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Multivariate Analysis and Selection to Enquire Genetic Variation Patterns in *Nigella sativa*

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Abstract

Thirty two accessions of *Nigella sativa* L. germplasm were evaluated under field conditions for three years during 2009-2011. Principal component analysis (PCA) was carried out to form six, three and five PCs during the year 2009, 2010 and 2011 which showed variability of 87.85%, 79.75% and 79.42% of germplasm, respectively. The subdivision of the variance into components assisted in the genetic resources conservation and future utilization of appropriate gene pool in crop improvement for specific plant attributes. Genetic dissimilarities revealed by un-weighed pair group method of arithmetic means (UPGMA) divided germplasm into eight clusters based on three years of field evaluation. Multivariate analysis revealed that genetic variation was independent of origin and many accessions from the same vicinity are grouped separately. Accessions present in specific clusters could be used for better exploitation of economic traits and could be used as parents for further study of genetic variation or to be incorporated for gene recombination.

Keywords: Black seeds; Kalongi; Clustering pattern; Germplasm

Introduction

The genus *Nigella* (*Ranunculaceae* or buttercup of order *Ranales*) contains about 20 domesticated species of annual herbs indigenous to the Mediterranean region through West Asia to Northern India (Weiss, 2008).

Nigella sativa L. one of the member, is rich in nutritional values and contains several chemical constituents having properties to cure various diseases (Iqbal *et al*., 2011). The whole seed or their extracts have antitumor (Khan *et al*., 2009), antidiabetics (Fararh *et al*., 2008), spasmolytic and bronchodilator (Boskabady *et al*., 2010), anti-inflammatory (Hajhashemi *et al*., 2010), antibacterial (Mashhadian and Rakhshandeh, 2011), galactogogue, antioxidant (Brutis and Bucar, 2000; Kanter *et al*., 2003) and insect repellent effects (Fisher, 2008).

Variation in highly heritable characteristics and qualitative traits provide an estimate of diversity while quantitative traits are exploited to determine the magnitude of genetic variation within germplasm (Iqbal *et al*., 2010). Various numerical taxonomic techniques have been successfully used to classify and measure the pattern of phenotypic variation in the relationship of germplasm collections in a variety of crops by many scientists as in black gram (Weir, 1990), soybean (Perry and McIntosh, 1991), lentil (Ahmad *et al*., 1997) and pea (Nisar *et al*., 2009). Moreover, evaluation of available genetic stocks to assess the genetic variation for economically important characteristics is a pre-requisite for combining desirable genes in a single accession (Palevitch, 2004).

Biochemical studies have been conducted to assess polymorphism through randomly amplified polymorphic DNA and revealed low level of genetic diversity. However, very few studies were carried out to determine the phenotypic diversity which seems to be high within germplasm of this species. Therefore there was need to evaluate the germplasm for phenotypic variation. Phenotypic variation is based on multiple factors (Iqbal *et al*., 2011). Considering these, we analyzed 32 accessions for assessing extent of genetic variation by evaluation and characterization for agro-morphic traits to identify promising generic lines for future use.

Materials and Methods

Planting Material

N. sativa germplasm consisting of 32 accessions were planted under field conditions of Plant Genetic Resources Program, National Agricultural Research Center, Islamabad, Pakistan for consecutively three years from (2008-2009, 2009-2010 and 2010 to 2011). This center is situated 33^{o} 44' N latitude and 73^{o} 08' E longitudes at an altitude of 540 masl. The 32 accessions evaluated during this study are presented in the Table 1.

Experiment Layout

N. sativa germplasm was planted in randomized complete block design (RCBD) with four replications. Three rows of

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each accession were planted in 4 meter bed length, 20 cm plant to plant distance, whereas row distance was 30 cm, fifty cm distance was kept to separate two accessions.

Evaluation of Morpho-physiological Traits

Morpho-physiological traits/agronomic data for days to first flower, days to 50% flowers, days to maturity, flowering duration were recorded on plot basis, while plant height, biological yield, number of branches, capsule weight, number of capsules, capsule length, capsule width, number of locules, root weight, root length, 1000 seed weight and grain yield were recorded on 10 randomly sampled plants from each accession. For 1000 seed weight, seeds from the single plant were bulked and counted. Five healthy capsules from each plant were selected at random for recording of capsule length, capsule width and number of locules. Harvest index was expressed as a ratio between grain yield and biological yield. The morpho-physiological traits (units, notions/codes, when measured and description of the traits) recorded are presented in the Table 2.

