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RESEARCH ARTICLE

Assessment of Genetic Diversity in Ajwain (*Trachyspermum ammi* L.) Genotypes using Morphological and Molecular Markers

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Twenty-eight genotypes of ajwain **(***Trachyspermum ammi* L.**)** consisting 25 genotypes and 3 checks were evaluated for genetic diversity using morphological and molecular markers (i.e RAPD markers). Out of 18 decamer RAPD primers, 10 primers were found polymorphic showed an average 5.16 numbers of polymorphic bands per primer. The similarity coefficient for different genotypes was in the range of 0.07 (GP-113 and GP-7) to 0.58 (Gujarat ajwain-1). The average similarity (0.32) across all the genotypes indicating a low level of genetic similarity among the genotypes and a high polymorphism (100%) and variability (78%) by RAPD and morphological analysis, respectively for all the genotypes under study. Though the genotypes *viz.,* GP-7, GP-113, Gujarat ajwain-1 and Pratap ajwain-1 showed moderate divergence in D^2 analysis, but found highly divergent in RAPD analysis and also had high *per se* performance for seed yield and its contributing characters, hence could be selected for the further breeding programme in ajwain.

Key Words: Ajwain, Germplasm, Genetic diversity, Morphological markers, RAPD markers.

Ajwain (*Trachyspermum ammi* L.) 2n=18 belongs to the family Apiaceae, a native of Egypt (Sayre, 2001) also known as Bishop's weed and Carum in English and cultivated mainly for its seed, herb and volatile oil in Iraq, Iran, Afghanistan, and India. In India, it is grown in Gujarat, Rajasthan, Madhya Pradesh, Bihar, Punjab, Tamil Nadu, West Bengal, Andhra Pradesh and Uttar Pradesh covering an area of 27000 ha with a production of 19000 mt and productivity of 703.70 kg/ha (Anonymous, 2014). It is highly valuable and medicinally important seed spice, widely grown in arid and semi-arid regions (Joshi, 2000) where the soil contains a high amount of salts (Munnas, 2002). It is an annual, aromatic and profusely branched herb having an erect straight stem which may grow up to 90-100 cm height. A number of chemical constituents have been reported from the herb. Carbohydrates (24.6%), fat (21.1%), protein (17.1%), fiber (11.9%), glycosides, tannins, saponins, flavones and other components (7.1%) involving iron, iodine, calcium, copper, phosphorous, cobalt, manganese, riboflavin, thiamine, and nicotinic acid are the phytochemical constituents of ajwain reported by Zarshenas *et al.* (2014). The seeds contain essential volatile oil responsible for its odor and taste. The main component of ajwain oil is thymol (~50%) which is a strong germicide and anti-spasmodic. The oil exhibits fungicidal (Singh *et al.,* 2000), antimicrobial (Sivropoulou *et al.,* 1996) and anti-aggregatory (Srivastava, 1988) effects on humans.

Despite the economic importance of ajwain, it is cultivated on marginal lands with poor fertility because of which, productivity is quite low (Meena *et al.,* 2015). Though the crop has domestic, medicinal as well as commercial value, it has altogether been neglected as far as genetic improvement is concerned. As a result, local types have low yield potential and are susceptible to diseases resulting in poor production. In our country improved ajwain varieties with good adaption are available in a limited number. Genetic variability available in the germplasm collection of a species is a basic requirement for its crop improvement programme. Genetic analysis of ajwain is essential to enhance the yield potential and maximum utilization of the desirable characters for development of any ideal genotypes. Detailed information on the genetic divergence in the available germplasm collection is necessary to ascertain the potential of the germplasm as a base material to sustain a varietal improvement programme. As already established, seed yield is the most important character and has complex inheritance, governed by a large number of genes and is greatly affected by environmental factors. Selections made in the field are not likely to be reliable as character, are subjected to large number

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of non-genetic factors. Molecular markers can provide an opportunity to measure genetic variability/genetic relationship among genotypes more precisely because these markers are potentially unlimited in number and are not affected by the environment.

DNA based molecular markers have applications in genetic diversity analysis among naturally occurring populations. Germplasm management could be brought about by conserving a minimum number of plants that show maximum diversity. For genetic relationships among genotypes and detecting and monitoring pedigree breeding record of inbred parents, RAPD is the first PCR-based molecular marker technique (Dongre and Parkhi, 2005) and has proved itself as an efficient method for varietal identification, study of polymorphism, gene mapping, biodiversity, genetic map construction, hybridization and phylogenetic relationships. The aim of the study was to observe the genetic diversity within twenty eight ajwain accessions by using morphological and random amplified polymorphic (RAPD) DNA markers and develop phylogenetic tree of different ajwain accessions by using bioinformatics tools.

