study the genetic variation based on RAPD. The experiments were conducted at Indian Grassland and Fodder Research Institute (IGFRI) Jhansi (U.P.).

DNA isolation and RAPD

Genomic DNA was isolated from the mature seeds of five accessions of *T. foenum-greacum* crushed with pestle and mortar in liquid nitrogen using the procedure

Table 1. Number of bands scored in *Trigonella* lines with different RAPD primers.

S/N	Primer	Primer Sequence	Total no. of bands	Polymorphic bands	% polymorphism	Fragment length (bp)
1	OPA 01	5‘ ACCTCAGCTC 3‘	10	8	80	3060- 900
2	OPA 07	5‘ GTGGTCCGCA 3‘	12	8	66.66	2880- 300
3	OPA 10	5‘ AACGCGTCGG 3‘	9	9	100	2760- 600
4	OPA 13	5‘ GGAGTGCCTC 3‘	4	4	100	1800-400
5	OPA 16	5‘ AAAGCTGCGC 3‘	7	7	100	2550-500
6	OPC 09	5‘ TTCCCCCCAG 3‘	6	5	83.33	2800-700
7	OPC 17	5‘ TGTCTGGGTG 3‘	6	6	100	2000-500
8	OPC 19	5‘ CAGGCCCTTC 3‘	8	6	75	1500-300
9	OPE 03	5‘ ACCCCCGAAG 3‘	6	6	100	1500-200
10	OPP 02	5‘ TCGGCACGCA 3‘	9	4	44.44	2400-200
11	OPE 08	5‘AGCCAGCGAA 3‘	3	3	100	2000-500
			80	66		Total

developed by Kang et al. (1998) with slight modification. RAPD analysis was performed using arbitary decamer primers procured from operon technologies. Initially, 25 decamer primers (from Operon technology) were screened against fresh genomic DNA of *Trigonella* accessions under study. Eleven out of the twenty five primers (Table 1) that produced reproducible and scorable amplifications were finally selected for the RAPD profiling. All the accessions under study were screened with each of the 11 random primers to generate banding profiles for the genetic diversity study. After quantification, DNA samples were diluted to working concentration of 10 ng/µl. Amplification was carried out in 25 µl reaction mixture containing about 25 ng genomic DNA, 0.2 mM of each dNTP, 0.2 µM primer, 2 mM of $MgCL_2$ and I U of Taq DNA polymerase (Fermentas, Life Sciences). DNA amplification was performed in a PTC 200 thermal cycler (MJ Research) according to the following thermal profile: initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 35°C for 1.5 min and extension at 72°C for 1.5 min, followed by final extension at 72°C for 3 min. Amplification products were separated in 1.5-2% agarose gel (Sigma) and detected by staining with ethidium bromide Sambrook et al. (1998). The gels were photographed under UV light.

Data analysis

The amplified fragment profiles were visually scored for the presence (1) or absence (0) of bands and entered into binary matrix. The variation in intensity was not taken into consideration to avoid confusion in scoring. Jaccard’s similarity coefficients were calculated and used to construct dendrogram based on unweighed pair group method with arithmetic averages (UPGMA). The computer package NTSYS-pc version 2.2 e (Applied Biostatistics, Inc.) was used for carrying out cluster analysis (Rohlf, 2004).

RESULTS AND DISCUSSION

The polymorphic bands and level of polymorphism of distinguishable RAPD bands in different cultivars of *Trigonella* are shown in Table 1. The amplification profiles were in the form of 80 bands out of which 66 were polymorphic with a polymorphism of about 82.50% .The amplified DNA fragments varied in size from 200-3060 bp (Table 1, Figure 1). The number of bands amplified from different accessions varied from 3-12 bands using all the 11 decamer primers with an average of 7.27 bands amplified per primer. Pairwise comparisons between the tested genotypes were used to calculate the genetic similarity (Table 2).

The similarity index (Table 2) revealed the maximum similarity between accessions IC-144225 (A1) with IC-144225 (A2) and IC-371755 (A4) with IC-433589 (A5), while distantly related varieties were IC-332236 (A3) and IC-433589 (A5).

The Bivariate 1-0 data matrix generated dendrogram shows that the genotypes analysed on DNA basis belong to two clusters (Figure 2). Cluster I includes two accessions IC-144225(A1) and IC-144225(A2). The major cluster-II included IC-371755(A4) and IC-433589 (A5). The accession IC-332236 (A3) was placed distantly from the studied accessions as revealed by the dendrogram constructed.

