

GENETIC DIVERGENCE (D^2) AND RELATIVE CONTRIBUTION ANALYSIS IN CORIANDER (*CORIANDRUM SATIVUM* L.) GENOTYPES UNDER DIFFERENT SEASONS IN TAMIL NADU, INDIA

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Abstract. An investigation to study the genetic divergence (D^2) and relative contribution analysis traits was carried out during three seasons viz., season I (June 2023 – August 2023), season II (October 2023 – December 2023) and season III (June 2024 – August 2024) at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. In the present study, Mahalanobis D^2 analysis was applied (i) to assess the genetic divergence among the genotypes (ii) to identify promising genotypes with more divergence to initiate crossing program (iii) to assess the contribution of different characters to genetic diversity. From the study, during first season, cluster I comprising of sixty-one genotypes had the highest intra cluster distance followed by cluster III with five genotypes. In the second season, cluster I comprising of sixty-eight genotypes had the highest intra cluster distance followed by cluster II with three genotypes. During the third season, also cluster I comprising at sixty-four genotypes had the highest intra cluster distance followed by cluster II and IV with three genotypes. During the first season, maximum contribution to genetic divergence was made by fresh weight of root (36.47%). In the second season, maximum contribution to genetic divergence was made by dry weight of stem (23.35%). During the third season, maximum contribution to genetic divergence was made by fresh weight of leaves (47.75%). Also, the mean performance of growth and yield was the highest for cluster X, V and VIII in first, second and third seasons. Keeping this in view, it appears that cluster X, (CS 101), V (CS 101, UD 685) and VIII (CS 101) would exhibit high heterosis as well as high level of production, involved in the hybridization program.

Keywords: coriander, green biomass yield, D^2 analysis, genotype, RAPD marker

Introduction

Coriander (*Coriandrum sativum* L.) is an annual herb, spice plant which belongs to the family Apiaceae. It is native to Mediterranean region, diploid, cross pollinated and medicinal plant (Tulsani et al., 2020). The major producers are Morocco, Canada, India, Pakistan, Romania and Russia. Other producers include Iran, Turkey, Egypt, Israel, China, Thailand, Myanmar, Poland, Bulgaria, Hungary, France, Netherlands, USA, Argentina and Mexico (Anonymous, 1999; Sriti et al., 2014). Coriander seeds and Dry coriander are highly useful for diarrhea and chronic dysentery remedial actions (Maroufi et al., 2010). Coriander fruits have various antistress activities such as antifungal, antibacterial, stomachic, anticancer, spasmolytic, carminative and antioxidant properties making it a valuable plant in Siddha, Allopathic and Ayurvedic industries (Duarte et al., 2016). Coriander oil has huge value and more importance in aroma industries (Kannan and Ganesh, 1993; Lopez et al., 2008). The green leaves and seeds of coriander contained major phytochemicals viz., carotenoids, chlorophylls, ascorbic acid, tocopherols, phenolics, flavonoids, tannins and anthocyanins by Dias et al. (2011).

Coriander plant is mainly used as spice, in perfumery, food, beverage and pharmaceutical industry, medicine such as antioxidant, treatment of nervous disorder, gut modulatory, blood pressure lowering agent and diuretic activities, anti-diabetic and antimicrobial agent (Isabelle et al., 2010; Qaiser et al., 2009). Due to cross-pollination, this crop has high level chances of molecular variability and scope for the development of improved varieties/genotypes and characterization of germplasm. Its rapid life cycle allow it to fit into different growing seasons and making it possible to grow the crop under a wide range of conditions by Diederichsen (1996). The knowledge, extent and magnitude of genetic variability/diversity of agronomic and quality trait is limited. With the innovations in molecular biology and biotechnology it is now possible to analyze large number of loci distributed throughout the genome of a plant. For coriander breeding, it is important to choose geographical fields, locations, climatic change for developing varieties. The main purpose of a coriander breeding program is to obtain genotypes that have high oil content and biometric attributes. Relationships between the coriander varieties and their contribution to seed, oil yield are highly important in carrying out thriving breeding programs. Differences in terms of coriander oil and seed yield of genotypes might result from the genotypic differences and ecological variations. Despite the selection of several desirable traits and resistance donors, Coriander varieties through breeding method is restricted to selection alone due to crossing technique absence. The reason mainly due to the herbaceous plants and delicate nature of the plant parts of umbels, umbellets and florets.

