# Evaluation of genetic variation in *Perilla* for agro-morphological and quality traits

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#### ABSTRACT

Perilla is an important underutilized oilseed crop cultivated by tribal farmers in hilly areas of north-eastern region of India. It is grown by tribal and marginalised farmers in the traditional form of agriculture. The crop has good oil quality and plays an important role in providing nutritional security to the tribal farmers. There is a need to evaluate the traditional landraces grown by tribal farmers for studying the important traits which can help develop high yielding variety with quality traits. For this purpose, 29 Perilla genotypes were evaluated to assess the genetic variation. Wide spectrum of variability was observed for all 16 characters. The genotypic variance was moderate to high for the characters resulting in high heritability and moderate to high genetic advance values. Seed yield per plant positively correlated with all the traits except days to 50% flowering, leaf width, petiole length and seed oil content. The Perillagenotypes were grouped into seven distinct clusters. An analysis of the percentage contribution of individual characters towards genetic diversity revealed that seed yield per plant, Mn content and Cu content were the major characters contributing to genetic diversity in Perilla. Based on the results it is concluded that Perilla genotypes exhibit a widerange of variability for most of the traits. Some genotypes possessed desirable genes for more than one character and hence could be utilized directly or included in hybridization programme.

Key words: Perilla, Quality traits, Seed oil content, Underutilized crop

Perilla frutescens (L.) Britton of family Lamiaceae is a self-fertilizing annual, bushy, aromatic herbaceous oilseed crop. It is mainly found in 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 300–3500 m. In India, it is cultivated in very small pockets in parts of North Eastern Hill Region (NEHR), hilly tract of Uttarakhand (Kumaon, Garhwal) and Himachal Pradesh. In NEHR, Perilla is mostly grown under jhum(shifting) cultivation or in kitchen garden.

Perilla crop includes two major varieties distinguished by their morphology and plant type. P. frutescens var. frutescens is used as an oil crop, whereas P. frutescens

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var. *crispa* is used as a medicine or vegetable crop. *Perilla* seeds contain 35–54% drying oil along with high protein content (Longvah and Deosthale 1991). The seeds are rich source of essential fatty acid such as omega-3 essential fatty acid, alpha-linolenic acid. Besides being source of edible oil, it is used in industries for making paints, varnishes, linoleum, printing ink, etc. Biochemical profiling of *Perilla* identified several potential chemotypes such as apigenin, ascorbic-acid, beta-carotene, caffeic-acid, citral, dillapiol, elemicin, limonene, luteolin, myristicin, perillaldehyde, protocatechuic-acid, quercetin, rosmarinic-acid, perilla ketone, elsholzia ketone, isoegomaketone, naginata ketone, and safrole (Asif 2012).

Variation in local agro-climatic conditions of NEHR offers wide range of genetic variability with respect to different agro-morphological and qualitative traits including seed yield, oil content and quality of oil. It is pertinent to decipher the nature and magnitude of genetic variability and other genetic parameters in the indigenous landraces. Estimation of genetic divergence among available landraces of *Perilla* could provide a rational basis for selection of parents in genetic improvement programme. The present study, was conducted to assess extent of diversity, genetic parameters of variability andassociation among various agro-morphological and quality traits.

