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Genetic Variations in *Coriandrum sativum* L. Varieties Detected By Random Amplified Polymorphic DNA Markers

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Abstract

Attempt is made to assess genetic divergence among 10 varieties of coriander (UD-29, 182, 262, 358, RCr-20, 41, 435, 436, 480, 684), 30 Random amplified polymorphic DNA markers were used and only 8 amplified. A total of 74 clear, consistent and strong bands were generated, out of which 43.2% were polymorphic. The total number of markers varied from 6 to 13 with a mean of 9.25 markers per primer. The number of polymorphic markers for each primer varied from 1 to 11 with a mean of 4 polymorphic markers per primer. Based on the UPGMA clustering method, a dendrogram was constructed which revealed two major clusters. Group-A consisted of one genotype 2, whereas Group-B could be further classified into five subgroups comprising remaining genotypes. Current study highlights meager genetic diversity among various varieties and is attributed to recent human selection pressure pertinent to few genes. Most recent introduction and secondary status of the crop appear to contribute to low genetic variability.

Keywords : RAPD, *Coriandrum sativum*, varieties, Jaccard, Genetic variations

Introduction

Coriandrum sativum (Fam.: Apiaceae) is an annual spice herb (Mengesha and Alemaw 2010). It is a diploid cross pollinated crop. The plant is a native to Mediterranean and near eastern region (Bhandsari and Gupta 1991) and is broadly cultivated in North Africa, Europe, India, China and Thailand. Spice is also employed in preparation of steam distilled essential oil and solvent extracted oleoresin for the aroma and flavor industries (Lopez et al. 2008).

Knowledge of germplasm diversity is valuable in crop improvement since it generates baseline data to guide selection of parental lines and designing of a breeding program. It is possible to screen desired genotypes for any trait of economic interest. With the innovations in biotechnology it is now possible to analyze large number of loci distributed throughout the genome of a plant. Among the numerous techniques available for assessing genetic variation and relationships among crop germplasm DNA based markers have proven to be very valuable and reliable. RAPD technique has been used for studying genetic variability and gene tagging in various plant species. The advantages of using RAPDs in genetic analysis are: it is sequence independent, easy, fast, cost-effective, and requires small amount of DNA (Haque et al. 2007). In the available studies some authors have determined variability in different coriander germplasm using AFLP markers (Lopez et al. 2008) and statistical tools to analyze 15 morphological and agronomic characters (Mengesha and Alemaw, 2010).

The present study was undertaken to capture the potential genetic diversity and genetic distance among 10 different indigenous *C. sativum* varieties, using RAPD marker which could facilitate the breeder for introducing agronomical important traits.

Materials and Methods

Plant material

Ten different genotypes of *C. sativum* were obtained from the Department of Plant Breeding & Genetics, SKN College of Agriculture, Jobner, Rajasthan Agricultural University, Bikaner.

DNA extraction

Total genomic DNA was extracted from 20 mg of powdered material using a common CTAB procedure (Doyle and Doyle 1990) modified by adding 2% 2-mercaptoethanol (v/v) to the extraction buffer. Extracted DNA samples were run on 0.8% agarose gel followed by quantification for confirmation of its good quality. The DNA samples were diluted to 25 ng/ìl and kept at -20°C until use.

RAPD analysis

RAPD/PCR reactions were initiated and optimized using an Applied Biometra thermal cycler

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programmed to repeat the thermal profile. Setting of the PCR program was based on three steps. Step one was initial denaturation at 94°C for 5 min. Step two comprised running for 45 cycles, each starting with denaturation at 94°C for 1 min., followed by annealing 42°C for 1 min. and ended by extension at 72°C for 1 min. Step three was a final extension cycle performed at 72°C for 7 min. The PCR machine was adjusted to hold the product at 4ºC. The product was mixed with 6µl of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol & 40% sucrose w/v) and spun briefly in a microcentrifuge before loading. The PCR products were electrophoresed using 1% agarose gel at 80 volts and followed by staining with ethidium bromide then separated fragments were visualized with a UV transilluminator (Biometra gel documentation system).

Data analysis

Amplification products were scored for the presence (1) and absence (0) of bands across the genotypes in order to obtain a binary data matrix. Genetic similarities for RAPD data were calculated using Jaccard's similarity coefficient (Jaccard 1908). Dendrogram showing genetic relationship among the ten varieties was constructed using Unweighted Pair Group Method with Arithmetic average (UPGMA). Data analysis was performed by software NTSYS-pc version 2.11.