The use of D^2 statistics of multivariate analysis gives an understanding of genetic diversity in the coriander was reported by many scientific people (Meena et al., 2014; Bhandari and Gupta, 1993). In the present study, Mahalanobis D^2 analysis was applied (i) to assess the genetic divergence among the genotypes; (ii) to identify promising genotypes with more divergence to initiate crossing program; (iii) to assess the contribution of different characters to genetic diversity. Hence the present study was undertaken to evaluate the genotypes for higher green biomass yield and quality.

Materials and methods

The present investigation was carried out during three seasons viz., season I (June 2022 – August 2022), season II (October 2022 – December 2022) and season III (June

2023 – August 2023) at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore which is located at 11°N latitude, 77°E longitude and at an altitude of 426.26 m above MSL. Seventy-five genotypes were raised in randomized block design with two replications.

Genetic diversity studies of coriander genotypes based on molecular markers

DNA from all the 75 genotypes was extracted using the protocol described by Shyamkumar et al. (2003) and molecular profiling of the selected genotypes was done using RAPD markers. RAPD markers OPZ (Operon Technologies Zenica)01, 03, 05, 06, 08, 09, 011, 012, 013 and 016 (Operon technologies, Alameda, California, USA; Table 2) were employed in this study. Amplification reactions were in the volumes of 15 µl reactions containing 10-20 ng of genomic DNA, 1.5 µl of 1.5 mM of assay buffer, 1.0 µl of 10.0 mM of dNTPs, 1.0 µl of 10 µM primer, 0.18 µl of 15 mM $MgCl_2$, 0.30 µl (1 unit) of Taq DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore) and 8.12 µl of sterile water. Amplifications were performed in PTC Thermal Cycler (MJ Research Inc.) that was programmed for 44 cycles of denaturation (60 s) at 94°C, 1 min annealing at 37°C and 2 min. and 72°C for extension than a final extension of 10 min at 72°C. PCR amplified products were detected by electrophoresis in a 1.2% agarose gel in 1X TAE buffer at 100 volts for 2 h using a horizontal gel electrophoresis unit (Bangalore Genei, Bangalore). The Ethidium bromide-stained gels were documented using the Alpha Imager TM 1200-Documentation (Alpha Innotech Corporation, USA). Sizes of the PCR amplicons were identified using a 100 bp ladder (Bangalore Genei, Bangalore) that was run simultaneously on the same gel.

Amplified DNA fragments were detected after electrophoretic separation in each genotype was scored for the presence (1) or absence (0) of clear and unambiguous bands. A data matrix was formed and this data matrix was subjected to NTSys analysis.

All possible $\{n(n-1)/2\}$ D^2 values between 75 genotypes were calculated utilizing the replicated values. The replicated data of the genotypes for the characters were subjected to analysis of variance using AGRES and D^2 statistic was employed using INDOSTAT and WINDOSTAT packages.

Computation of D^2 values

All possible $\{n(n-1)/2\}$ D^2 values between 75 genotypes were calculated utilizing the replicated values. The replicated data of the genotypes for the characters were subjected to analysis of variance using AGRES and D^2 statistic (Mahalonobis, 1936) was employed using INDOSTAT and WINDOSTAT packages. Packages available at Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

Determination of group constellations or clusters

A relatively simple criterion suggested by Tocher (Rao, 1960) was adopted in the determination of clusters. The criterion grouping was that, any two populations belonging to the same cluster on the average should show smaller D^2 than those belonging to the different cluster. Starting with two closely associated varieties, a third variety having the smallest average D^2 from the first two was added. Similarly, the fourth one was chosen to have the smallest D^2 from the first three and so on and at any stage the average D^2 of a group, from already included appeared to be high, it was considered that the group does not fit in with the former cluster and hence taken to be

outside the first cluster. The groups of the first cluster were then omitted and the vest treated in the similar way.

