Genetic Divergence Analysis in Perilla
[*Perilla frutescens* (L.) Britton]

SAFIQUL HUSSAIN[*], S. CHANGKIJA[1], D. K. HORE[2]

ABSTRACT

Divergence analysis using Mahalanobi's D^2 statistic was carried out in Fifty Perilla (*Perilla frutescens* (L.) Britton) accessions, collected from different parts of northeastern region of India including one exotic collection from Australia. All the fifty genotypes were grouped into nine clusters depending on their genetic distances. Interestingly, the cluster-I comprised of forty two genotypes, while the remaining eight genotypes were distributed over eight different clusters. The clustering pattern of the genotypes indicates that genetic diversity does not necessarily relate to geographical diversity. Inter cluster distances varied from 11.16 between cluster-IV and cluster-V to 34.02 between cluster-III and cluster-VIII. The contribution of individual traits to the divergence reveals that 1000-seed weight contributed maximum (31.18%) towards genetic divergence followed by days to maturity (19.84%) and number of leaves per plant (18.69%).

Key words: Perilla, germplasm, divergence analysis, genetic diversity, geographic diversity

INTRODUCTION

Perilla frutescens (L.) Britton (Lamiaceae) is a self pollinated annual, bushy and aromatic herbaceous oilseed crop. According to Godin and Spensley (1971), the crop is a native of India and China. Although, the wild ancestral species of the cultivated *Perilla* is unknown, Makino (1961) suggested that the crop probably originated in China because China is the main area of diversity of *Perilla* (Zeven and de Wet, 1982) and the history of cultivation of this crop is very old in China (Li, 1969). The species is distributed in the humid tropical, sub-temperate and temperate climates of the Himalayan region of India, Nepal, Southeast Asia, China, Korea, Japan and Taiwan within the altitude range of 300 m to 3500 m. In India, it is cultivated in an unorganized manner to a very limited scale in the northeastern hill region, Kumaon, Garhwal and Himachal Pradesh. The local hilly people of these regions grow *Perilla* in certain pockets under *jhum* (shifting) cultivation or in kitchen garden to use as condiments.

Perilla seeds contain 35-54% of a drying oil, similar to linseed oil, which is a rich source of protein and fat (Longvah and Deosthale, 1991). The seed oil is used as edible oil as well as it has got some industrial uses in the manufacture of paints, varnishes, linoleum, printing ink, etc. Being a rich source of oil and protein, the seeds are much relished by the hill people of this region in the form of *chutney* (sauce). In Nagaland state, it is traditionally used for dying purpose.

Genetic divergence provides a rational basis for selection of parents in any hybridization programme. Mahalanobi's D^2 analysis appears to be the best tool for assessing genetic divergence. Hence, an experiment was conducted to assess the genetic divergence in fifty *Perilla* accessions.

MATERIALS AND METHODS

The materials for the present investigation comprised of fifty *Perilla* germplasm accessions collected from different parts of northeastern region

* Regional Agricultural Research Station, Karimganj- 788710, Assam
[1] Department of Genetics and Plant Breeding, Nagaland University, Medziphema- 797112, Nagaland
[2] NBPGR Regional Station, Umroi Road, Umiam, Meghalaya-793103
*Corresponding author's Email: safiqulh@gmail.com

of India including one exotic collection from Australia (Table 1). The experiment was conducted in two consecutive years using a Randomized Block Design (RBD) with two replications. Seeds of all the fifty accessions were sown on 4th May during both the years of experimentation i.e., 2005 and 2006. A plot consisted of four rows of 2 m length, spaced 50 cm apart. Subsequently, the crop was thinned out to maintain a plant-to-plant distance of 20-25 cm. Well rotten compost was applied @ 5 t

Table 1: List of *Perilla* genotypes included in the present investigation along with their place of collection