Statistical Analysis

Principal component analysis was performed by computer software "SPSS" version 12 and Cluster analysis by "Statistica" version 6.0 for windows XP Professional. Genetic distances based on Euclidean distances between pairs of accessions and cluster analyses were obtained using Number Cruncher Statistical system, NCSS 2000 (Hintze, 2000).

Results

Multivariate Analysis

Principal component analysis: Six components with Eigen values >1 contributed 87.85% of the variability among 32 accessions evaluated for 17 morpho-physiological traits during 2009 (Table 3). Traits that showed positive contribution to PC_1 were days to first flower, days to 50% flowers, days to maturity, capsule weight, root length and grain yield, while seed weight and flowering duration contributed negatively. Biomass and harvest index contributed high variability for PC_2. Root weight exhibited high contribution towards PC_3, while capsule width showed negative contribution. Plant height and number of capsules contributed positively, while capsule length exhibited negative contribution for PC_4. For PC_5, number of locules contributed high to this principal component and number of branches (0.74) contributed high for PC_6.

First three components with eigen values >1 contributed 79.75% of the variability amongst 32 accessions evaluated for 17 morpho-physiological traits during 2010 (Table 4). Days to first flower, days to 50% flowers, days to maturity, flowering duration, number of branches, capsule length, root weight, and root length showed positive contribution to the variability in PC_4. The plant height, biomass, capsule weight, number of capsules, capsule width, number of locules, 1000 seed weight, and total yield contribution was high in PC_2. Harvest index exhibited highly positive contribution for PC_3.

During 2011, five principal components with eigen values >1 contributed 79.42% of the variability among 32 accessions (Table 5). Traits that showed positive contribution to PC_1, were flowering duration, number of branches, capsule weight, number of capsule, and grain yield. PC_2 represents the contribution of days to 50% flowers, capsule width, number of locules, root weight, and root length. For PC_3, 60.07% of the variability was contributed by traits days to first flower, plant height and capsule length (0.62), while for PC_4, biomass and harvest index exhibited the highest contribution. Traits days to maturity and seed weight exhibited the highest contribution to PC_5.

Principal component analysis revealed six PCs for 2009, three PCs for 2010 and five PCs for 2011 (Table 6). Flowering duration was common for all the three years while days to first flower, days to 50% flowers, days to maturity and root length were common for 2009 and 2010. On the other hand capsule weight and grain yield were common for 2009 and 2011. The traits plant height and harvest index were found to be inconsistent throughout three years that might be due to variation in temperature, rainfall and also the soil conditions. So selection on the basis of these traits requires more investigation in further trials.

Cluster Analysis

Cluster pattern based on Euclidean distances using unweighed pair group arithmetic mean of averages (UPGMA) were studied and 8 clusters were observed for 17 traits for the year 2009 (Fig. 1). Cluster 1, 2, 3, 4, 7 and 8 consisted of 4 accessions in each case whereas cluster 5 consisted of five accessions and in cluster 6, three accessions were present. Table 7, presented the grouping of various accessions in eight clusters. Cluster 1 could be selected for days to first flower (158.77±1.12), days to 50% flowers (166.94±0.89), days to maturity (192.83±1.44) and plant height (68.28±2.75). Whereas cluster 2 could be selected for capsule weight (10.00±1.15), number of capsules (54.01±2.98), root weight (1.59±0.28) and root length (9.91±0.35). Flowering duration (44.34±2.99), number of locules (6.19±0.14) and 1000 seed weight (5.25±0.32) performed best for cluster 3. The members of this cluster were of longer duration hence could be used for selection of long duration but with highest 1000 seed weight. Late maturing cultivars with highest yield might be used in hybridization programs with early high yielding maturing lines which contain high quantity of oil. For cluster 6, biomass (27.04±1.29), grain yield (7.62±1.24) and harvest index (33.66±0.9) showed the highest values for these respective traits. Selection could be made for cluster 7, for capsule length (15.98±0.43) recorded, which was the

Table 1: Black cumin (*Nigella sativa* L.) germplasm collection with genebank numbers and provinces, collection sites and altitudes, collected during 2002-2003