The results obtained during the present investigation seems to be consistent with the studies of Dangi et al. (2004) who reported 70.12% polymorphism in the case of *Trigonella foenum-greacum* and 94.83% polymorphism in *Trigonella caerulea* using 40 and 10 decamer primers,

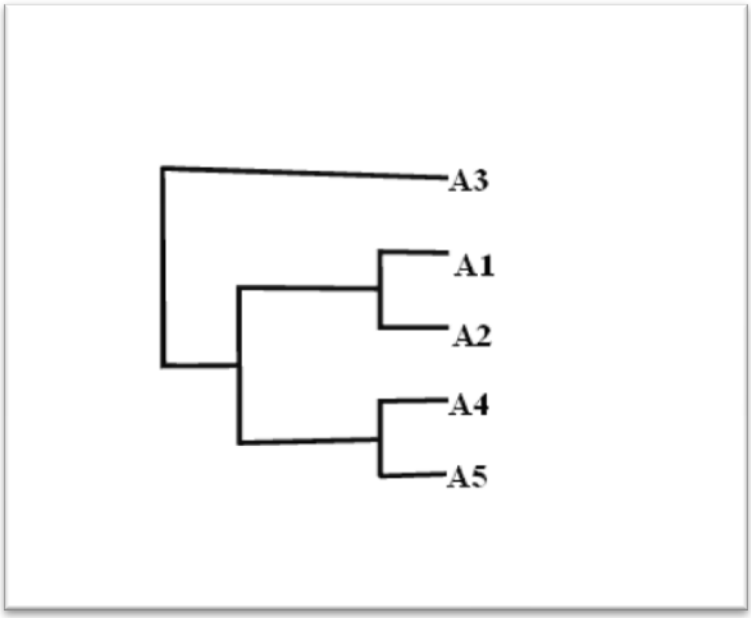

Figure 1. Dendrogram showing the result of clustering on five *Trigonella* accessions.

Table 2. Jaccard's similarity coefficient.

	A1	A2	A3	A4	A5
A1					
A2		0.54545			
A3		0.26667	0.50000		
A4		0.42857	0.46154	0.40000	
A5		0.35714	0.50000	0.42857	0.61538

while 64% polymorphism was reported by Kakani et al. (2011) in the case of *Trigonella* by using 10 decamer primers. Moreover different workers worked on different plant species and reported almost similar pattern of polymorphism. For example Rao et al. (2006) reported 77.8% polymorphism in chick pea by using 10 decamer primers. On the other hand, 78.8% polymorphism was reported by Thomas et al. (2006) in wheat upon utilizing 50 random decamer primers. Patra and Chawla (2010) also found 76.5% polymorphism in eighteen Basmati rice varieties by utilizing 12 random primers. Malviya and Yadav (2010) also observed the same extent of polymorphism (74.7%) in pigeon pea by using 17 random decamer primers. Reby et al. (2011) also observed 72.27% polymorphism in rice genotypes from Kerala by using twenty 10-mer random primers. The detection of high level genetic diversity observed in *Trigonella* genotypes confirms the findings of Welsh and McClelland (1990) and Dos Santos et al. (1994) who demonstrated that RAPD markers are effective for visualizing high level of polymorphism in plant species. The high level of polymorphic products generated by certain products might be attributed to the fact that in RAPD, even small divergence between two cultivars can result in distinct pattern as polymorphism may be the result of any of the various reasons: a) Single nucleotide change within the primer binding site. b) Insertion or deletion with the amplified region so that part of the primer binding site in one of the strand is missing. 3) Complete absence of complimentary sites. 4) The region between the binding sites on the opposite strand is beyond the normal amplifiable length.

RAPD similarity coefficients ranged from 0.26 to 0.61 with 78.79% polymorphism. Similar results were obtained by Muhammad et al. (2010) in wheat. RAPD marker system may amplify similar size fragments from different genomic regions, resulting in underestimation of genetic diversity. Molecular markers such as RAPD and seed storage protein analysis and subsequent banding pattern should be included in the testing of advanced breeding lines. This will not only help in the development of varieties with wider genetic base, but will also generate fingerprints of such varieties.