Results

Level of polymorphism

A set of 30 random primers were tested out of which 8 showed amplification with ten individual DNA samples producing a total of 74 loci. The number of bands obtained for each primer ranged from six (GCC-134) to thirteen (GCC-81) with a mean of 9.25 bands per primer. The monomorphic bands were excluded from analysis and a total of 32 polymorphic bands were finally deciphered. Each primer produced 1 to 11 such bands with a mean value of 4 polymorphic bands per primer. The percentage of polymorophic bands averaged 43.24%. Highest number of polymorphic loci was 11 obtained with primer GCC 81 (84.6%) and lowest was 1 with GCC 73 (12.5%). The discrimination power of each locus was estimated by the PIC (Polymorphism Information Content) value. It ranged from 0.05 to 0.22 with a mean of 0.12.

Genetic similarity matrix and cluster analysis

Thus a dendrogram based on UPGMA analysis of the genetic similarity matrix was constructed. All the 10 varieties were clustered into two major groups. Group-A consisted of one genotype 2, whereas Group-B could be further classified into five subgroups, subgroup 1st (genotype 1), subgroup 2nd (genotype 3), subgroup 3rd (genotype 4 & 5), subgroup 4th (genotype 6, 7 & 8), subgroup 5th (genotype 9 & 10). In general varieties with similarity were grouped together. Similarity coefficients for all the 10 varieties ranged from 0.70 to 0.95 with a mean of 0.81 (Average genetic similarity). The smallest value of 0.70 was observed between variety 2 and 3 and the maximum value of 0.95 was observed between 6 and 7.

Discussion

RAPD technique is a well proven tool which is commonly employed in genetic studies. It is a convenient method to detect total genetic variation within and among populations. The RAPD process has been effectively used in several taxonomic and genetic diversity studies (Ranade et al. 2008, Sahasrabudhe and Deodhar 2010). The ease of employing this technique has facilitated its use in the analysis of genetic relationship in several instances (Malviya and Yadav 2010, Gupta et al. 2010).The major concern regarding RAPD generated phylogeny includes homology of bands showing the identical rate of migration, causes of variation in fragment mobility and origin of sequence in the genome. Despite these limitations, RAPD marker has distinct advantage in its ability to scan across all regions of the genome, hence highly pertinent for phylogenetic studies at species level (Nair et al. 1999). In the current investigation, the marker technology was employed to detect genetic variation within *C. sativum* varieties. *C. sativum* showed a high percentage of genetic polymorphism of 43.24%, which is higher than that of *Dacydium pierrei* (33.3%) (Su et al. 1999) and *Cathaya argrophylla* (32%) (Wang et al. 1996). Similarly, the genetic diversity index was also highly variable from 0.05 to 0.22 in case of *C.sativum* varieties.

The discrimination power of each locus was estimated by the PIC (Polymorphism Information Content) value which is an estimate of the polymorphism for a marker locus (Dubreuil et al. 2003). Variation for PIC value was observed within populations and the mean for them was 0.21 with a range of from 0.05 to 0.22. Interestingly, at least 50% of the populations had PIC values below 0.15, suggesting that some coriander varieties may be homogenous, either because of their breeding

history or because of small sample sizes during acquiring or regeneration.

It may be added that the feasibility of naturally occurring genetic cross and gene flow should be extremely low among varieties growing far from each other. Present study highlights that the high genetic diversity among varieties could be attributed to artificial selection, and not natural genetic differentiation.

The level and distribution of genetic diversity detected by RAPD are in overall agreement with recent studies in India (Malviya and Yadav, 2010, Sujatha et al. 2010). RAPD, being a multi-locus marker with the simplest and fastest technique, has been successfully employed for the determination of intra-specific genetic diversity in several plant species (Gupta et al. 2010). In *C. sativum* two samples did not group with any other variety in dendrogram indicating its genetic distinctness from other varieties in our study.

Lopez et al. (2008) failed to detect any parallelism between phenotypic and genetic traits. Multiplexing RAPD reactions are suitable to characterize coriander varieties. Phenotypic and genetic classifications in coriander did not match since coriander is a relatively young crop species. Since *Coriandrum* is a secondary crop, it has retained many characteristics of original plants, e.g. indeterminate flowering, differential seed maturity, fruit shattering, and uneven seed germination. The impact of human selection, under various environmental conditions, is discernable phenotypically but selection possibly concerned relatively few genes for very short period limiting completion of the genetic assimilation process. Another possibility of mismatch between phenotypic and genetic classifications is the wide exchange of coriander fruit as a spice around the world and the relatively recent introduction of this species into many countries. Thus a fairly common genetic background is maintained among populations. In these ways, no clear differentiation was observed among coriander populations at the molecular level, even when a clear differentiation was observed at the phenotypic level.

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