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Full Length Research Paper

Characterization of coriander (*Coriandrum sativum* L.) varieties using SDS-PAGE and RAPD markers

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In an experiment, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA (RAPD) were evaluated separately as a tool for characterizing coriander varieties. The SDS-PAGE electrophoresis of total soluble seed proteins revealed a total of 7 bands with Rm value ranging from 0.347 to 0.926. A polymorphism of 71.4% was recorded among 20 varieties of coriander. The maximum dissimilarity value was shown by Sindhu. Varieties like Sadhana, Sindhu, RCr-436 and RCr-684 were observed different from the rest of the coriander varieties whereas, RCr-436 and RCr-684 could not be differentiated from each other. Twenty random primers were used for polymorphism studies based on DNA fingerprinting analysis of eighteen coriander varieties. These primers gave amplification and a total of 9 bands were produced with an average of 4.5 bands per primer. A total of 73 polymorphic bands and 17 monomorphic bands were observed thus, showing 81.1% polymorphism among all the varieties. The highest number of bands was generated by the primer OPC-3 and OPC-6 followed by OPC-2, OPC-7 and OPC-20 while the lowest number of band was produced by OPC-19. The variety GC-2 is highly diverse from other varieties. The dendrogram based on protein electrophoresis grouped the varieties into two major clusters whereas RAPD analysis showed clear-cut difference among coriander varieties. Both techniques grouped coriander varieties like Swathi, CS-6, H.Surbhi and RCr-446 in one cluster.

Key words: *Coriandrum sativum* L., SDS-polyacrylamide gel electrophoresis, RAPD, varietal characterization.

INTRODUCTION

Coriander (*Coriandrum sativum* L) is an annual herb (2n=22), which belongs to the family Apiaceae and generally grown in winter season as main crop in India. It shows broad adaptation as crop, growing well under many different types of soil and weather conditions (Simon, 1990), even at extreme latitudes and elevations. All parts of the plant are edible, but the fresh leaves and dried seeds are most commonly used in cooking. The dried seeds are mainly used either whole or in ground form as spice for adding taste and flavour in different food stuffs whereas, green leaves are sprinkled to garnish a variety of dishes. The essential oil extracted from seed is included among the twenty major essential oils in the world market (Lawrence, 1992) and its commercial value depends on its physical properties, chemical composition and aroma (Smallfield et al., 2001). The essential oil content of the dried seeds varies from 0.03% to 2.7% (Purseglove et al., 1981; Bandara et al., 2000). Linalool is the main constituent of essential oil which constitutes more than 50% of the total essential oil.

The importance of cultivar/variety identification was recognized long back with the increased number of

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Table 1. List of coriander varieties and their source.

Name of varieties	Source	Maturity
Gujarat Coriander-1	Spices Research Station, Jagudan, (Gujarat)	Early
Gujarat Coriander-2	-do-	Early
Jawahar Dhaniya-1	JNKV, Jabalpur (MP)	Medium
CS-6	-do-	Early
NRCSSACr-1	NRCSS, Ajmer (Rajasthan)	Late
Sindhu	Guntur (AP)	Early
Sudha	-do-	Early
Sadhana	-do-	Early
Swathi	-do-	Early
Hisar Anand	CCS HAU, Hisar (Haryana)	Medium
Hisar Bhoomit	-do-	Late
Hisar Surbhi	-do-	Medium
Hisar Sugandh	-do-	Medium
RCr-20	SKN College, Jobner (RAU), Rajasthan	Early
RCr-41	-do-	Late
RCr-435	-do-	Early
RCr-436	-do-	Early
RCr-446	-do-	Medium
RCr-480	-do-	Medium
RCr-684	-do-	Medium

varieties, which were expected to further increase in future due to ever changing objectives to meet the current and the future demands of the producers and consumers. The basic objective of varietal identification is to confirm the occurrence of traits that helps in identifying a particular variety when grown in different environmental conditions and generations (Flenner and Smith, 1983).