Sl. No.	Accession No.	IC/EC No.	Date of collection	Place of collection	Entry No.
1.	BDS-1647	IC-006444	28-03-1991	Tuensang Nagaland	1
2.	BDS-1650	IC-006447	21-03-1991	Wokha, Nagaland	2
3.	BDS-1644	IC-006441	21-03-1991	Khonoma, Kohima, Nagaland	3
4.	H-1756	IC-003913	19-11-1990	Tuikhuralu, Mizoram	4
5.	H-3944	IC-211608	20-12-1997	Tidding, Lohit, Arunachal Pradesh	5
6.	H-1796	IC-003942	21-11-1990	Kawnpui, Mizoram	6
7.	BDS-1649	IC-006446	19-03-1991	Phek, Nagaland	7
8.	BDS-1643	IC-006440	16-03-1991	Kiruphema, Kohima, Nagaland	8
9.	H-1696	IC-003865	17-11-1990	Khawzawl, Saiha, Mizoram	9
10.	H-1748	IC-003908	19-11-1990	Seling, Mizoram	10
11.	BDS-1646	IC-006443	18-03-1991	Chizami, Phek, Nagaland	11
12.	H-3639	IC-204185	19-11-1996	Bomdila, West Kameng, Arunachal Pradesh	12
13.	BDS-1645	IC-006442	18-03-1991	Phek, Nagaland	13
14.	H-1812	IC-003955	21-11-1990	Kolasib, Mizoram	14
15.	H-2216	IC-012640	1991	Nagaland	15
16.	H-664	IC-521282	02-10-1988	Anutangree, Phek, Nagaland	16
17.	H-1143	IC-521283	27-03-1989	Mizoram	17
18.	H-621	IC-521284	01-10-1988	Lephori, Phek, Nagaland	18
19.	H-1099	IC-521285	27-03-1989	Mizoram	19
20.	H-556	IC-521286	06-10-1988	Akhegowa, Phek, Nagaland	20
21.	GP-178	IC-521287	Sept., 1988	Meghalaya	21
22.	NH-6/10	IC-521288	23-11-1986	Mokokchung, Nagaland	22
23.	DRLT-1524	IC-416861	19-12-2003	Mariyang, Upper Siang, Arunachal Pradesh	23
24.	FDI/RS-250	IC-419606	22-12-2003	Pungro, Kiphire, Nagaland	24
25.	FDI/RS-243	IC-419598	21-12-2003	Pungro, Kiphire, Nagaland	25
26.	FDI/RS-132	IC-419475	18-12-2003	Solumi, Kiphire, Nagaland	26
27.	FDI/RS-05	IC-419701	15-12-2003	Kiphire, Kiphire, Nagaland	27
28.	FDI/RS-212	IC-419564	20-12-2003	Pungro, Kiphire, Nagaland	28
29.	FDI/RS-134	IC-419477	18-12-2003	Solumi, Kiphire, Nagaland	29
30.	FDI/RS-046	IC-419706	16-12-2003	Chomi, Kiphire, Nagaland	30
31.	H-794	IC-521289	06-10-1988	Wokha, Nagaland	31
32.	BDS-837	IC-521290	Dec., 1988	Balek, Arunachal Pradesh	32
33.	RS-12/96	IC-204210	09-12-1996	Lunglei, Mizoram	33
34.	H-746	IC-521291	04-10-1988	Tuensang, Nagaland	34
35.	H-529	IC-521292	29-09-1988	Chipiketo, Nagaland	35
36.	EC-216268	EC-216268	-	Australia	36
37.	RS-72/99	IC-335408	17-12-1999	Lawngthlai, Mizoram	37
38.	RS-66/99	IC-335402	11-12-1999	Lunglei, Mizoram	38
39.	USK-415	IC-330441	03-12-2001	Shanshak, Ukhrul, Manipur	39
40.	USK-419	IC-330445	03-12-2001	Shanshak, Ukhrul, Manipur	40
41.	FDI/AP-01	IC-334313	12-01-2001	Disi village, West Siang, Arunachal Pradesh	41
42.	VRB-MA-2029	IC-521293	29-09-1988	Chipiketo, Nagaland	42
43.	RD-89	IC-374609	19-12-2002	Chaural, Saiha, Mizoram	43
44.	RD-71	IC-374590	18-12-2002	Newlaty, Saiha, Mizoram	44
45.	RD-117	IC-374494	20-12-2002	Thlatlang, Saiha, Mizoram	45
46.	RD-74	IC-374593	18-12-2002	Newlaty, Saiha, Mizoram	46
47.	RD-29	IC-374543	17-12-2002	Zwangling, Chintuipui, Mizoram	47
48.	RD-134	IC-374513	21-12-2002	Darzo, Lunglei, Mizoram	48
49.	RS/SB/BP-61	IC-369352	19-11-2002	Zote, Champhai, Mizoram	49
50.	RS/SB/BP-59	IC-369349	19-11-2002	Champhai, Mizoram	50