Materials and Methods

Germplasm used and Experimental Plan

Twenty-eight genotypes originating from different places were selected for this study (Table 1). These accessions were grown at the Agricultural farm, Rajasthan College of Agriculture, MPUAT, Udaipur, India in RBD with three replications during *rabi* 2014-15. In each replication, the genotype was sown in 2 row plot of 4-meter row length keeping row to row distance of 30 cm. All the recommended agronomical practices and plant protection measures were adopted to raise a healthy crop to attain maturity. Fertilizers were applied @ 20 kg N: 20 kg P_2O_5 at the time of sowing as basal dose while 20 kg N/ha was top-dressed in two split doses in thirty and sixty days, respectively. The crop was irrigated 6 times during the crop season. First irrigation was given immediately after sowing and there after irrigation was given at an interval of 20-25 days.

Morphological Characterization

Observations were taken on each accession along with the checks for 10 morphological and one biochemical trait *viz.,* days to 50 per cent flowering (DF), days to 75 per cent maturity (DM), plant height (PH), primary branches (PB), secondary branches (SB), umbels per plant (UPP), umbellates per umbel (UPU), seed yield per plant (YPP), biological yield per plant (BY), harvest index (HI) and seed oil content (SO), respectively.

Statistical Analysis

For calculating Genetic Divergence for eleven characters, Mahalanobis D^2 Statistics (1936) was used.

DNA Isolation and quantification

Total genomic DNA was isolated from fresh and healthy leaves using the CTAB method (Doyle and Doyle, 1990) with few modifications. Briefly, 2.0 g of leaves were ground in liquid nitrogen to get a fine powder. The powder was added to 3 mL of extraction buffer (100 mM Tris–HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% (wv-1) CTAB, 2% 2–mercaptoethanol and incubated at 65°C for 30 min. DNA was extracted with C:I (24:1)

Table 1. Twenty eight diverse ajwain (*Trachyspermum ammi* L.) genotypes

S.No.	Name of Genotypes	Origin	State	S. No.	Name of Genotypes	Origin	State
1.	GP-7	Udaipur	Rajasthan	15.	GP-90	Chittorgarh	Rajasthan
2.	GP-28	Chittorgarh	Rajasthan	16.	GP-113	Chittorgarh	Rajasthan
3.	GP-29	Chittorgarh	Rajasthan	17.	GP-125	Chittorgarh	Rajasthan
4.	GP-30	Chittorgarh	Rajasthan	18.	GP-127	Neemauch	Madhya Pradesh
5.	GP-32	Chittorgarh	Rajasthan	19.	GP-131	Neemauch	Madhya Pradesh
6.	GP-41	Chittorgarh	Rajasthan	20.	GP-141	Neemauch	Madhya Pradesh
7.	GP-48	Chittorgarh	Rajasthan	21.	GP-149	Radhapur	Gujarat
8.	GP-53	Chittorgarh	Rajasthan	22.	GP-168	Banaskantha	Gujarat
9.	GP-63	Pratapgarh	Rajasthan	23.	GP-169	Banaskantha	Gujarat
10.	GP-66	Pratapgarh	Rajasthan	24.	GP-175	Banaskantha	Gujarat
11.	GP-70	Bhilwara	Rajasthan	25.	GP-191	Banaskantha	Gujarat
12.	GP-71	Bhilwara	Rajasthan	26.	Gujarat Ajwain-1	Banaskantha	Gujarat
13.	GP-83	Chittorgarh	Rajasthan	27.	Pratap Ajwain-1	Banaskantha	Gujarat
14.	GP-87	Chittorgarh	Rajasthan	28.	Local check	Banaskantha	Gujarat

and precipitated by the addition of equal volume of 70% chilled isopropanol, with step by step centrifugation. After washing with 70% ethanol and dissolved in 100 µl of HPLC water. DNA was quantified by spectrophotometer by using a comparison of the optical density values of the solution at A260/A280 wavelengths. Stock DNA samples were stored at -20°C.

RAPD Analysis

A total of 18 decamer oligonucleotide RAPD primers of arbitrary sequence obtained from Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, MPUAT, Udaipur, were tested for PCR amplification. The sequences of these primers were selected from literature and purchased from Banglore Genei Pvt. Ltd. Bengaluru. PCR reaction was performed in a final volume of 20 µl containing 1X reaction buffer, 1 unit of Taq DNA polymerase, 200 mM each dNTPs, 0.5 µM/reaction primer and 50 ng of template DNA. DNA amplification was carried out in advanced Thermal cycler using the following conditions: initial denaturation of 94°C for 4 minutes, followed by 35 cycles comprising 30 sec at 94°C, 1 min at 36°C and 2 min at 72°C. An additional cycle of 7 min at 68°C was used for final extension. Amplified products were separated by electrophoresis at 50 Volts for 3-4 hrs in 1.2 per cent agarose gel prepared in 1X TAE buffer (Sambrook *et. al.,* 1989) containing 0.5 µg/ml of ethidium bromide. Gels were photographed under UV light with the help of gel documentation system (Bio Rad Gel DOC).