Evaluation of phenotype based morphological characters is traditional method of characterization, which is sometimes too limited and inadequate to allow for variety discrimination. In coriander, most investigations of variation within and among coriander population have been based on morphological traits although, many of those traits are environmentally influenced and genotype × environment interactions is common (Bhandari and Gupta, 1991; Angelini et al., 1997 and Ali et al., 1999).

In this respect, other procedures have been proposed in addition to the morphological approach like protein electrophoresis and Random Amplified Polymorphic DNA (RAPD) which can be very useful in variety identification, particularly when low morphological variability is present in the cultivated types of crop. Molecular markers possess ideal characteristics since they analyze genetic diversity at the DNA level, which is not affected by environmental variations.

Among the several classes of molecular markers, RAPD is very interesting due to their time and cost effectiveness and because they are easy to perform and comparatively less expensive and do not require radioactive isotopes. Considering these aspects, the present study was conducted with the objectives to evaluate the potential of protein electrophoresis and RAPD markers for varietal identification in coriander and to study the relationship among various genotypes.

MATERIALS AND METHODS

Experimental materials

The material used in present investigation was obtained from various research centers including Department of Vegetable Science, CCS Haryana Agricultural University, Hisar. The detail list is given in Table 1.

SDS-PAGE electrophoresis of proteins

Sound seeds from each variety were taken separately and ground in a mortar and pestle after removing the seed coat and defatted by a mixture of Choloroform, Methanol and Acetone (2 :1 :1). One hundred milli gram of defatted seed powder was taken in eppendorf tube and 0.5 ml of 2X sample buffer [1M Tris HCl (pH 6.8) 2.5ml, SDS 0.4 g, β-mercaptoethanol 1.0 ml, glycerol (20%) 2.0 ml and bromophenol blue (1%) 0.4 ml added to distilled water to make final volume 10.0 ml] was added to it. The contents were thoroughly mixed and kept overnight in the refrigerator. The tubes were taken out and contents were mixed properly and then it was subjected to

centrifugation at 10 000 rpm for 10 min. The supernatant was taken into separate glass tubes. These samples were then boiled in water bath for 10 min, cooled and finally used as protein source for electrophoresis. After the completion of electrophoresis, the gel was removed from the glass plates, transferred to 15% tri-chloro acetic acid (TCA) solution and after 15 min, again transferred to staining solution (100 ml of 15% TCA solution, 15 ml of 1% CBBR) for overnight. Excess stain was removed by destaining solution (Methanol, acetic acid and water mixed in ratio of 50:70:880 (v/v/v), respectively). Gels were photographed and stored in ten per cent acetic acid solution. The relative mobility (Rm) of each band in the protein gel was calculated by the following formula:

$$Rm = \frac{\text{Distance travelled by the protein band}}{\text{Distance travelled by the tracking dye}}$$

The protein bands were numbered based on increasing Rm values.

DNA isolation

Total genomic DNA was isolated from young leaves of coriander varieties following Cetyl Trimethyl Ammonium Bromide (CTAB) method of Saghai-Maroof et al., (1984) with minor modifications. The ground leaves sample was incubated in water bath at 65°C for 45 min. After purification, DNA pellet was dissolved in appropriate volume of TE buffer and stored at -20°C until use. The quality and quantity of DNA was estimated by using spectrophotometer and also by running the genomic DNA on agarose gel (0.8%) along with undigested λ DNA.