Accessions*	Province	Collecting sites	Altitude	Accessions*	Province	Collecting sites	Altitude
Pk-020545	NWFP	Haripur (Hattar)	580	Pk-020742	AJK	Mirpur	950
Pk-020561	Punjab	Lahore	290	Pk-020749	Punjab	Chakwal	525
Pk-020567	Punjab	Faisalabad	230	Pk-020766	Punjab	Multan	125
Pk-020576	Punjab	Faisalabad	230	Pk-020780	Punjab	Lahore	290
Pk-020585	Punjab	Faisalabad	230	Pk-020781	Punjab	Bahawalpur	190
Pk-020592	Punjab	Faisalabad	230	Pk-020783	Punjab	Attock	430
Pk-020609	NWFP	Peshawar	500	Pk-020867	Punjab	Lahore	290
Pk-020620	NWFP	Peshawar	500	Pk-020868	Punjab	Lahore	290
Pk-020631	Punjab	Narowal	290	Pk-020871	AJK	Muzaffarabad	810
Pk-020646	NWFP	Kohat	510	Pk-020872	Punjab	Mianwali	250
Pk-020654	Punjab	Gujranwala	270	Pk-020873	Punjab	Rawalpindi (MT)	530
Pk-020662		Ukrain		Pk-020874	Punjab	Rawalpindi (Saddar)	521
Pk-020663		Pakistan		Pk-020875	Capital	Islamabad (Aabpara)	550
Pk-020699	NWFP	D.I. Khan	230	Pk-020876	Capital	Islamabad (Karachi Co.)	550
Pk-020720	NWFP	D.I. Khan	230	Pk-020877	Punjab	Rawalpindi (Kohuta)	600
Pk-020729	NA	Chilas	1450	Pk-020878	Punjab	Lahore	290

Pk-020662 and Pk-020663 were received from United States of America; D. I. Khan, Dera Ismail Khan; NA, Northern Areas; AJK, Azad Jammu and Kashmir; MT, Muslim Town

*Accessions enlisted are preserved in National Gene bank of Plant Genetic Resources Program, National Agricultural Research Center, Islamabad, Pakistan

Table 2: Plant morpho-physiological traits studied in 32 accessions of *Nigella sativa* L. germplasm

Traits	Units	Notions/codes	When measured	Description of the traits
Days to first flower	days	D1F	After the appearance of first flower	Days from the time of planting to the appearance of first flower
Days to 50% flowers	days	D50%F	After the appearance of 50% flowers	Days from the time of planting to the appearance of 50% flowers
Days to maturity	days	DM	At physiological maturity	At the stage of capsule maturity
Flowering duration	days	FD	Days between days to first flower and 50% flowering	Days between days to first flower and 50% flowers
Plant height	cm	PH	At physiological maturity	The length of the upper part above the ground (length of the plant stem)
Biomass	g	BM	After maturity	The weight of the whole plant except roots
Number of branches	Number	NB	After harvesting	The total number of branches
Capsule weight	g	C wt	After maturity and harvesting	The weight of the capsules
Number of capsule	Number	NC	After harvesting	The total number of capsules present on the particular plant/plants
Capsule length	cm	CL	After harvesting	Length of capsules
Capsule width	mm	CW	After harvesting	The width of the capsules
Number of locules	Number	NL	After harvesting	The total number of locules (where seeds are formed/placed)
Root weight	g	RW	After harvesting	The weight of the root
Root length	cm	RL	After harvesting	The length of the root
Seed weight	g	1000 SW	After thrashing	The weight of the seeds (the 1000 seeds weight)
Grain yield	g	GY	After thrashing	The production/weight of the grains/seeds for particular plant
Harvest index	%	HI	After thrashing	The difference between grain yield over biomass, multiplied by 100

highest among other traits. Cluster 8, can also be selected for number of branches (7.80±0.55) and capsule width (10.04±0.05). While cluster 4 and 5 showed poor performances as compared to other clusters because of the low values of the traits, and hence suggested to be exploited as much as possible.The traits showed best performance in a particular cluster will be helpful in the selection and choice of cultivars existed in that cluster, due to genetic variability in the sense of similarities or dissimilarities.