Scoring the RAPD Products

RAPD bands were designated on the basis of their molecular size ranging between 100-1000 bp. The molecular size of PCR products was estimated by referencing to a 100 bp DNA ladder. Presence of a band was denoted by score of '1' while its absence was denoted by '0'. Only prominent bands were considered for scoring.

Statistical Analysis for Similarity Coefficient

The pair-wise association coefficients were calculated from data matrix using Jaccard's similarity coefficient (Jaccard, 1908). The equation for calculating Jaccard's similarity coefficients 'F' between two samples A and B is:

f = nxy / (n1 – nz)

nxy = Number of bands common to sample A and sample B.

n1 = Total number of bands present in all samples.

nz = Number of bands not present in sample A or B, but found in other samples.

For genetic distance, cluster analysis was then conducted by using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method (Sneath and Sokal, 1973). The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genotypes using computer program NTSYSpc version 2.02 (Rohlf, 1998).

Result and Discussion

Per se Performance

The present experimental material showed a wide range of variability for almost all the characters including seed yield (Table 2). Days to 50 per cent flowering ranged from 72.33 to 80.00 days with a mean value of 76 days. Values for primary branches and secondary

Table 2. Analysis of variance along with *per se* and coefficient of variation (CV) for eleven characters in ajwain

Characters	Replication	Genotypes	Error	Mean ± SE	Range	CV (%)	Best Genotypes on the basis of per se
	[2]	[27]	[54]				
Days to flowering	12.76	12.32**	5.43	76.76±1.35	72.33-80.00	3.04	GP- 41 (72.33 days)
Days to maturity	14.89	40.18*	23.14	164.14±2.78	156.67-169.33	2.93	GP-87, GP-113 (156 days)
Plant height	43.80	83.73**	15.92	98.02± 2.30	82.00-107.00	4.07	GP-83 (107 cm)
Primary branches	0.90	2.12**	0.45	10.10±0.39	8.67-11.33	6.63	GP-149, GP-169, GP-191 (11)
Secondary branches	35.51*	67.42**	10.78	59.87± 1.90	49.67-71.00	5.49	Local check (71)
Umbels/plant	112.40	676.84**	40.42	116.02±3.67	72.33-160.67	5.48	GP-41 (160.67)
Umbellates/ umbel	1.65	2.11**	0.79	10.19±0.51	8.33-12.00	8.73	GP-191 (12)
Seed yield /plant	1.45	5.32**	0.50	10.33±0.41	8.14-12.39	6.89	GP-191 (12.39 g)
Biological yield/plant	13.16	43.63**	4.54	33.84±1.23	27.68-42.58	6.30	Pratap ajwain-1 (42.58 g)
Harvest index	0.61	22.34**	3.56	30.62±1.09	25.48-36.63	6.17	GP-125 (36.63 %)
Oil content	0.02	0.52**	0.01	3.62±0.06	2.72-4.03	3.01	GP-191 (4.03 %)

branches ranged from 8.67 to 11.33 and 49.67 to 71.00, respectively. Umbels/plant showed a mean value of 116.02 umbels, while each umbel has an average 10.19 umbellates, with a range of 8.33-12.00 umbellates/umbel. Seed yield /plant was ranged from 8.14-12.39 g with the mean of 10.33. Oil content showed a mean value 3.62 % and ranged from 2.72-4.03 %. Similar trends of results for seed oil content were found by Kole *et al.* (2002). Genotypes GP-191, GP-175, Gujarat ajwain-1 and Pratap ajwain-1 displayed high *per se* for both seed yield per plant and seed oil content. Therefore, these entries could be gainfully utilized in breeding programmes.

D^2 Analysis

Twenty-eight varieties were grouped into 5 clusters on the basis of observed similar D^2 values among genotypes within a cluster as compared to genotypes in another cluster (Table 3). Cluster II includes a maximum number of genotypes i.e. 14 followed 9 in cluster V, 3 in cluster I and cluster III, IV were monogenotypic. The clustering pattern revealed that, in general varieties from the same origin showed no tendency to be in the same cluster.

Looking at the pattern of varietal distribution in different clusters, it appeared that geographical distance between the varieties had no relation with the genetic divergence as the varieties from same source had fallen into different clusters as well as the same cluster contained varieties from different sources. These findings are in close agreement to earlier reports of Mathur (1992), Banerjee *et al.* (2004), Jain *et al.* (2006) and Kole and Saha (2009) in fenugreek.