PCR amplification and RAPD analysis

A total of twenty 10-base random oligo-nucleotide primers were used to find out polymorphism among the eighteen varieties of coriander. The Polymerase Chain Reaction (PCR) was carried out in 20 µL of reaction mixture containing 20 ng of template DNA, 1X PCR buffer, 2.0 mM $MgCl_2$, 200 µM dNTP mix, 0.7 µM of primer and 1.0 Unit of Taq DNA polymerase (Fermentas, USA). Amplification was carried out in Eppendorf Thermo Cycler. The PCR conditions for RAPD analysis included an initial denaturation step of 3 min at 94°C and following 40 cycles. Each cycle was programmed for 94°C for 1 min (Denaturation), 36°C for 1 min (Annealing) and 72°C for 1 min (Extension) with a final extension step at 72°C for 5 min. The electrophoresis was carried out at a constant voltage until the dye moved three quarter of the gel. The amplified products were visualized in a 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide and documented by a gel documentation system (BIORAD, USA). Molecular size of different fragments was determined by using 100 base pair gene ruler (Fermentas, USA). The reproducible banding patterns from RAPD analysis for each primer were scored by visual observation. Polymorphic and monomorphic bands were recorded in each amplification and percent polymorphism was calculated for each primer.

Data analysis

Protein and RAPD electrophoresis from coriander varieties were scored as absent (-) or present (+). Squared Euclidean distance was calculated by using SPSS for Windows ver.16 programme. A dendrogram was constructed based on the squared Euclidean distance dissimilarity matrix by the average linkage method.

RESULTS

Protein electrophoresis

The SDS-PAGE electrophoresis on the basis of total soluble seed protein is used for cultivar identification in the present study. The protein profile of twenty coriander varieties resulted into seven protein fragments (Figures 1 and 2). The variety Sindhu show minimum number of protein fragments (3) whereas; maximum number (7) was recorded in RCr-436 and RCr-684. The two bands (3 and 6) with Rm value (0.558 and 0.842), respectively were monomorphic while the other 5 bands gave a total of 78% polymorphism. Dissimilarity index was calculated to have an idea of genetic relationship among the varieties under study and maximum dissimilarity value was observed with variety Sindhu followed by Sadhana, RCr-684 and RCr-436. Rest varieties were found similar to each other. Based on dendrogram analysis, all coriander varieties were grouped into two major cluster including Sindhu and Sadhana in one cluster and rest of the varieties in another cluster. Again the larger cluster divided into two sub clusters including RCr-436 and RCr-684 in one cluster and rest varieties in other cluster (Figure 3).

RAPD analysis

In the present study, twenty random decamer primers obtained from Operon Technologies^{TM}, USA, having 60 per cent or more (G + C) content were used for RAPD analysis of different coriander varieties. The DNA amplification and polymorphism generated among various coriander varieties are presented in Table 2. A total of 90 bands were obtained from twenty random primers and the majority of bands were polymorphic (73 bands, 81.1%) and only 17 bands (18.9%) were monomorphic (Table 2). The maximum polymorphism (100%) was scored with OPC-1, OPC-4, OPC-6, OPC-9 and OPC-20. The highest numbers of bands (8) was generated by primer OPC-3 and OPC-6 followed by OPC-2, OPC-7 and OPC-20, which generated six bands. The band size obtained after amplification by RAPDs varied in size from 250 to 3000 bp (base pair). The numbers of amplified bands ranged from 1 to 8 for various primers with an average of 4.5 bands per primer. The dissimilarity matrix was calculated based on the bands obtained from RAPDs. The highest dissimilarity value was obtained for GC-2 variety. The coriander varieties like H.Surbhi, Swathi, Sadhana and RCr-446 were found closely related. Based on the squared Euclidean dissimilarity matrix, the dendrogram was constructed. The constructed dendrogram grouped the varieties into five clusters showing clear cut divergence (Figure 4).