The highest genetic distances were recorded (85.16) between Pk-020729 and Pk-020878 followed by 84.74 between Pk-020720 verses Pk-020878. While the shortest genetic distances were (9.83) recorded between Pk-020766 with Pk-020875 and this was followed by 9.95 between Pk-020872 and Pk-020875, whereas accession Pk-020545 was present at the distance of 77.31 with Pk-020720 followed by 76.08 between Pk-020561 and Pk-020720. These accessions will also be a good choice for future utility depending on genetic variation reflecting distances.

Four clusters were formed on the basis of cluster analysis constructed by UPGMA (Fig. 2) in 32 accessions for 2010. In cluster 1, two accessions were present, while in cluster 2, four accessions were present. Cluster 3 consisted of 6 accessions and cluster 4, 20 accessions. Variation within clusters showed the performance of different traits with respect to different accessions (Table 8). For cluster 1, flowering duration (51.63±9.02), biomass (80.69±20.35), number of branches (23.13±0.88), capsule weight (25.76±3.10), number of capsules (151.38±21.39), root weight (4.33±1.34), root length (16.00±2.05) and grain

Table 3: Principal components (PCs) for 17 morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2009

		Component Matrix					
		PC1	PC2	PC3	PC4	PC5	PC6
Eigen value		5.59	3.02	2.15	1.96	1.15	1.02
Proportion of variation		32.87	17.74	12.63	11.50	6.74	6.36
Cumulative variance		32.87	50.61	63.24	74.75	81.49	87.85
Communality				Eigen Factors			
Days to first flower	0.98	0.89	-0.33	-0.28	-0.11	0.00	0.00
Days to 50% flowers	0.99	0.86	-0.31	-0.29	-0.25	0.05	0.04
Days to maturity	0.93	0.72	-0.63	-0.08	-0.03	-0.05	-0.08
Flowering duration	0.88	-0.79	-0.15	0.45	0.15	-0.03	-0.09
Plant height (cm)	0.79	0.40	-0.29	-0.01	0.58	0.22	0.41
Biomass (g)	0.96	0.05	0.87	-0.19	-0.39	-0.10	-0.04
Number of branches	0.97	-0.15	-0.15	-0.19	-0.04	-0.58	0.74
Capsule weight (g)	0.87	0.81	0.25	0.29	0.18	-0.14	0.06
Number of capsule	0.91	0.59	0.34	-0.03	0.60	-0.23	-0.22
Capsule length (cm)	0.93	-0.07	-0.10	0.42	-0.72	0.38	0.27
Capsule width (mm)	0.85	-0.24	0.59	-0.62	-0.09	-0.21	0.09
Number of locules	0.71	-0.09	0.04	-0.39	0.39	0.57	0.27
Root weight (g)	0.80	0.52	-0.13	0.65	0.05	-0.29	-0.08
Root length (cm)	0.71	0.63	0.35	0.40	-0.01	0.08	0.16
Seed weight (g)	0.89	-0.63	0.25	0.44	0.44	0.01	0.21
Grain yield (g)	0.90	0.57	0.55	0.38	-0.26	0.08	0.25
Harvest index (%)	0.89	0.52	0.71	-0.05	0.21	0.25	-0.04

Table 4: Principal components (PCs) for 17 morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2010

		Component Matrix		
		PC1	PC2	PC3
Eigen value		6.13	5.60	1.83
Proportion of variation		36.06	32.93	10.75
Cumulative variance		36.06	69.00	79.75
Communality			Eigen Factors	
Days to first flower	0.88	-0.76	0.54	0.12
Days to 50% flowers	0.90	-0.76	0.57	0.04
Days to maturity	0.78	-0.65	0.51	-0.31
Flowering duration	0.60	0.52	-0.33	-0.47
Plant height	0.90	0.01	0.87	-0.39
Biomass	0.96	0.60	0.78	0.02
Number of branches	0.86	0.80	0.44	-0.14
Capsule weight	0.95	0.52	0.80	0.19
Number of capsule	0.97	0.60	0.77	0.13
Capsule length	0.84	-0.73	0.51	0.19
Capsule width	0.61	-0.41	0.58	0.33
Number of locules	0.59	-0.33	0.58	-0.38
Root weight	0.84	0.88	0.25	0.11
Root length	0.87	0.85	-0.18	0.35
Seed weight	0.28	0.07	-0.41	0.34
Grain yield	0.93	0.45	0.79	0.33
Harvest index	0.80	-0.40	-0.13	0.79