After establishing the group constellations or clusters, the average inter and intra clusters, divergences were worked out taking into consideration all the component D^2 values possible among the members of the two clusters taken for consideration. The square root of the D^2 values gave the distance (D) between the clusters.

Ranking of characters to D^2 values

Ranking of individual D^2 values, contributed by each character, was done as the highest contributor taking rank 1. The character with the least rank total was taken to contribute the maximum to genetic divergence and the one with highest rank total, the least to genetic divergence.

Results and discussion

Genetic divergence

The genetic divergence within the genotypes was tested by Wilks Criterion and was found to be significant. Thus, the analysis of genetic divergence among the genotypes taken for the study was considered to be relevant.

D^2 analysis

The square of the distance (D^2 values) between the genotypes, calculated as the sum of squares of the difference between the mean values of all the variables were used for final grouping of the genotypes.

By the application of clustering technique, the seventy-five genotypes were grouped into different clusters during different seasons. The constituents of different clusters are furnished in *Tables 1–3*.

During the first season, the first cluster, the biggest cluster included 61 genotypes. The second, fourth, fifth, seventh, eighth, ninth and tenth clusters consisted one genotype each. Cluster three included five genotypes and sixth included two genotypes (*Table 1; Figs. 1 and 2*).

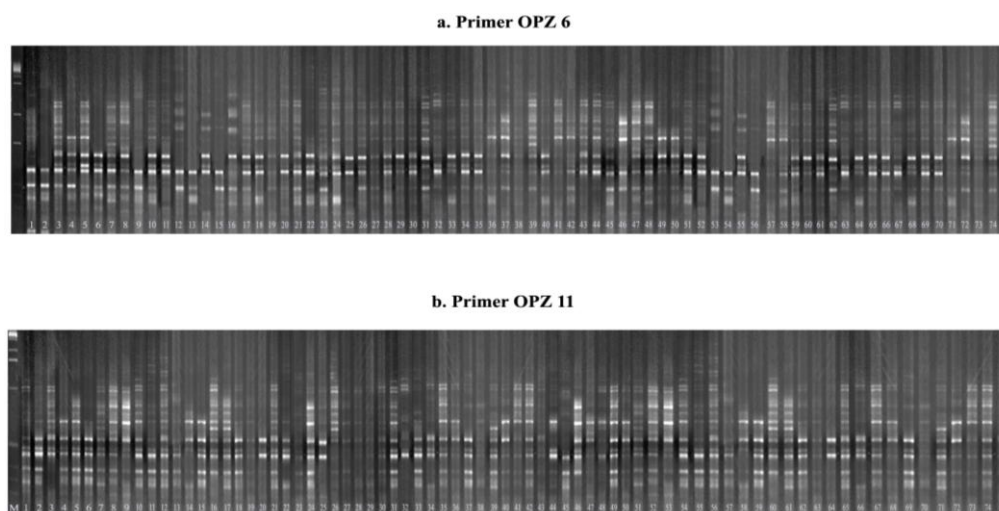


Figure 1. RAPD marker profiles of seventy-five coriander genotypes

Table 1. Constitution of D^2 cluster and Relative contribution of characters towards divergence of 75 genotypes of coriander during season I