DISCUSSION

The banding pattern obtained from total seed protein

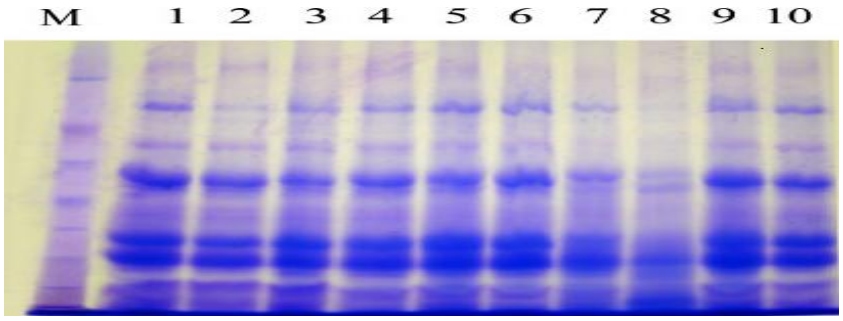

Figure 1. Electrophoretic pattern of total soluble seed protein in different coriander varieties. [Marker (M), GC-1 (1), GC-2 (2), JD-1 (3), CS-6 (4), NRCSSACr-1(5), Swathi (6), Sadhana (7), Sindhu (8), Sudha (9), H.Anand (10)].

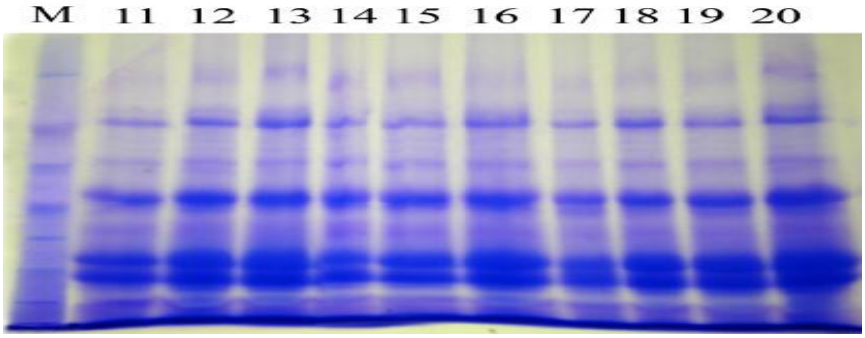

Figure 2. Electrophoretic pattern of total soluble seed protein in different coriander varieties. Marker (M), H.Surbhi (11), H.Sugandh (12), H.Bhoomit (13), RCr-20 (14), RCr-41 (15), RCr-435 (16), RCr-436 (17), RCr-446 (18), RCr-480 (19) and RCr-684 (20).

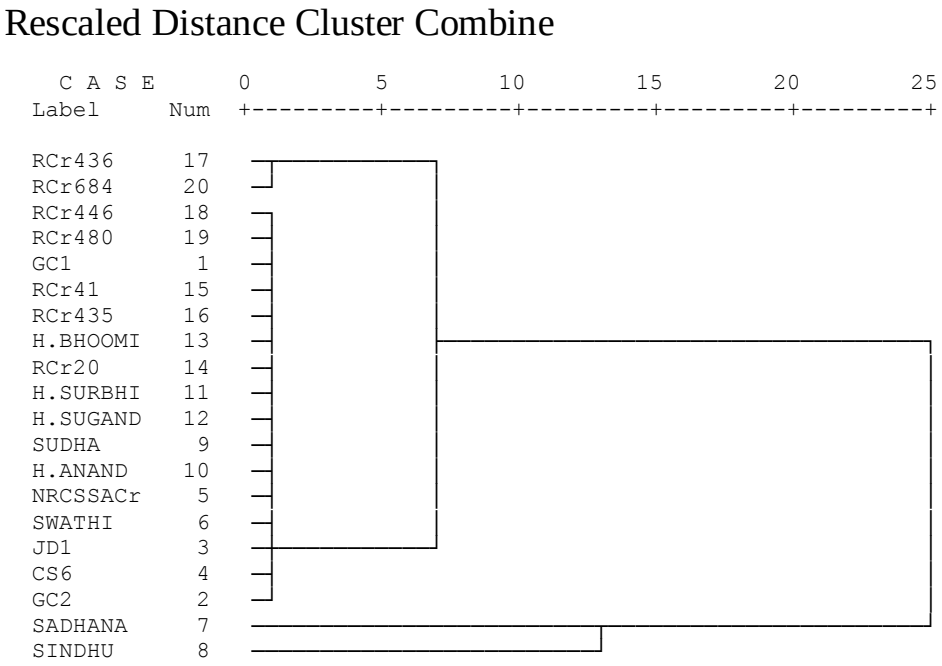

Figure 3. Dendrogram of coriander (*Coriandrum sativum* L.) varieties based on protein electrophoresis.