Average inter cluster values were found maximum between cluster III and IV (89.65), whereas maximum intra cluster values were recorded for cluster V (10.12) followed by cluster II (9.18) and cluster I (6.61) (Fig. 2). The inter-cluster distances were greater than intra-cluster distance revealing a considerable amount of genetic diversity among the genotypes (Table 4). Therefore, the genotypes falling in these clusters appeared to be divergent and might have genetic origin hence could be gainfully utilized in ajwain improvement programme. These findings were in agreement with Banerjee *et al.* (2004) and Swami *et al.* (2012).

Table 3. Ajwain genotypes included in each cluster

Clusters	Number of Genotypes	**Genotypes**
I	3	GP-53, GP-87, GP-168
II	14	GP-63, GP-66, GP-70, GP-71, GP-83, GP-90, GP-113, GP-125, GP-127, GP-131, GP-141, GP-149, GP-169 and Gujarat Ajwain-1
III	1	GP-29
IV	1	GP-41
V	9	GP-7, GP-28, GP-30, GP-32, GP-48, GP-175, GP-191, Pratap Ajwain-1 and Local check

Contribution of Different Traits towards Total Genetic Divergence

The importance of genetic divergence and its use in the manifestation of heterosis is obvious. The maximum amount of heterosis and variability will be manifested in a cross involving parent belonging to the most divergent cluster. The perusal of the comparison of contribution of different characters towards genetic diversity was estimated based on ranking method. As evident from Table 5, seed oil content contributed maximum (42.03 %) followed by umbels/plant (14.13 %), and Seed yield/ plant (13.94 %). The characters like days to 50 per cent flowering, days to maturity, umbellates/umbel and harvest index was found to be least contributing traits. These findings are in close agreement with Kole *et al.* (2002), Banerjee *et al.* (2004) and Pathak *et al.* (2014) in other seed spice.

To conclude that seed oil content, seed yield per plant and umbels per plant had high estimates of all variability parameters and were strongly correlated with

Table 4. Average intra and inter-cluster D^2 values in twenty eight genotypes of ajwain

Clusters	I	II	III	**IV**	**V**
I	**6.61**	12.13	39.86	52.50	21.92
II		**9.18**	40.29	50.48	16.42
III			**0.00**	89.65	55.52
IV				**0.00**	37.36
V					**10.12**

Bold number = intra-cluster distance

Table 5. Contribution of characters to divergence

Characters	Contribution percent	Parent I rank	Total rank
Days to 50 per cent flowering	1.92	0.00	2829
Days to maturity	2.05	0.53	2805
Plant height	4.50	6.61	2346
Primary branches/plant	4.26	2.38	2328
Secondary branches/plant	5.78	2.91	2241
Umbels/plant	14.13	15.08	1950
Umbellates/umbel	2.32	0.26	2725
Seed yield/plant	13.94	20.90	1538
Biological yield/plant	6.66	7.14	2065
Harvest index	2.42	1.59	2720
Seed oil content	42.03	42.59	1399

each other having maximum direct effects towards seed yield appeared promising to contribute genetic diversity in ajwain. Further, these genotypes were grouped into 5 clusters and cluster III and IV had maximum inter cluster distance, therefore, the genotypes belonging to these clusters namely GP-29 and GP-41 showed high *per se* performance for oil content, umbels per plant and exhibited earliness and hence, could be utilized in breeding programmes.

RAPD Analysis

RAPD markers are superior in terms of simplicity and cost, and are used for analysis of genetic diversity and reliability in the identification of cultivars due to their high resolution in several types of plant material such as natural population in breeding programme and cultivar collections. RAPD analyses does not require any prior sequence information because arbitrary DNA sequence can be used as primer which target random genomic sequences to generate a genetic profile. RAPD (Random Amplified Polymorphic DNA) markers have the advantage of detecting polymorphism simply and quickly (Demeke *et al.,* 1996). Khan *et al.* (2008) mentioned that RAPD is a simple and fast technique to compare the genetic relationship and pattern of variation among the gene pool in mustard crop.

Genetic Polymorphism among Ajwain Accessions

All the 28 varieties of ajwain cultivars were examined for DNA polymorphism using 18 decamer primers (OPERON) showing high (G+C) content. Out of 18 primers 10 primers were found polymorphic. The RAPD primer OPA-07, OPA-08, OPA-10 generated 9 bands with 100% polymorphism and other primers *viz.,* OPA-14, OPB-03 and OPB-06 gave 11 bands and showed 100% polymorphism (Plate 1). Whereas primer OPB-07, OPA-05, OPA-11 and OPA-09 showed 7, 8, 8, 10 bands respectively with 100% polymorphism (Table 6). The average numbers of polymorphic bands per primer were 5.16. Similar results were observed by Srivastava *et al.* (2011) observed 97.26% polymorphism in black gram (*Vigna mungo*) while, Kameswari *et al.* (2014) in chrysanthemum genotypes obtained 97.4% polymorphism.