ha^{-1} one month before final land preparation. Inorganic fertilizers were applied @ 40:40:20 kgha^{-1} of N:P$_2$O$_5$:K$_2$O. Five randomly chosen plants from each entry in each replication were tagged for recording observations on twelve morphological and yield traits. The data were pooled and subjected to Multivariate analysis using Mahalanobi's (1936) D^2 statistic, which was further described by Rao (1952). The criterion used in clustering was that any two genotypes belonging to the same cluster must, at least on the average show a smaller intra cluster distance than those belonging to two different clusters, so they would vary very little from one another with regard to the aggregate of characters.

RESULTS AND DISCUSSION

Genetic diversity provides a rational basis for selection of parents in hybridization programmes. In the present investigation all the fifty genotypes were grouped into nine clusters depending on their genetic distances indicating the presence of considerable amount of genetic diversity in the materials under investigation. Cluster composition and intra cluster D^2 are presented in Table 2 and inter cluster distances in Table 3. The statistic distances among the fifty genotypes based on square root of average D^2 have been presented diagrammatically in Fig. 1.

Table 2: Clustering pattern of genotypes on the basis of genetic divergence in *Perilla*

Clusters	D^2	No. of genotypes	Genotypes	Place of collection of the genotypes with number
I	13.60	42	IC-006444, IC-006441, IC-003913, IC-211608, IC-003942, IC-006440, IC-003865, IC-003908, IC-006443, IC-204185, IC-006442, IC-003955, IC-012640, IC-521282, IC-521283, IC-521284, IC-521285, IC-521286, IC-521287, IC-521288, IC-416861, IC-419606, IC-419598, IC-419475, IC-419701, IC-419564, IC-419477, IC-521289, IC-521290, IC-204210, IC-521291, IC-521292, EC-216268, IC-335408, IC-335402, IC-330441, IC-330445, IC-334313, IC-521293, IC-374609, IC-374494, IC-374513	Arunachal Pradesh (5) Manipur (2) Meghalaya (1) Mizoram (13) Nagaland (19) Unknown (1) Exotic (1)
II	0	1	IC-006447	Nagaland (1)
III	0	1	IC-006446	Nagaland (1)
IV	0	1	IC-369349	Mizoram (1)
V	0	1	IC-369352	Mizoram (1)
VI	0	1	IC-419706	Nagaland (1)
VII	0	1	IC-374543	Mizoram (1)
VIII	0	1	IC-374590	Mizoram (1)
IX	0	1	IC-374593	Mizoram (1)
Total		50		

Table 3: Intra (bold) and inter cluster distances among different clusters in *Perilla*