Table 5: Principal components (PCs) for 17 morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2011

		Component Matrix				
		PC1	PC2	PC3	PC4	PC5
Eigen value		4.53	3.49	2.19	1.87	1.42
Proportion of variation		26.63	29.55	12.89	11.00	8.36
Cumulative variance		26.63	47.18	60.07	71.07	79.42
Communality			Eigen Factors			
Days to first flower	0.89	-0.43	0.13	0.60	0.53	-0.20
Days to 50% flowers	0.89	-0.48	0.70	0.34	-0.21	0.11
Days to maturity	0.85	0.52	0.18	0.44	-0.01	-0.60
Flowering duration	0.86	0.72	0.01	-0.26	-0.48	-0.22
Plant height	0.93	0.42	-0.12	0.66	-0.53	-0.10
Biomass	0.80	0.62	-0.07	-0.02	0.63	0.05
Number of branches	0.62	0.62	0.46	0.04	0.07	-0.14
Capsule weight	0.96	0.91	-0.07	0.03	0.01	0.34
Number of capsule	0.91	0.82	0.29	-0.13	0.32	0.21
Capsule length	0.81	0.13	-0.61	0.62	-0.20	-0.02
Capsule width	0.85	-0.10	-0.88	0.20	0.05	0.14
Number of locules	0.34	-0.07	-0.42	-0.22	0.18	0.29
Root weight	0.58	0.06	0.70	0.23	0.17	0.04
Root length	0.66	-0.39	0.66	0.06	0.24	-0.03
Seed weight	0.85	-0.13	-0.01	0.64	0.07	0.65
Grain yield	0.82	0.81	0.22	0.21	0.06	0.27
Harvest index	0.90	-0.13	0.58	-0.14	-0.60	0.41

yield (14.34±1.88) showed best to be selected for future use. The traits plant height (95.57±2.21) and number of locules (6.99±0.21) is preferred for cluster 2. For cluster 3, days to first flower (176.40±5.50), days to 50% flowers (188.56±5.96), days to maturity (212.50±3.21), capsule length (15.96±0.79), capsule width (12.08±0.50), and harvest index (22.50±3.95) recorded to be best in response within this group. Cluster 4 showed highest potential towards 1000 seed weight (3.16±1.01). The highest genetic distances recorded were 155.48 between Pk-020654 and Pk-020878 followed by 151.27 between Pk-020545 and Pk-020654. Shortest genetic distances were recorded as 16.24 between Pk-020876 verses Pk-020877 and 17.18 between Pk-020576 and Pk-020663, respectively.

Eight clusters were formed on the basis of UPGMA by Euclidean distances presented in (Fig. 3) during 2011. Clusters 1, 2, 3, 4, 6 and 7 consisted of four accessions each, while three accessions were present in cluster-5 and five in cluster-8, respectively (Table 9). In Cluster-1 selection can be made for highest plant height (111.27±0.70), capsule length (13.56±0.45), capsule width (10.20±0.45) and 1000 seed weight (3.35±0.48) as presented in the Table 9. In case of cluster-2, highest capsule weight (4.08±0.32) and number of locules (5.87±0.20) were observed and hence choice can

Table 6: Principal components formed for morpho-physiological traits for three years 2009, 2010 and 2011

Principal components	2009	2010	2011
PC_1	Days to first flowers, days to 50% flowers, days to maturity, flowering duration, capsule weight, root length, grain yield, 1000 seed weight	Days to first flowers, days to 50% flowers, days to maturity, flowering duration, Number of branches, capsule length, root weight, root length	Flowering duration, Number of branches, capsule weight, number of capsules, grain yield
PC_2	Biomass, harvest index	Plant height, biomass, capsule weight, number of capsules, capsule width, number of locules, 1000 seed weight, grain yield	Days to 50% flowers, capsule width, number of locules, root weight, root length
PC_3	Root weight, capsule width	Harvest index	Days to first flowers, plant height, capsule length
PC_4	Plant height, number of capsules, capsule length		Biomass, harvest index
PC_5	Number of locules		Days to maturity, 1000 seed weight
PC_6	Number of branches		