Plate-1

RAPD Profile of Ajwain genotypes generated with primers OPB-06 and OPB-07

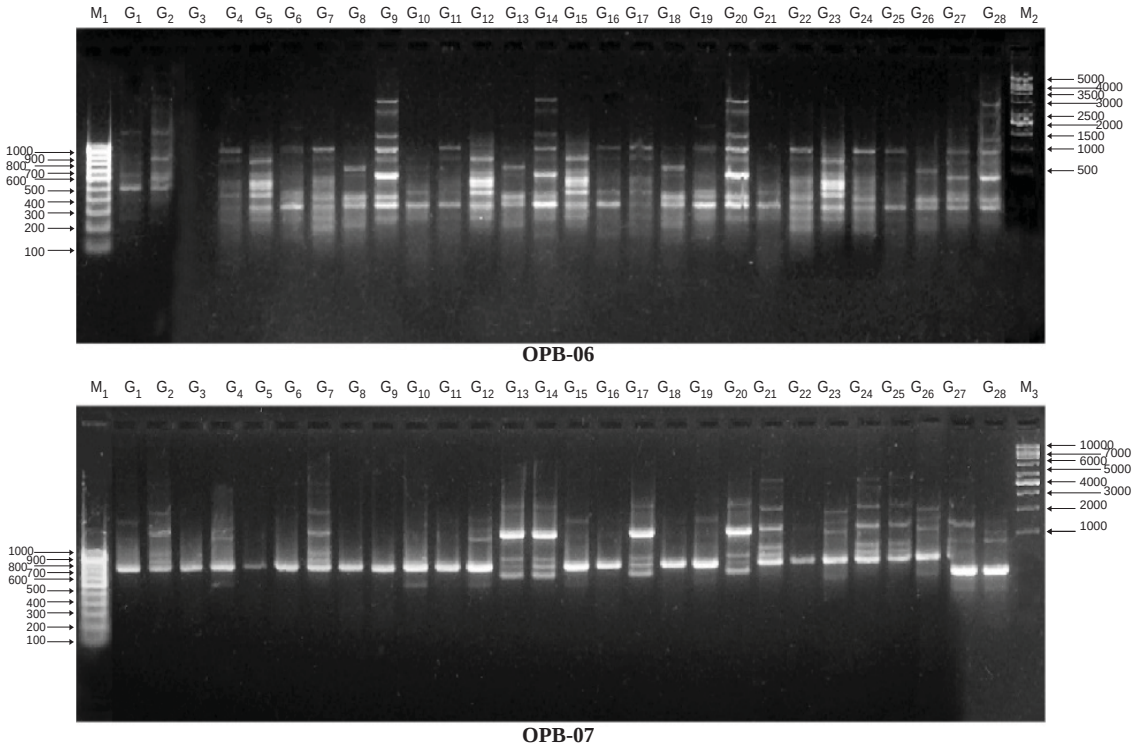

Plate 1. G-1= GP-7, G-2= GP-28, G-3= GP-29, G-4= GP-30, G-5= GP-32, G-6= GP-41, G-7= GP-48, G-8= GP-53, G-9= GP-63, G-10= GP-66, G-11= GP-70, G-12= GP-71, G-13= GP-83, G-14= GP-87, G-15= GP-90, G-16= GP-113, G-17= GP-125, G-18= GP-127, G-19= GP-131, G-20= GP-141, G-21= GP-149, G-22= GP-168, G-23= GP-169, G-24= GP-175, G-25= GP- 191, G-26= Gujarat Ajwain-1, G-27= Pratap Ajwain-1, G-28= Local check.

Table 6. Polymorphism information of RAPD primers analyzed

S. No.	Primers code	Sequence 5' -3'	Base pair	Total No. of bands (a)	Total No. of polymorphic bands (b)	Polymorphism % (b/a × 100)
1.	OPA-05	AGGGGTCTTG	400-2000	8	8	100
2.	OPA-07	GAAACGGGTG	300-3000	9	9	100
3.	OPA-08	GTGACGTAGG	300-4000	9	9	100
4.	OPA-09	GGGTAACGCC	200-2000	10	10	100
5.	OPA-10	GTGATCGCAG	500-3000	9	9	100
6.	OPA-11	CAATCGCCGT	800-7000	8	8	100
7.	OPA-14	TCTGTGCTGG	400-6000	11	11	100
8.	OPA-15	TTCCGAACCC	NA	NA	NA	0
9.	OPA-16	AGCCAGCGAA	NA	NA	NA	0
10.	OPB-02	TGATCCCTGG	NA	NA	NA	0
11.	OPB-03	CATCCCCCTG	200-2500	11	11	100
12.	OPB-04	GGACTGGAGT	NA	NA	NA	0
13.	OPB-05	TGCGCCCTTC	NA	NA	NA	0
14.	OPB-06	TGCTCTGCCC	200-3000	11	11	100
15.	OPB-07	GGTGACGCAG	600-4000	7	7	100
16.	OPB-08	GTCCACACGG	NA	NA	NA	0
17.	OPB-10	CTGCTGGGAC	NA	NA	NA	0
18.	OPB-11	GTAGACCCGT	NA	NA	NA	0
Total				93	93	100
Average				5.16	5.16	–