Similar works have been carried out by many researchers both in India and elsewhere. Rout and Das (2002) studied the genetic integrity of *Plumbago zeylanica* using RAPD markers. Gilani et al. (2009) reported the genetic diversity of 7 populations of *Withania coagulans* from the districts of Kohat and Karak in

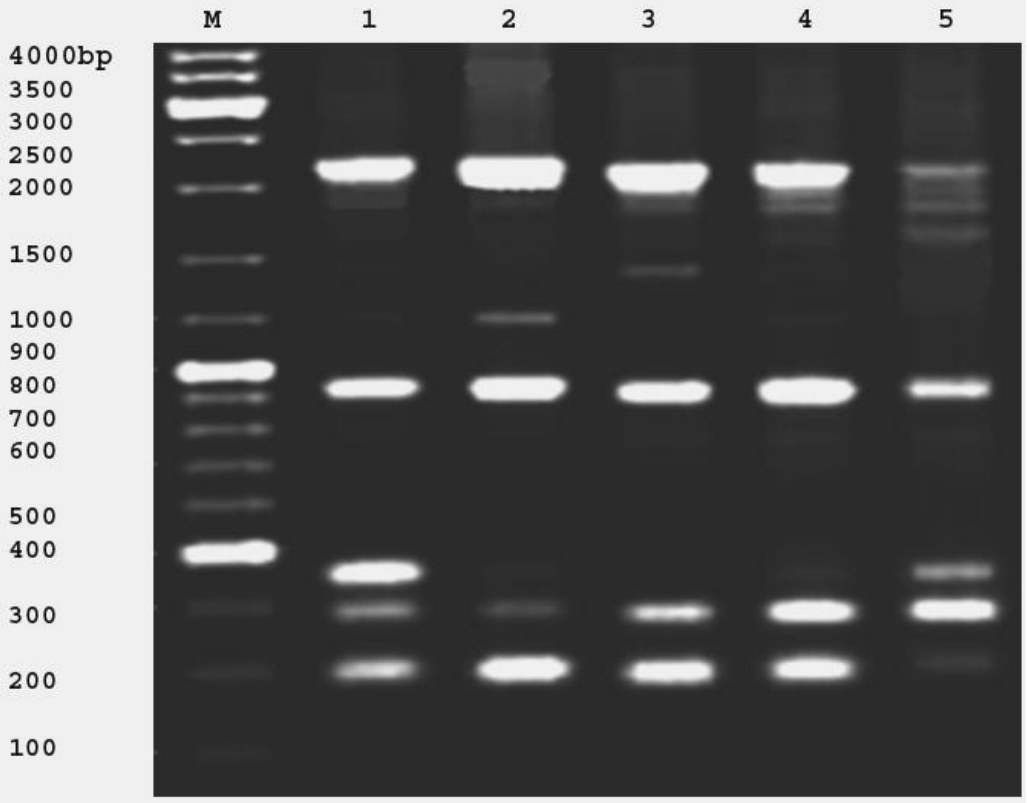

Figure 2. RAPD profiles from genomic DNA of five *Trigonella* accessions using primer opp-02 (5' TCG GCA CGC A 3').

Pakistan using molecular markers. This finding showed higher diversity within population and lower diversity among population. Bilal et al. (2010) analyzed the intraspecific variation of *Withania somnifera* using molecular markers. Analysis of the genetic diversity provided evidence that wild and cultivated genotypes are not different only in morphological and chemical basis. This type of investigation gives information regarding genetic relatedness within species for conservation, and an understanding of the level and partitioning of genetic variation within the species would provide an important input to determine the appropriate management strategies. Smita and Keshavachandran (2006) characterized 18 phenotypically and biochemically distinct *Gymnema sylvestre* accessions representing different geographical regions of Kerala using RAPD markers. Overall, molecular fingerprinting revealed the existence of considerable genetic variations. Padmalatha and Prasad (2006) studied the molecular variations in *Rauvolfia tetraphylla* collected from different locations of Andhra Pradesh, India. Cluster analysis indicated the high levels of differentiation among accessions which existed independent of geographical distance. Vivek et al. (2009) investigated the genetic relationships among 19 populations of *Senna tora* and they reported that such genetic studies in genetic relationships may be useful to assign new and unclassified accessions of specific taxonomic groups and to reclassify species. According to Jayaram and Prasad (2008) and Chengxin et al. (2003), the study of distributive patterns of genetic variation would provide baseline data for conservation and collection strategies of the species. Artyukova et al. (2004) reported that the mean genetic heterozygosity may be helpful in estimating gene diversity of the populations of rare and endangered species. Rashmi et al. (2004) suggested that an understanding of a level and partitioning of genetic variability within the species would provide an important input to determine the appropriate management strategies.

Conservation of genetic diversity is an essential aspect of the management of threatened and endangered species. Genetic diversity is vital for population viability. In the short term, low levels of diversity can result in inbreeding depression, increasing the probability of

population extirpation or reducing population fitness (Saccheri et al., 1998; Puurtinen et al., 2004).

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