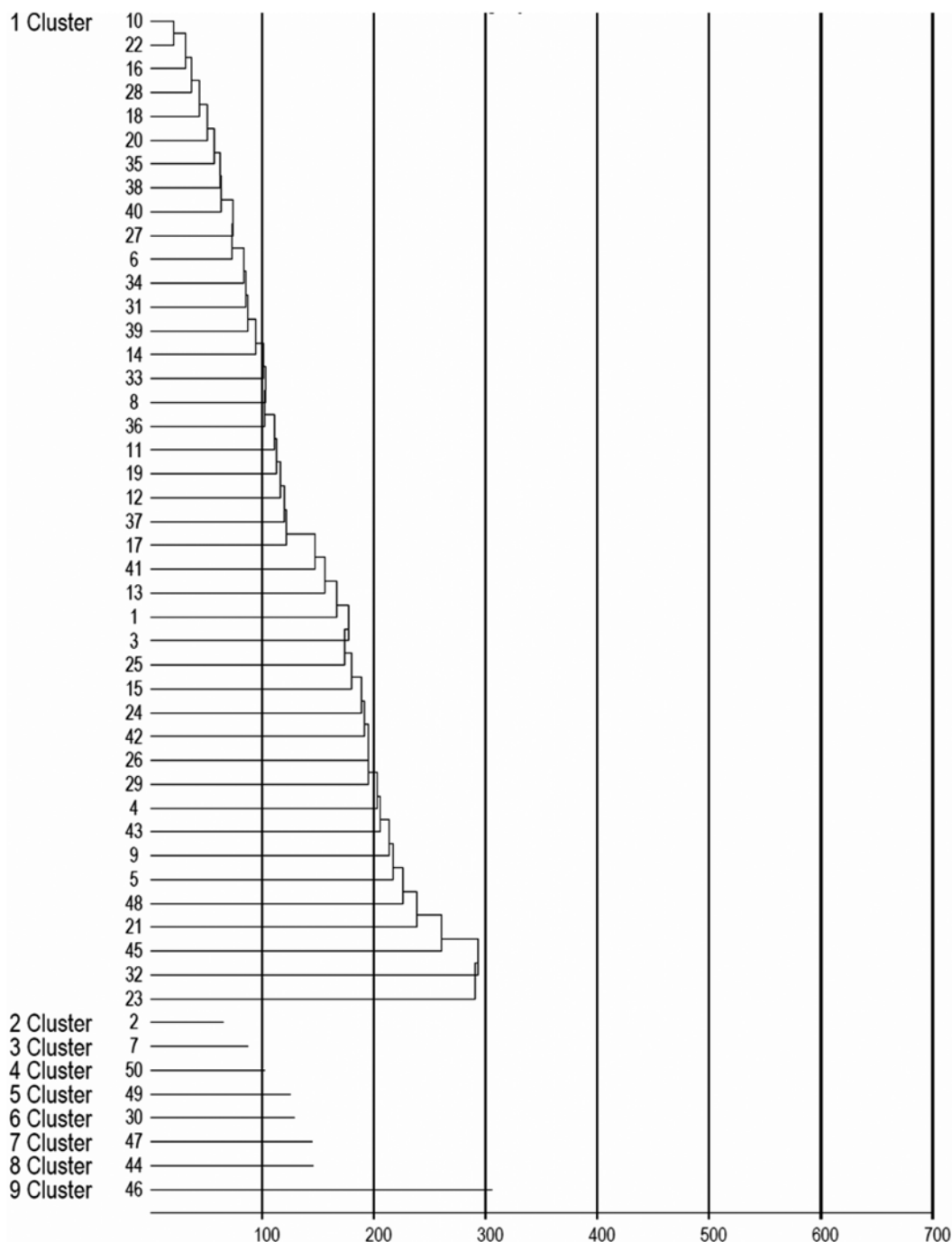| Clusters	I	II	III	IV	V	VI	VII	VIII	IX
I	**13.60**	21.29	19.08	17.54	20.55	20.86	19.17	23.02	24.21
II		**0.00**	15.81	26.26	27.57	23.17	28.30	32.87	20.04
III			**0.00**	26.66	29.65	18.04	28.83	34.02	26.63
IV				**0.00**	11.16	22.69	12.32	13.75	22.57
V					**0.00**	28.20	15.18	12.05	20.39
VI						**0.00**	30.46	31.79	31.39
VII							**0.00**	12.76	19.80
VIII								**0.00**	21.13
IX									**0.00**

Fig. 1: Clustering by Tocher method- A diagrammatic representation

The results of the present investigation indicated that cluster-I comprised of forty two genotypes collected from different parts of northeastern region of India including the only exotic collection (EC-216268), while the remaining eight genotypes were distributed over eight different clusters. Contrary to this, the genotypes from same geographical area were grouped in different clusters as revealed by the clusters II, III and VI. The single genotypes included in these three clusters namely IC-006447, IC-006446 and IC-419706, respectively were collected from the state of Nagaland. Similarly, the genotypes IC-369349, IC-369354, IC-374543, IC-374590 and IC-374593 belonging to clusters IV, V, VII, VIII and IX, respectively were from the state of Mizoram. The clustering pattern of the genotypes

indicates that genetic diversity does not necessarily relate to geographical diversity, which was also reported by Park *et al.* (2007) and Nitta *et al.* (2003) in *Perilla*; Paliwal and Jain (2006) in ajowan (*Trachyspermum ammi*); Murty and Quadri (1966) in *Brassica*; Murty and Arunachalam (1966) in a number of crop plants, etc.