** NA= Not Amplified

Genetic relationship among Ajwain Genotypes and Cluster Analysis based on RAPD

Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis. The similarity coefficient matrix generated for the primers was subjected to algorithm UPGMA (Unweighted Pair Group Method with Arithmetic averages) and dendrogram was generated using NTSYS-pc 2.02 programme (Rohlf, 1997).

The RAPD data were used to obtain a similarity matrix (Table 7). The similarity coefficient for different genotypes was in the range of 0.07 to 0.58. The average similarity across all the genotypes was found to be 0.32 indicating a low level of genetic similarity among the genotypes. This indicated a broad genetic base of tested cultivars.

The maximum similarity coefficient (0.58) was observed between local check and Pratap ajwain-1 followed by GP-149 and GP-131, GP-87 and GP-83 showed similarity of 0.48 and 0.47 respectively. The minimum similarity coefficient (0.07) was observed between GP-113 and GP-7 and Gujarat ajwain-1 and GP-70. The results obtained were in conformity with the earlier report by Javan *et al.,* 2012 who evaluated the genetic variation among eight species of Salvia using the RAPD markers. The pair-wise Jaccard genetic similarity varied from 0.07 to 0.35 for RAPD.

RAPD Dendrogram

The dendrogram generated on the basis of Jaccard's similarity coefficient, clearly indicated two main clusters. The clustering results based on RAPDs did not match with those based on morphological traits. The result suggested the high level of genetic diversity.

Cluster I was the major cluster which included 26 genotypes out of 28 genotypes. This cluster was divided into 2 sub clusters IA and IB at the similarity coefficient of 0.202. Sub-cluster IB included only one genotype *viz.,* GP-29. Sub cluster IA was divided into two sub clusters IA-1 and IA-2. Both had the similarity coefficient of 0.240. The sub clusters IA-1 included two genotypes Pratap ajwain-1 and local at the similarity coefficient of 0.58. Sub cluster IA-2 included 23 genotypes and so on shown in Fig 1. Genotypes Pratap ajwain-1 and local showed highest similarity co-efficient 0.580 for traits like days to 50 % flowering, primary branches per plant, umbels per plant, umbellates per umbel, seed yield per plant, harvest index and seed oil content. Cluster II was the minor cluster which included two genotypes *viz.,* GP-7 and GP-28 at the similarity coefficient of 0.471.

Table 7. Jaccard similarity coefficient for ajwain genotypes based on RAPD analysis