Table 2. Random primers showing polymorphism among coriander varieties.

Primer	Sequence	No. of varieties amplified	Total bands	Polymorphic bands	Monomorphic bands	Percent polymorphism
OPC-1	TTCGAGCCAG	10	4	4	0	100
OPC-2	GTGAGGCGTC	14	6	5	1	83.3
OPC-3	GGGGGTCTTT	10	8	6	2	75.0
OPC-4	CCGCATCTAC	14	5	5	0	100
OPC-5	GATGACCGCC	5	5	4	1	80.0
OPC-6	GAACGGACTC	10	8	8	0	100
OPC-7	GTCCCGACGA	12	6	5	1	83.3
OPC-8	TGGACCGGTG	12	4	3	1	75.0
OPC-9	CTCACCGTCC	11	5	5	0	100
OPC-10	TGTCTGGGTG	12	4	3	1	75.0
OPC-11	AAAGCTGCGG	10	4	3	1	75.0
OPC-12	TGTCATCCCC	12	3	2	1	66.6
OPC-13	AAGCCTCGTC	11	5	4	1	80.0
OPC-14	TGCGTGCTTG	10	3	1	2	33.3
OPC-15	GACGGATCAG	12	4	3	1	75.0
OPC-16	CACACTCCAG	12	2	1	1	50.0
OPC-17	TTCCCCCCAG	7	4	3	1	75.0
OPC-19	ACTTCGCCAC	11	1	0	1	0.0
OPC-20	GGGTAACGCC	12	6	6	0	100
OPA-9	GGGTAACGCC	15	3	2	1	66.6
Total	-	-	90	73	17	81.1

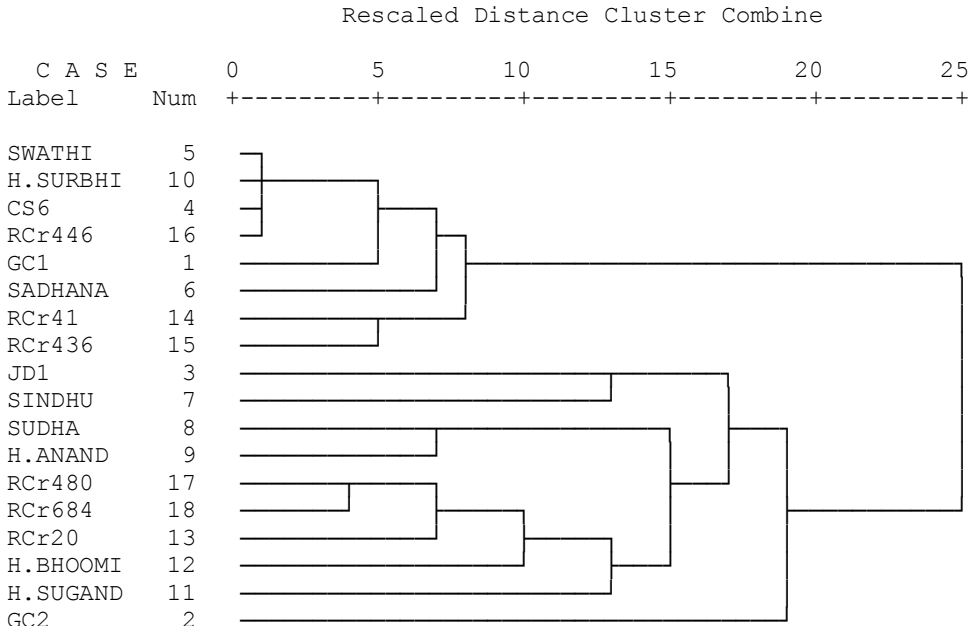

Figure 4. Dendrogram of coriander (*Coriandrum sativum* L.) varieties based on RAPD analysis.