#### MATERIALS AND METHODS

Twenty nine genotypes of *Perilla* were evaluated during post rainy season for two consecutive years (2015-17) at the experimental farm of ICAR Research Complex for NEH Region, Manipur Centre. The experiment was laid out in a randomized block design (RBD) with three replications. Plants were grown in a gross plot size of 5 m × 6 m with a spacing of  $50 \text{ cm} \times 40 \text{ cm}$ . The recommended agronomic practices and plant protection measures were followed to ensure a normal crop growth. Observations were recorded for plant height, leaf length, leaf width, petiole length, number of primary branches, days to 50% flowering, number of inflorescence per plant, inflorescence length, days to 80% maturity, seed yield per plant, 100 seed weight, seed oil content, Cu content, Fe content, Zn content and Mncontent on ten randomly selected plants in each treatment across all replications. Mean values were taken for analysis of variance as per Panse and Sukhatme (1978). Phenotypic and genotypic variance of the genotypes were estimated as described by Burton and Devane (1953), heritability as per Hanson et al. (1956) and genetic advance was estimated using the formula suggested by Johnson et al. (1955). The genotypic and phenotypic correlation coefficient and path coefficient were estimated as suggested by Dewey and Lu (1959). The data were subjected to analysis of genetic divergence through D<sup>2</sup> statistic (Mahalanobis 1936) to measure genetic divergence as suggested by Rao (1952), while Tocher's method was used to form clusters.

## RESULTS AND DISCUSSION

Genetic variability, heritability and genetic advance: The exploration of variability is a pre-requisite for effective screening of superior genotypes. The yield and its contributing characters of any crop are polygenically controlled, environmentally influenced and determined by the magnitude and nature of their genetic variability. Hence, it is essential to partition the overall variability into its heritable and non-heritable components with the help of genetic parameters like genetic coefficient of variation, heritability and genetic advance. Analysis of variance revealed that genotypic differences were significant for all the characters. The estimates of range, mean, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance are presented in Table 1. Considerable range of variation was observed for all the traits under study indicating enough scope for bringing about improvement in the desired direction. Maximum oil content (47.89%) was observed in genotype IC-0615390, while minimum (33.78%) in genotype IC-0615367. The number of inflorescence per plant ranged from 16-206. A range of 4-13 cm was observed among genotypes for the length of inflorescence. The results showed a significant content of zinc and iron. Manganese was also found in more quantity as compared to other oilseed crops which assists the body in metabolizing protein and carbohydrates. Fe content in seeds among genotypes varied from 3.8 to 74.5 ppm whereas Mn content ranged from 21.05-52.85 ppm.

Table 1. Variation in *Perilla* genotypes for agro-morphological and quality traits

Character	Mean	GCV	PCV	h²	GAM
				(b)	(5%)
Leaf length (cm)	10.18	16.56	17.69	0.88	31.92
Leaf width (cm)	11.45	15.19	16.01	0.90	29.67
No.of primary branches	8.72	37.61	40.54	0.86	71.89
Petiole length (cm)	6.55	13.45	15.68	0.74	23.77
Days to 50% flowering	88.38	14.77	15.04	0.97	29.89
Inflorescence length (cm)	8.46	19.78	20.71	0.91	38.92
No.of inflorescence/ plant	69.74	51.96	55.13	0.89	46.89
Plant height (cm)	116.84	14.52	17.47	0.69	24.87
Days to 80% maturity	132.75	4.60	4.65	0.98	9.35
Seed yield/plant (g)	10.94	51.08	53.19	0.91	42.14
100-seed weight (g)	0.17	19.85	20.07	0.98	40.46
Seed oil content (%)	42.01	8.57	8.63	0.99	17.53
Cu content (ppm)	12.42	12.76	12.85	0.99	26.09
Fe content (ppm)	26.74	32.60	38.48	0.72	56.90
Zn content (ppm)	32.04	8.77	8.84	0.99	17.95
Mn content (ppm)	33.68	21.11	21.24	0.99	43.21

\*GAM, Genetic advance as percent of mean.

IC-0615367 that had highest Fe content may be used as parent in future varietal development programme. In earlier study by Verma *et al.* (2008), significant difference was recorded in days to 50% flowering, leaf length, leaf width, number of primary branches, petiole length, inflorescence length, plant height, days to maturity, seed yield/plant and 100-seed weight. Bahuguna *et al.* (2014) also observed wide variation for days to 50% flowering, maturity duration, plant height, number of primary branches plant, 1000 seed weight and seed yield per plant in *Perilla*.