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28
G1	1																											
G2	0.42	1																										
G3	0.08	0.24	1																									
G4	0.13	0.29	0.37	1																								
G5	0.14	0.25	0.26	0.45	1																							
G6	0.15	0.23	0.20	0.31	0.41	1																						
G7	0.14	0.30	0.20	0.35	0.34	0.39	1																					
G8	0.17	0.27	0.29	0.38	0.40	0.35	0.41	1																				
G9	0.10	0.17	0.17	0.33	0.31	0.33	0.36	0.36	1																			
G10	0.16	0.26	0.36	0.37	0.36	0.38	0.34	0.41	0.26	1																		
G11	0.11	0.15	0.19	0.28	0.34	0.42	0.33	0.40	0.30	0.27	1																	
G12	0.18	0.21	0.12	0.24	0.37	0.27	0.32	0.25	0.23	0.17	0.37	1																
G13	0.13	0.27	0.19	0.31	0.32	0.30	0.46	0.31	0.27	0.39	0.19	0.25	1															
G14	0.15	0.28	0.23	0.40	0.35	0.37	0.37	0.30	0.23	0.39	0.26	0.26	0.48	1														
G15	0.35	0.26	0.18	0.37	0.29	0.27	0.31	0.34	0.22	0.33	0.17	0.27	0.30	0.23	1													
G16	0.07	0.17	0.21	0.30	0.29	0.30	0.37	0.30	0.41	0.27	0.31	0.24	0.30	0.23	0.35	1												
G17	0.13	0.21	0.13	0.34	0.28	0.27	0.39	0.25	0.24	0.27	0.24	0.17	0.33	0.32	0.22	0.36	1											
G18	0.18	0.16	0.19	0.35	0.37	0.28	0.42	0.41	0.41	0.37	0.22	0.22	0.37	0.24	0.31	0.28	0.30	1										
G19	0.22	0.22	0.14	0.32	0.20	0.31	0.42	0.50	0.50	0.30	0.28	0.28	0.26	0.19	0.41	0.33	0.27	0.35	1									
G20	0.25	0.30	0.12	0.31	0.23	0.24	0.37	0.31	0.31	0.22	0.29	0.40	0.39	0.40	0.33	0.26	0.33	0.25	0.37	1								
G21	0.17	0.30	0.20	0.38	0.27	0.35	0.51	0.32	0.32	0.29	0.29	0.28	0.36	0.33	0.29	0.33	0.34	0.38	0.49	0.37	1							
G22	0.10	0.24	0.24	0.44	0.43	0.41	0.47	0.47	0.47	0.32	0.34	0.26	0.32	0.29	0.27	0.35	0.47	0.40	0.41	0.38	0.43	1						
G23	0.18	0.20	0.18	0.22	0.32	0.21	0.32	0.28	0.28	0.33	0.18	0.30	0.36	0.27	0.37	0.30	0.27	0.40	0.22	0.22	0.29	0.33	1					
G24	0.14	0.26	0.15	0.33	0.28	0.23	0.42	0.39	0.39	0.31	0.20	0.29	0.37	0.40	0.27	0.26	0.35	0.33	0.33	0.34	0.41	0.47	0.44	1				
G25	0.17	0.33	0.20	0.33	0.20	0.30	0.33	0.27	0.27	0.38	0.26	0.18	0.32	0.37	0.24	0.26	0.26	0.25	0.36	0.32	0.44	0.31	0.27	0.42	1			
G26	0.10	0.20	0.15	0.35	0.20	0.19	0.32	0.23	0.15	0.25	0.07	0.17	0.26	0.27	0.31	0.25	0.28	0.32	0.23	0.29	0.38	0.33	0.40	0.39	0.24	1		
G27	0.14	0.17	0.11	0.25	0.26	0.18	0.24	0.23	0.14	0.21	0.17	0.21	0.26	0.30	0.22	0.21	0.24	0.29	0.19	0.22	0.26	0.27	0.34	0.30	0.30	0.32	1	
G28	0.11	0.17	0.11	0.27	0.25	0.14	0.20	0.27	0.18	0.24	0.16	0.11	0.20	0.29	0.29	0.21	0.18	0.27	0.24	0.25	0.20	0.25	0.30	0.31	0.31	0.27	0.58	1

G-1= GP-7, G-2= GP-28, G-3= GP-29, G-4= GP-30, G-5= GP-32, G-6= GP-41, G-7= GP-48, G-8= GP-53, G-9= GP-63, G-10= GP-66, G-11= GP-70, G-12= GP-71, G-13= GP-83, G-14= GP-87, G-15= GP-90, G-16= GP-113, G-17= GP-125, G-18= GP-127, G-19= GP-131, G-20= GP-141, G-21= GP-149, G-22= GP-168, G-23= GP-169, G-24= GP-175, G-25= GP- 191, G-26= Gujarat Ajwain-1, G-27= Pratap Ajwain-1, G-28= Local check.