Clusters number	Number of genotypes	Name of the genotypes	Relative contribution of characters towards divergence		
			Characters	Number of first rank	Percentage of contribution towards divergence
1	61	CS 10, CS 45, CS 27, CS 156, UD 273, DH 221, CS 20, CS 66, CS 88, Velachikulam (local), CS 108, CS 170, CS 3, CS 110, CS 198, CS 119, CS 187, CS 63, CS 845, UD 744, CS 152, CS 169, J Co-387, CS 91, CS 106, DH 208, CS 131, CS 83, ND Cor-2, DH 230, CS 68, CS 194, CS 146, CS 144, CS 40, UD 686, CS 142, CS 13, CS 497, CIMPO-S-33, UD 158, CS 180, DH 259, CS 89, CS 33, CS 39, CS 200, CS 177, CS 32, CS 176, UD 209, CS 36, CS 177, CS 74, DH 226, CS 70, ATP 72, DH 232, RCR 144, CS 49, CS 18	X1	94	3.39
2	1	CS 65	X2	77	2.76
3	5	CS 52, CS 37, UD 120, CS 62, CS 26	X3	8	0.29
4	1	DH 266	X4	266	9.59
5	1	CS 142	X5	70	2.52
6	2	UD 685, CS 136	X6	35	1.26
7	1	CS 25	X7	0	0
8	1	CS 71	X8	1012	36.47
9	1	CS 745	X9	208	7.5
10	1	CS 101	X10	1005	36.22
			X 11	0	0
			Total	2775	100

X1 - Plant height; X2 - Number of branches; X3 - Number of leaves; X4 - Fresh biomass yield; X5 - Fresh weight of leaves; X6 - Fresh weight of stem; X7 - Dry herbage yield; X8 - Fresh weight of root; X9 - Dry weight of leaves; X10 - Dry weight of stem; X11 - Dry weight of root

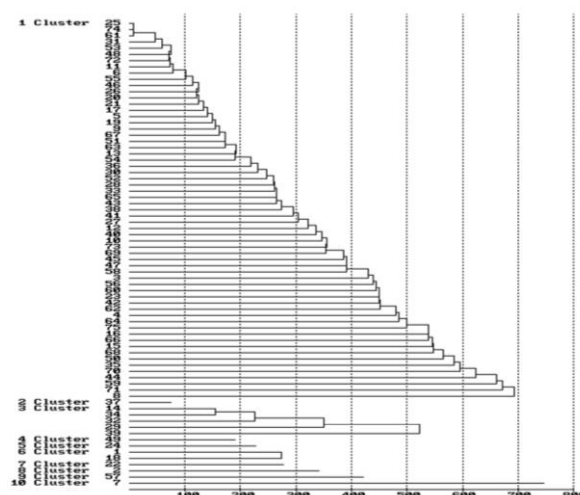


Figure 2. Cluster diagram during first season

In the second season, the first cluster also, the biggest cluster included 68 genotypes. The third and fourth clusters consisted of one genotype each. The second cluster included three genotypes and fifth cluster included two genotypes (Table 2; Figs. 1 and 3).

Table 2. Constitution of D^2 cluster and Relative contribution of characters towards divergence of 75 genotypes of coriander during season II

Clusters number	Number of genotypes	Name of the genotypes	Relative contribution of characters towards divergence		
			Characters	Number of first rank	Percentage of contribution towards divergence
1	68	CS 497, CS 108, CS 177, DH 266, CS 36, UD 158, CS 131, CS 146, CS 45, CS 68, CS 152, DH 221, CS 13, CS 49, CS 74, ND Cor-2, CS 20, CIMPO-S-33, CS 200, DH 259, CS 10, CS 198, J Co-387, CS 142, CS 91, Velachikulam (local), DH 208, CS 27, CS 187, DH 230, CS 32, CS 106, ATP 72, UD 744, CS 83, DH 226, RCR 144, CS 180, CS 170, CS 142, CS 3, CS 119, CS 52, CS 88, UD 209, CS 33, CS 37, CS 65, UD 273, CS 25, CS 71, CS 110, CS 26, CS 845, CS 144, CS 18, CS 39, CS 156, UD 686, CS 194, CS 176, CS 745, CS 169, CS 66, CS 63, CS 89, CS 40, UD 120	X1	244	8.79
2	3	CS 70, DH 232, CS 177	X2	463	16.68
3	1	CS 62	X3	319	11.5
4	1	CS 136	X4	66	2.38
5	2	UD 685, CS 101	X5	67	2.41
			X6	236	8.51
			X7	0	0
			X8	570	20.54
			X9	162	5.84
			X10	648	23.35
			X 11	0	0
			Total	2775	100