The PCV was high (>20%) for number of primary branches, number of inflorescence per plant, seed yield per plant, Fe content and Mn content, and moderate (10-20%) for leaf length, leaf width, petiole length, days to 50% flowering, inflorescence length, plant height, seed yield per plant, 100seed weight and Cu content. Highest coefficients of variation were observed for seed yield per plant followed by number of inflorescence per plant. Working with 25 accessions of Perilla, Sharma and Hore (1994) observed higher coefficient of variation for seed yield per plant (51. 51%). They observed low coefficient of variation for days to maturity and length of inflorescence. A high coefficient of variation for number of inflorescence/plant was also reported by Wei et al. (2017). Days to 80% maturity (4.6%) showed lowest PCV. Genotypic coefficients of variation closely followed the phenotypic coefficients of variation in all characters except yield/plant. This indicated the suppression of phenotypic expression under the influence of environment. High heritability was observed in all the characters except plant height. GAM was also found to be

Table 2 Correlation coefficients among 16 characters of Perilla

Character		2	3	4	5	9	7	8	6	10	11	12	13	14	15 S	Seed yield
Leaf length (cm)	Ь	0.479**	-0.176	0.509**	-0.391**	0.351**	-0.098	-0.117	-0.102	0.336	0.054	-0.026	-0.142	-0.210	0.544**	0.283*
	G	0.452	-0.287	0.480	-0.412	0.384	-0.150	-0.191	-0.109	0.373	0.057	-0.030	-0.155	-0.225	0.586	0.303
Leaf width (cm)	Ь		-0.295**	0.639**	0.195	0.004	-0.436**	0.240	-0.029	0.546	0.090	0.022	0.114	-0.005	0.083	-0.109
	G		-0.397	0.684	0.211	0.007	-0.526	0.239	-0.029	0.587	0.095	0.027	0.188	0.004	0.088	-0.113
No of primary branches	Ь			-0.148	-0.189	0.209	0.630**	0.375	0.174	0.009	-0.317	0.295	0.267	0.309	0.230*	0.562**
	Ŋ			-0.333	-0.199	0.236	0.627	0.396	0.187	0.015	-0.351	0.313	0.356	0.334	0.245	0.607
Petiole length (cm)	Ь				0.062	0.031	-0.435**	0.182	0.001	0.619	0.017	-0.100	-0.305	-0.107	0.125	-0.129
	G				0.080	0.030	-0.589	0.157	0.003	0.748	0.003	-0.122	-0.375	-0.123	0.146	-0.149
Days to 50% flowering	Ь					-0.706**	-0.333*	0.367	0.576	-0.065	0.404	0.064	-0.021	0.299	-0.335*	-0.489**
	Ð					-0.753	-0.358	0.455	0.597	-0.076	0.412	0.062	-0.047	0.304	-0.341	-0.499
Inflorescence length (cm)	Ь						0.308*	-0.065	-0.389	0.024	-0.196	-0.001	-0.111	-0.229	0.320*	0.291**
	Ŋ						0.364	-0.087	-0.404	0.020	-0.199	-0.005	-0.155	-0.250	0.339	0.303
No of inflorescence/plant	Ь							-0.053	0.223	-0.286	-0.129	0.195	0.087	0.154	0.311*	0.558**
	Ð							-0.128	0.234	-0.307	-0.139	0.192	0.132	0.160	0.318	0.594
Plant height (cm)	Ь								0.343	0.060	0.018	0.260	0.237	0.249	-0.022	0.012
	Ð								0.414	0.072	0.017	0.316	0.376	0.307	-0.032	0.013
Days to 80% maturity	Ь									-0.097	0.153	0.265	0.127	0.340	0.096	0.055
	G									-0.092	0.154	0.272	0.163	0.340	0.095	0.061
100 seed weight (g)	Ь										-0.280	0.108	0.149	-0.051	0.273*	0.323**
	Ð										-0.286	0.106	0.168	-0.053	0.281	0.325
Seed oil content (%)	Ь											-0.350	-0.441	-0.220	-0.227*	-0.396
	Ð											-0.356	-0.536	-0.223	-0.232	-0.400
Cu content (ppm)	Ь												0.433	0.650	0.274*	0.457
	Ö												0.511	0.653	0.274	0.462
Fe content (ppm)	Ь													0.482	0.138	0.384
	Ö													0.561	0.189	0.445
Zn content (ppm)	Ь														0.108	0.233
	Ð														0.109	0.235
Mn content (ppm)	Ь															0.659**
	Ŋ															0.665
,																