electrophoresis of 20 coriander varieties revealed that a total of 7 bands were present but the number of bands varied in some varieties. The band no. 1 and 5 were present in all varieties except Sindhu, which enabled it to differentiate from rest of the coriander varieties. The band no. 3 and 6 were present in all coriander varieties. Sindhu and Sadhana differentiated from rest of the varieties as both did not show band no. 2 and 7 while these bands

were present in rest of the varieties. The band no. 4 was absent in all varieties except Sadhana, Sindhu, RCr-436 and RCr-684 and could discriminate them from the rest of the varieties based on the banding pattern. Based on the results, seed protein electrophoregrams can be used to discriminate coriander varieties and to identify them by comparing the unknown electrophoregrams of known genotypes in a variety data bank. Therefore, this method can be employed for genotype fingerprinting. Similarly, many workers have also reported polymorphism in total protein of different crops like hybrids of sunflower and their parents (Varier et al., 1992), potato (Mishra et al., 2005) and lentil (Singh et al., 2006).

A total of 90 bands were observed in 18 coriander varieties, which indicate an overall 81.1% polymorphism. The majority of bands were polymorphic (73 bands, 81.1%) and only 17 bands (18.9%) were monomorphic. Out of twenty primers only four primers gave 100 per cent polymorphism. The high level of polymorphism obtained was due to the fact that coriander is often cross pollinated crop. The cross pollination has resulted in the maintenance of high level of genetic variability in the gene pool.

The number of amplified bands obtained for various primers with an average of 4.5 bands per primer ranged from 1 to 8. The size of DNA bands obtained in investigation ranged from 250 to 3000 base pairs. The variation in the number of bands for each primer may be due to the dependence of efficiency of the primer on the primary sites available. It is speculated that the efficiency of the primer on primers amplification varied as a result of the absence of the suitable sites in the template DNA (Devos and Gale, 1992). Therefore, variation in the amplification patterns and polymorphism was observed for different primers. Polymorphism in other crops has also been reported by different researchers. Betal et al. (2004) evaluated 14 cultivars of mungbean using 14 primers of RAPD and obtained 121 bands which were comprised of 76 polymorphic (63.17%) and 45 monomorphic (37.17%) bands. Karuppanapandian et al. (2006) reported that all the 20 primers used to study genetic diversity among mungbean landraces generated polymorphism.

The genetic dissimilarity indices between different cultivars ranged from 0 to 1. The maximum dissimilarity value of 1 was observed between varieties RCr-446 and GC-2. The data on hierarchical cluster analysis showed that the varieties could be divided mainly in five major clusters differentiating Swathi, H. Surbhi, RCr-446 and CS-6 in one cluster, GC-1, Sadhana, RCr-41 and RCr-436 in second cluster, JD-1, Sindhu, Sudha and H. Anand in third cluster and RCr-480, RCr-684, RCr-20, H. Bhoomit, H. Sugandh in fourth cluster and only GC-2 in fifth cluster. Varieties in one cluster were more similar to one another compared to varieties of other clusters. The variety GC-2 was found most diverse than other varieties. The least diverse varieties were Swathi, H. Surbhi, CS-6 and RCr-446, which were grouped into one cluster. The varieties like Swathi, H.Surbhi, CS-6 and RCr-446 were grouped in one cluster in both the techniques which showed reliability of seed protein electrophoresis for varietal identification. The pair wise dissimilarity of 20 coriander varieties revealed the closeness among the different varieties that may be either due to common parentage or accumulation of similar genes from different parents in the development of varieties as well as germplasm lines.

Thus, on the basis of the results of these studies, we can conclude that SDS-PAGE of total soluble seed proteins and RAPD based DNA fingerprinting or polymorphism can differentiate the coriander varieties. This methodology can be used in further genetic diversity studies for genotype identification and characterization which can be helpful in breeding programmes for crop improvement. The polymorphism of the total seed protein can serve as supplementary and RAPD analysis as confirmatory for cultivar identification of coriander genotypes.

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