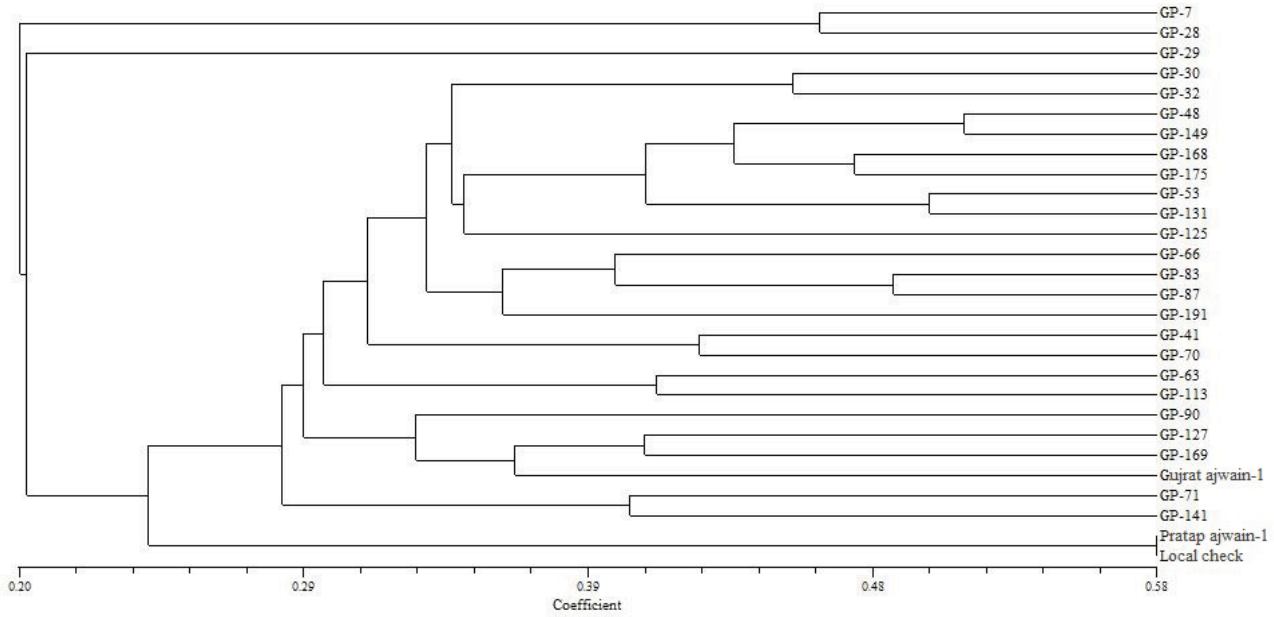

Fig. 1. Dendrogram generated for ajwain genotypes using UPGMA cluster based on Jaccard similarity coefficient (RAPD analysis)

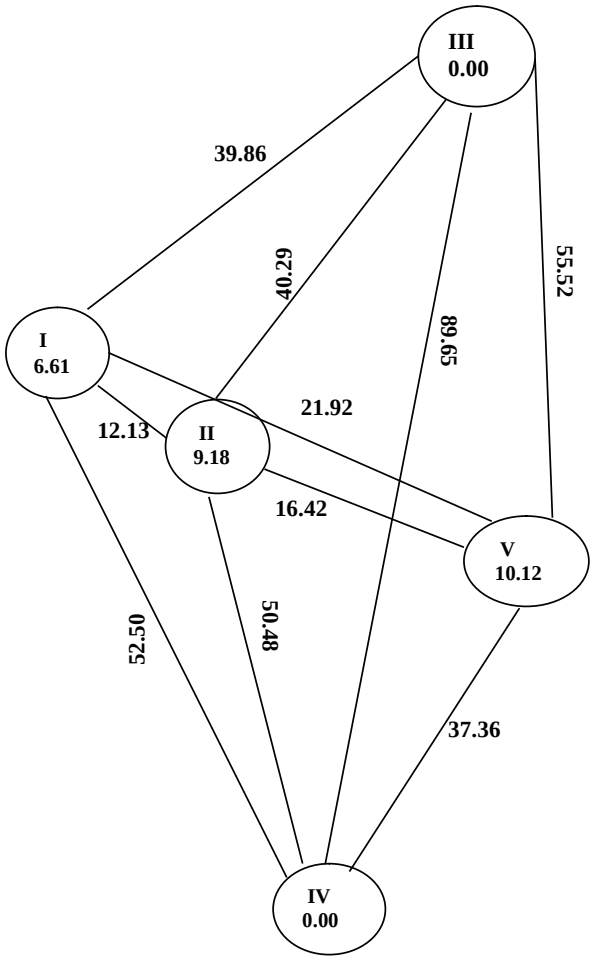

Fig. 2. Cluster diagram for 28 genotypes of ajwain based on Mahalonobis D^2 analysis

The results of the present investigation could also be used as a stepping stone for evolving a well-defined approach based on evaluation and characterization of genetic variation in ajwain, which is one of the important minor seed spices crop.

On the basis of results, it might be concluded that RAPD profile of ajwain genotypes *viz.,* GP-7, GP-28, GP-113, GP-29, GP-70, Gujarat ajwain-1, Pratap ajwain-1 and local can be used for the diversity studies. Further, the groups/clusters obtained by dendrogram could also be distinguished by similarity coefficient. The most dissimilar genotypes GP-113, GP-7, Gujarat ajwain-1 and GP-70. (similarity co-efficient 0.07) could be utilized to breeding programmes.

RAPD analysis revealed that 28 ajwain genotypes had high polymorphism (100%) and morphological analysis also had high variability (78%) and both analyses conclude that high variability could be used for seed yield improvement.

Conclusion

To conclude, characters like umbellates per umbel, secondary branches, and biological yield per plant had high estimates of all variability parameters and were strongly correlated with each other having maximum direct effects towards seed yield appeared promising to contribute genetic diversity in ajwain.

On the basis of D^2 analysis, it was found that genotypes *viz.,* GP-29 and GP-141 were highly divergent whereas these genotypes had low *per se* performance for seed yield and its contributing characters. Genotypes *viz.,* GP-7, GP-113, Gujarat ajwain-1 and Pratap ajwain-1 showing moderate divergence in D^2 analysis, were found high divergent in RAPD analysis and also had high *per se* performance for seed yield and its contributing characters like umbellates per umbel, secondary branches, and biological yield per plant. Results obtained through RAPD analysis appeared highly precise and accurate hence the genotypes identified can be selected for further breeding programme in ajwain.

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