Significance levels: 0.05= \*; 0.01=\*\*

high and moderate for majority of the characters except for days to 80% maturity, seed oil content and Zn content. High heritability and genetic advance for the traits suggest the least effect of environment and probably prevalence of additive gene action. Such characters would be responsive to selection (Burton 1952, Johnson *et al.* 1955). Higher estimates of heritability coupled with high to moderate estimates of genetic advance as percentage of mean were observed for different traits in *Perilla* by Hussain *et al.* (2014).

Correlation coefficients and path coefficient analysis: Correlation coefficients were estimated among 16 traits in 29 genotypes of *Perilla* (Table 2). Seed yield per plant was positively correlated with all traits except days to 50% flowering, leaf width, petiole length and seed oil content. The association of number of seed yield per plant was significantly positive with inflorescence length, number of inflorescence per plant, 100 seed weight and Mn content while it was negative with days to 50% flowering. Negative association of number of inflorescence per plant with days to 50% flowering, petiole length and leaf width were observed. Seed oil content had negative association with inflorescence length, number of inflorescence per plant, 100 seed weight. Nam et al. (2004) observed positive correlations of 1000seed weight with plant height, number of stem nodes, branches, flower clusters and inflorescence length.

In the present investigation, the path coefficient analysis was performed to estimate the direct and indirect contribution of various plant characters to grain yield per plant. Path analysis is standardized partial regression coefficient which splits the correlation coefficient into the measures of direct and indirect effects of a set of independent variables on the dependent variable. Further compartmentalization of correlation coefficients into direct and indirect effects discerned the true nature of association between observed characters. Except petiole length, days to 50% flowering, inflorescence length and Fe content, all other characters exhibited positive direct effect on seed yield per plant (Table 3).

Seed weight had maximum direct effect followed by Mn content and number of primary branches. Leaf width had positive direct effect on seed yield per plant but its effect through petiole length made its association with seed yield per plant negative. Inflorescence length had negative direct effect on seed yield but it had significant negative correlation with days to 50% flowering which resulted in positive association with seed yield per plant. The correlation between yield per plant and a character, due to the direct effect of the character reflects a true relationship between them and selection may be practised for such a character in order to improve yield. But if the correlation is mainly due to the indirect effect of the character through another component trait, the selection should be practised for the latter trait through which indirect effect is exerted. Results of the correlation and path analysis suggest that selection of genotypes may reliably be done based on leaf width, number of seeds per pod, number of pods per plant, average

Table 3 Path coefficient showing direct (bold) and indirect effect of observed characters on seed yield/plant