The result also reveals considerably smaller estimates of intra-cluster distances than the inter-cluster distances (Table 2 and Table 3), which confirmed the genuineness of clustering pattern. Cluster-I showed an intra-cluster distance of 13.60, while the other clusters comprised of single genotype. Inter cluster distances varied from 11.16 between cluster-IV and cluster-V to 34.02 between cluster-III and cluster-VIII. The inter cluster distances were also higher between clusters II and VIII (32.87), VI and VIII (31.79), VI and IX (31.39), VI and VII (30.46), III and V (29.65), etc. The selection of diverse genotypes from the above clusters showing higher inter cluster distances would produce a broad spectrum of variability for morphological and yield traits which may enable selection and improvement in this crop.

The estimates of cluster means of the twelve characters (Table 4) considered for the investigation can be utilized to identify diverse and agronomically superior genotypes. The genotype (IC-006447) included in cluster-II exhibited maximum seed yield and number of leaves per plant, whereas, the genotype (IC-006446) included in cluster-III produced tallest plants with longest main inflorescence and the boldest seeds. Similarly, the genotype (IC-369352) included in cluster-V exhibiting maximum number of primary branches

and inflorescences per plant with earliest flowering and maturity. It can therefore, be concluded that hybridization among genotypes of these cluster combinations is not only expected to enhance variability in *Perilla* for the targeted traits but will also provide an opportunity to select superior recombinants for various traits in this crop.

The contribution of individual traits to the divergence assessed on the basis of number of times it appeared first is presented in Table 5, which reveals that 1000-seed weight contributed maximum (31.18%) towards genetic divergence followed by days to maturity (19.84%) and number of leaves per plant (18.69%). Hence, selection may be practiced based on these characters in order to realize maximum gain under selection.

CONCLUSION

Genetic divergence, assessed through Mahalanobi's D^2 statistic, grouped the experimental materials into nine clusters. The clustering pattern of the genotypes indicates that genetic diversity does not necessarily relate to geographical diversity. The result also reveals considerably smaller estimates of intra-cluster distances than the inter-cluster distances, which confirmed the genuineness of the clustering pattern. Maximum inter-cluster distance of 34.02 was observed between cluster-III and cluster-VIII, followed by between cluster-II & cluster-VIII (32.87), cluster-VI & cluster-VIII (31.79), cluster-VI & cluster-IX (31.39), cluster-VI & cluster-VII (30.46), cluster-III & cluster-V (29.65), etc. The estimates of cluster means revealed

Table 4: Estimates of cluster means of twelve characters in *Perilla* based on the data pooled over two years, 2005 and 2006

Clusters	Days to 50% flowering	Plant height (cm)	No. of leaves / plant	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	No. of primary branches / plant	No. of inflore-scences / plant	Length of main inflores-cence (cm)	Days to maturity	1000 seed weight (g)	Seed yield / plant
I	160.55	217.92	102.18	11.67	9.74	6.02	19.05	152.64	8.08	201.65	1.39	19.38
II	157.25	237.34	263.67	12.12	10.26	6.90	21.75	169.58	7.56	200.00	1.40	26.82
III	167.75	255.50	174.34	12.81	10.38	6.01	21.09	139.83	10.61	206.00	1.66	14.57
IV	161.00	168.42	79.50	11.40	9.40	5.62	24.34	151.67	7.13	201.00	0.83	10.41
V	156.75	180.76	121.25	9.60	7.79	4.40	26.34	203.00	7.01	193.75	0.90	22.85
VI	169.25	190.09	93.01	13.78	12.28	7.05	22.59	173.42	8.73	218.25	1.54	10.66
VII	160.75	186.67	80.50	10.75	8.11	4.17	17.59	125.84	6.31	198.75	0.68	12.80
VIII	159.25	173.09	61.25	11.00	9.05	4.28	17.00	191.92	6.40	196.50	0.66	12.80
IX	158.00	195.67	227.26	10.37	8.37	4.07	15.17	195.50	6.11	196.25	0.75	16.96