X1 - Plant height; X2 - Number of branches; X3 - Number of leaves; X4 - Fresh biomass yield; X5 - Fresh weight of leaves; X6 - Fresh weight of stem; X7 - Dry herbage yield; X8 - Fresh weight of root; X9 - Dry weight of leaves; X10 - Dry weight of stem; X11 - Dry weight of root

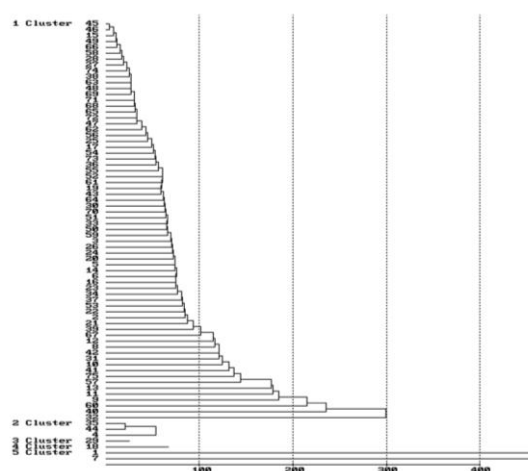


Figure 3. Cluster diagram during second season

During the third season, the first cluster the biggest cluster included 64 genotypes. Clusters second and fourth included three genotypes and third, fifty, sixth, seventh and eight consisted of one genotype each (Table 3; Figs. 1 and 4).

Table 3. Constitution of D^2 cluster and Relative contribution of characters towards divergence of 75 genotypes of coriander during season III

Clusters number	Number of genotypes	Name of the genotypes	Relative contribution of characters towards divergence		
			Characters	Number of first rank	Percentage of contribution towards divergence
1	64	CS 170, CS 89, CS 45, CS 176, CS 71, CS 180, CS 91, CS 33, CS 152, CS 187, CS 497, Velachikulam (local), CS 65, CS 70, CS 10, CS 13, CS 169, DH 232, CS 194, CS 36, CS 156, CS 27, UD 158, CS 198, DH 221, DH 266, ATP 72, CS 52, CS 110, CS 20, CS 845, UD 744, DH 259, CS 63, CS 18, CS 131, CIMPO-S-33, CS 108, CS 146, CS 142, CS 39, DH 230, CS 83, CS 88, CS 200, UD 273, CS 25, J Co-387, UD 686, CS 144, ND Cor-2, CS 3, UD 209, CS 177, CS 49, CS 40, DH 226, CS 66, DH 208, CS 745, CS 74, CS 119, CS 37, RCR 144	X1	0	0
2	3	CS 32, CS 142, CS 106	X2	310	11.17
3	1	CS 26	X3	1	0.04
4	3	CS 136, UD 120, UD 685	X4	1325	47.75
5	1	CS 68	X5	39	1.41
6	1	CS 62	X6	756	27.24
7	1	CS 177	X7	0	0
8	1	CS 101	X8	32	1.15
			X9	29	1.05
			X10	279	10.05
			X11	4	0.14
			Total	2775	100

X1 - Plant height; X2 - Number of branches; X3 - Number of leaves; X4 - Fresh biomass yield; X5 - Fresh weight of leaves; X6 - Fresh weight of stem; X7 - Dry herbage yield; X8 - Fresh weight of root; X9 - Dry weight of leaves; X10 - Dry weight of stem; X11 - Dry weight of root