											1				
Character	TT	ΓM	NPB	PL	DFF	IL	Z	PH	DM	SW	0	CO	FE	ZN	MN
Leaf length (cm); LL	0.116	0.056	-0.020	0.059	-0.045	0.041	-0.011	-0.014	-0.012	0.039	900.0	-0.003	-0.016	-0.024	0.063
Leaf width (cm); LW	0.019	0.039	-0.011	0.025	0.008	0.000	-0.017	0.009	-0.001	0.021	0.004	0.001	0.004	0.000	0.003
No of primary branches; NPB	-0.041	-0.068	0.232	-0.034	-0.044	0.049	0.146	0.087	0.040	0.002	-0.073	0.068	0.062	0.072	0.053
Petiole length (cm); PL	-0.222	-0.279	0.065	-0.437	-0.027	-0.014	0.190	-0.080	0.000	-0.270	-0.007	0.044	0.133	0.047	-0.055
Days to 50% flowering; DFF	0.237	-0.119	0.114	-0.038	-0.606	0.428	0.202	-0.223	-0.349	0.039	-0.245	-0.039	0.013	-0.181	0.203
Inflorescence length (cm); IL	-0.083	-0.001	-0.049	-0.007	0.167	-0.236	-0.073	0.015	0.092	-0.006	0.046	0.000	0.026	0.054	-0.076
No.of inflorescence/plant;NI	-0.012	-0.055	0.080	-0.055	-0.042	0.039	0.126	-0.007	0.028	-0.036	-0.016	0.025	0.011	0.020	0.039
Plant height (cm); PH	-0.010	0.021	0.033	0.016	0.032	-0.006	-0.005	0.087	0.030	0.005	0.002	0.023	0.021	0.022	-0.002
Days to 80% maturity; DM	-0.016	-0.005	0.028	0.000	0.091	-0.061	0.035	0.054	0.158	-0.015	0.024	0.042	0.020	0.054	0.015
100 seed weight (g); SW	0.170	0.277	0.005	0.314	-0.033	0.012	-0.145	0.031	-0.049	0.508	-0.142	0.055	0.076	-0.026	0.139
Seed oil content (%); O	900.0	0.010	-0.036	0.002	0.046	-0.022	-0.015	0.002	0.018	-0.032	0.115	-0.040	-0.051	-0.025	-0.026
Cu content (ppm); CU	-0.004	0.004	0.049	-0.017	0.011	0.000	0.032	0.043	0.044	0.018	-0.058	0.165	0.072	0.107	0.045
Fe content (ppm); FE	0.012	-0.009	-0.022	0.025	0.002	0.009	-0.007	-0.019	-0.010	-0.012	0.036	-0.036	-0.082	-0.040	-0.011
Zn content (ppm); ZN	-0.027	-0.001	0.039	-0.014	0.038	-0.029	0.020	0.032	0.043	-0.007	-0.028	0.083	0.061	0.127	0.014
Mn content (ppm); MN	0.138	0.021	0.058	0.032	-0.085	0.081	0.079	-0.006	0.024	0.069	-0.058	0.070	0.035	0.027	0.254
Seed yield/plant (g)	0.283	-0.109	0.562	-0.129	-0.489	0.291	0.558	0.012	0.055	0.323	-0.396	0.457	0.384	0.233	0.659

pod weight and plant height as these traits having positive association with yield are also directly contributing to yield.

Genetic divergence analysis: The 29 Perilla genotypes were grouped into seven clusters (Table 4). Maximum number of genotypes were retained in cluster I (21) followed by cluster IV (3). The contribution of individual traits to the divergence revealed that seed yield per plant contributed maximum (42.12%) towards genetic divergence followed by Mn content (16.75%) and Cu content (14.53%). Mean

performance of different characters for yield and yield contributing traits in *Perilla* in different cluster has been presented in Table 5. The results of D<sup>2</sup> analysis could be utilized in identifying the best parental combination for generating variability with respect to various traits under study. For creating wide spectrum of variability and improving the grain yield the genotypes in cluster I and II would be crossed with genotypes in cluster IV and VII. Accession IC- 0615390 present in cluster I has highest

Table 4 Inter- and intra- (bold) cluster distances (D2) and distribution of 29 genotypes of Perilla to different clusters