Table 5: Contribution of different characters in cluster formation in *Perilla*

Source	Times Ranked 1st	Contribution (%)
Days to 50% flowering	12	0.98
Plant height (cm)	79	6.45
No. of leaves/ plant	229	18.69
Leaf length (cm)	4	0.33
Leaf breadth (cm)	42	3.43
Petiole length (cm)	44	3.59
No. of primary branches/ plant	61	4.98
No. of inflorescences/ plant	46	3.76
Length of main inflorescence (cm)	36	2.94
Days to maturity	243	19.84
1000-seed weight (g)	382	31.18
Seed yield per plant (g)	47	3.84
Total		100.01

that cluster-II accommodated the genotype (IC-006447) with maximum seed yield and number of leaves per plant; cluster-III included the genotype (IC-006446) with maximum plant height, length of main inflorescence and 1000-seed weight. Similarly, the genotype (IC-369352), included in cluster-V, exhibited maximum number of primary branches and inflorescences per plant with earliest flowering and maturity. The results of the present investigation revealed maximum contribution of 1000-seed weight, maturity duration and number of leaves per plant towards genetic divergence. It can therefore, be concluded that hybridization among genotypes of these cluster combinations is likely to enhance variability for targeted traits as well as provide an opportunity to select superior recombinants for various traits in this crop.

REFERENCES

Godin, N. J. and Spensley, P. C. (1971). Oils and Oilseeds. *In. Crop and Product Digests No. 1*, Tropical Products Institute, pp. 104-105.

Li, H. L. (1969). The vegetables of ancient China. *Economic Botany,* 23: 235-260.

Longvah, T. and Deosthale, Y. G. (1991). Chemical and nutritional studies on Hanshi (*Perilla frutescens*), a traditional oilseed from northeast India. *Journal of the American Oil Chemists' Society,* 68 (10): 781-784.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proceeding of National Institute of Science (India),* 2: 49-55.

Makino, T. (1961). In. *Makino new illustrated flora of Japan, Hokuryu-kan Publi., Tokyo.*

Murty, B. R. and Arunachalam, V. (1966). The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet.,* 26A (Spl. No.): 188-198.

Murty, B.R. and Quadri, M.T., 1966, Analysis of divergence in some self compatible forms of *Brassica campestris* L. var. Brown sarson. *Indian Journal of Genetics and Plant Breeding,* 26: 45-58.

Nitta, M.; Lee, J. K. and Ohnishi, O. (2003). Asian *Perilla* crops and their weedy forms: their cultivation, utilization and genetic relationships. *Economic Botany,* 57 (2): 245-253.

Paliwal, R. V. and Jain, U. K. (2006). Genetic divergence analysis in ajowan [*Trachyspermum ammi* (L.) Sprague]. *Journal of Spices and Aromatic Crops,* 15(2): 105-107.

Park, Y.; Dixit, A.; Ma, K.; Lee, J.; Lee, M.; Chung, C.; Nitta, M.; Okuno, K. Kim, T.; Cho, E. and Rao, V. R. (2007). Evaluation of genetic diversity and relationships within an on-farm collection of *Perilla frutescens* (L.) Britt. using microsatellite markers. *Genetic Resources and Crop Evaluation, published online dated 18th July 2007.*

Rao, C. R. (1952). In. *Advanced Statistical Methods in Biometrical Research, John Wiley & Sons, New York.*

Zeven, A. C. and De Wet, J. M. J. (1982). In. *Dictionary of cultivated plants and their regions of diversity.* Cent. for Agric. Publishing and Documentation, Wageningen, the Netherlands.