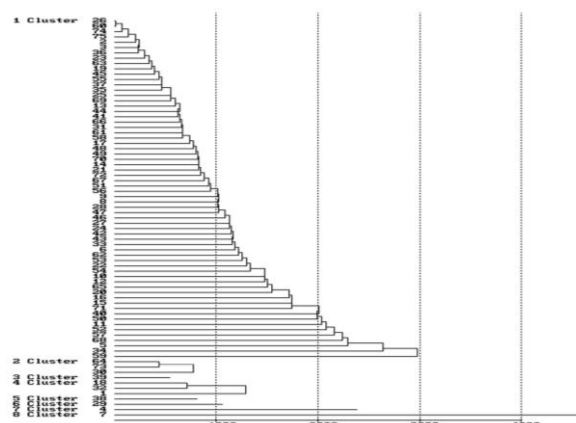


Figure 4. Cluster diagram during third season cluster

Molecular tools developed in the past few years provide easy, less laborious means to characterize known and unknown plant taxa. Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity assessment within and between species and populations by revealing different classes of variation (Gronau and Mora, 2007; Powell et al., 1996). The techniques include either or both DNA and enzyme-based system (Packia et al., 2000). It is correlated with the genome

fraction surveyed by each kind of molecular marker, their distribution throughout the genome and the extent of DNA target which is analyzed by each specific assay (Davila et al., 1999b). RAPD (Random amplified polymorphic DNA) is a PCR based technique developed by Williams et al. (1990) and detect nucleotide polymorphism in a DNA amplification-based assay using a short single synthetic primer of arbitrary nucleotide sequence. The advantages of using RAPD in genetic analysis are that it is sequence independent, easy, fast, cost-effective, efficient and requires small amount of DNA for cultivar identification and diversity analysis (Haque et al., 2007).

RAPD marker have been efficiently used for the study of molecular diversity in coriander genotypes (Tomar et al., 2014; Singh et al., 2013, 2012) and in various seed spice crops like cumin, fenugreek and fennel (Choudhary et al., 2013, 2015, 2018).

Genetic divergence and varietal identification in vegetable plants have been investigated in several studies using SSR and SNP markers, isozyme, and protein polymorphism reported by Taher and Solberg (2017). In addition, other molecular approaches including restriction fragment length polymorphism (RFLP) and PCR-based markers have been extensively used for the authentication of closely related horticultural and medicinal plant varieties by Wang and Sun (2010). Hints, the RFLP-markers show a high level of polymorphism and stability but this approach may raise limitations due to high cost and complexity Kratochwil and Kautt (2022).

Basic knowledge on genetic variability available in the germplasm is a prelude for potential crop improvement. Genetic divergence is mainly employed to assess the proximity of genotypes with each others thus facilitating to classify them in different clusters and this would be also useful for the easy identification of diverse genotypes for higher biomass yield. The success in obtaining highly variable genotypes and creating greater variability for efficient selection in a breeding program depends to a larger measure in the degree of divergence. One such tool is the D^2 statistic that has been found best for estimating genetic divergence. The importance of the choice of characters has been stressed since they reflect the usefulness of D^2 analysis.

With regard to the contribution of different characters towards the genetic divergence, it was found that the maximum contribution to the genetic divergence was accounted by fresh weight of root, fresh weight of stem and weight of leaves. The low contribution to genetic divergence by other characters may be due to the fact that selection towards uniformity in these characters could have caused an eroding effect of genetic diversity. The Jaccard Similarity coefficients approach provide similarities of sparse binary data sets while the it receipts the common or split components of in two sets reported by Baharav and Kamath (2020).

Information on molecular diversity and relationship among individuals, population, plant varieties and species are important to plant breeders for the improvement of crop plants. Molecular 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 and molecular improvement (Duran et al., 2009). Various types of markers such as morphological, biochemical and molecular are used for this purpose (Barwar et al., 2008).

Relative contribution

The relative contribution made by each character to genetic divergence is presented in *Tables 1–3*.

During the first season, maximum contribution to genetic divergence was made by fresh weight of root (36.47%) followed by dry weight of stem (36.22), fresh biomass yield (9.59%), dry weight of leaves (7.50%), plant height (3.39%), number of branches (2.77%), fresh weight of leaves (2.52%), fresh weight of stem (1.26%) and number of leaves (0.29%) (Table 1).