Cluster	I	II	III	IV	V	VI	VII	No.of	Genotypes
Ī	385.2	585.7	698.8	1706.5	672.0	949.8	1407.9	genotypes 21	IC-0615362 (RC Thoiding-1), IC-0615365 (RC Thoiding-4), IC-0615370 (RC Thoiding-9), IC-0615372 (RC Thoiding-11), IC-0615373 (RC Thoiding-12), IC-0615374 (RC Thoiding-13), IC-0615375 (RC Thoiding-14), IC-0615377 (RC Thoiding-16), IC-0615378 (RC Thoiding-17), IC-0615379 (RC Thoiding-18), IC-0615380 (RC Thoiding-19), IC-0615383 (RC Thoiding-22), IC-0615384 (RC Thoiding-23), IC-0615385 (RC Thoiding-24), IC-0615386 (RC Thoiding-25), IC-0615387 (RC Thoiding-26), IC-0615389 (RC Thoiding-28), IC-0615390 (RC Thoiding-29), IC-0615391 (RC Thoiding-30), IC-0615392 (RC Thoiding-31), IC-0615393 (RC Thoiding-32)
II		0	705.7	1918.9	1278.2	1630.8	1948.6	1	IC-0615368(RC Thoiding-7)
III			0	1475.9	943.6	767.4	740.1	1	IC-0615371(RC Thoiding-10)
IV				396.2	1217.0	1062.2	705.1	3	IC-0615364(RC Thoiding-3), IC-0615367(RC Thoiding -6), IC-0615376(RC Thoiding-15)
V					0	729.7	903.1	1	IC-0615381(RC Thoiding-20)
VI						0	441.9	1	IC-0615382(RC Thoiding-21)
VII							0	1	IC-0615369(RC Thoiding-8)

Table 5 Mean performance of different characters for yield and yield contributing traits

Character				Cluster			
	I	II	II	IV	V	VI	VII
Leaf length (cm)	9.98	7.5	8.5	10.67	12.75	11.5	13.5
Leaf width (cm)	11.6	8.5	10.5	12.17	15	8.5	9.5
No. of primary branches	8.12	10.0	9.5	11.33	11	7.5	10.5
Petiole length (cm)	6.61	5.5	5.0	7.17	8.5	5.5	5.25
Days to 50% flowering	90.03	99.33	95.0	82.33	96	60	75
Inflorescence length (cm)	8.35	6.00	10.0	8.5	9.75	9.25	9.5
No. of inflorescence/plant	59.43	67.5	106	76.33	98.5	148.5	125
Plant height (cm)	117.36	109.5	138.5	114.83	129.5	95.5	106.5
Days to 80% maturity	131.57	139.33	137.33	134.11	137	137.67	133
Seed yield/plant (g)	8.4	6.32	15.56	21.22	13.36	18.43	23.71
100 seed weight (g)	0.17	0.13	0.14	0.23	0.22	0.15	0.16
Seed oil content (%)	42.56	40.59	47.65	35.79	46.46	38.36	44.06
Cu content	11.96	14.03	13.87	15.22	10.32	10.88	14.2
Fe Content	24.35	33.85	28.45	42.04	24.35	24.68	26.77
Zn content	31.32	38.58	32.73	35.31	34.07	28.28	31.82
Mn Content	31.58	21.63	31.08	42.03	43.15	40.5	51.08

seed oil content and should be used in future breeding programmes for developing variety with high oil content. Accession IC-0615382 (cluster VI) may be used for breeding early variety in *Perilla*.

PCA is a powerful technique for data reduction which removes interrelationships among components. Results reported by various researchers showed multivariate analysis as a valid system to deal with germplasm collection. Smith *et al.* (1995) conducted average linkage cluster and principal component analyses, and reported the utility of these results in preservation and utilization of germplasm. In present study, principal components analysis performed on quantitative traits revealed that the first three most informative components accounted for 66.26% variance among 29 genotypes of *Perilla*.

In present study, *Perilla* genotypes exhibited a wide range of variability for most of the traits. Some genotypes possessed desirable genes for more than one character, hence, these could be utilized directly or included in hybridization programmes for the developing of variety suitable for North East Hill Region to ensure oilseed security among tribal farmers.

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