In the second season, maximum contribution to genetic divergence was made by dry weight of stem (23.35%) followed by dry weight of leaves (20.54%), number of branches (16.68%), number of leaves (11.50%), plant height (8.79%), fresh weight of stem (8.50%), dry biomass yield (5.84), fresh weight of leaves (2.41%) and fresh biomass yield (2.38%) (Table 2).

During the third season, maximum contribution to genetic divergence was made by fresh weight of leaves (47.75%) followed by fresh weight of stem (27.24%) number of branches (11.17%), dry biomass yield (10.05%), fresh biomass yield (1.41%), dry weight of leaves (1.15%), dry weight of root (0.14%), dry weight of stem (1.05%) and number of leaves (0.04%) (Table 3). The low contribution to genetic divergence by other characters may be due to the fact that selection towards uniformity in these characters could have caused an eroding effect of genetic diversity. This is in conformation with earlier works by Reddy (1987) and Shanmugasundaram (1998) in turmeric and Singh et al. (2000) in ginger. However, a contradictory report is established by Jalgaonkar et al. (1990) in turmeric. This may be due to the fact that character expression might have been affected by a difference in environment conditions and background of material used. Similar to the present finding Choudhary et al. (2013) obtained high level of polymorphism of 57.66% among Indian fenugreek varieties. The data obtained in this study also confirmed the efficiency of the RAPD technique for the determination of genetic distances and relatedness among different eggplant varieties by Gronau and Moran (2007).

The seventy-five genotypes studied for genetic divergence resolved into ten clusters (Season I), five clusters (Season II) and eight clusters (Season III), through mahalanobis D^2 analysis and the largest cluster consisted of sixty-one genotypes (season I), sixty-eight genotypes (season II) and sixty-four (season III). The maximum contribution to the genetic divergence was accounted by weight of root, weight of stem and weight of leaves.

In our study in coriander, the primers were differentially amplified in different coriander genotypes but showed low polymorphism at molecular level. The low degree of polymorphisms indicates there was low divergence among the coriander genotypes. However, the percentage distribution of RAPD marker always indicate a distribution level, frequency of rare alleles which are either similar or not to commercial of landrace accessions by Carelli and Gerald (2006).

Conclusion

Based on similarity matrix dendrogram, results indicate polymorphism among most of the coriander genotypes. Hence, RAPD marker is suitably proven in characterization of coriander genotypes. Genetic divergence analysis, During first season, cluster I comprising of sixty-one genotypes had the highest intra cluster distance. In the second season, cluster I comprising of sixty-eight genotypes had the highest intra cluster distance. During the third season, also cluster I comprising at sixty-four genotypes had the highest intra cluster distance followed by cluster II and IV with three genotypes.

During the first season, maximum contribution to genetic divergence was made by fresh weight of root (36.47%). In the second season, maximum contribution to genetic divergence was made by dry weight of stem (23.35%). During the third season, maximum contribution to genetic divergence was made by fresh weight of leaves (47.75%). These genotypes could be efficiently utilized in crop genetic improvement and breeding programs. Keeping this in view, it appears that cluster X, (CS 101), V (CS 101, UD 685) and VIII (CS 101) would exhibit high heterosis as well as high level of production, involved in the hybridization program. Thus, the result of present study could be useful to facilitate the phylogenetic relationship for crop improvement and breeding programs.

Author contributions. Palanikumar Muniyandi, Arularasu Palanichamy and Sundharaiya Kalangiyam and Anand Gurusamy designed the study, executed the field research, data collection and in writing and improving paper critically, Rajarathinam Palanivel, Krishnasurender Karuppasamy, Manikandaboopathy Narayanan and Jaiganesh Vaikundaperumal carried out few laboratory analysis, literature collection, improving paper critically and finalize the paper, was involved in basic idea of the paper, statistical analysis of data and critical review of the paper. All these authors have substantial contributions to the finalize manuscript and approved this submission.

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Conflict of interests. The authors declare that there is no conflict of interests.

Data availability. The data presented in this study are available on request from the corresponding author. The data are not publicly available since these data are published for the first time. The authors have no problems providing them on request.

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