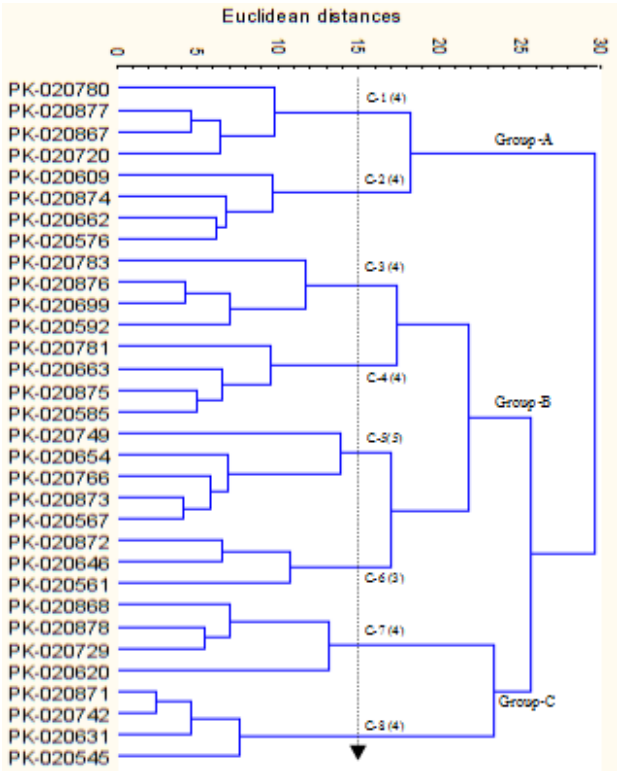

Fig. 1: Cluster pattern for morpho-physiological traits constructed by UPGMA in 32 genotypes of *Nigella sativa* L. germplasm for 2009

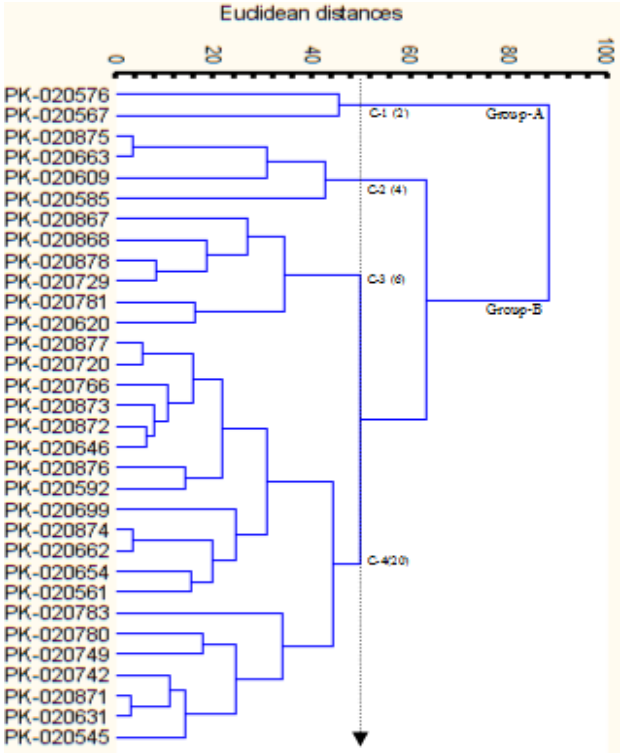

Fig. 2: Cluster pattern for morpho-physiological traits constructed by UPGMA in 32 genotypes of *Nigella sativa* L. germplasm for 2010

be made for accessions with high number of locules and high capsule weight as compared to other accessions. In cluster-3, flowering duration was highest (60.58±0.31), the accessions present in this cluster were late in maturity, which might affect the oil yield of *N. sativa*. Traits days to 50% flowers, days to maturity, root weight, root length and harvest index were observed with high average values for cluster-4. Cluster-5 could be selected for highest biomass (20.70±4.92), number of capsules (30.77±3.30) and highest grain yield (2.73±0.30) showed the potential for the accessions present in this cluster. This is suggested to exploit more without any doubt for high biomass and yield potential. Cluster-6 took 155.92±1.45 days for days to first flower and cluster-7 contributed for high number of branches. The highest genetic distances recorded were (87.74) between Pk-020592 and Pk-020729 followed by 83.60 between Pk-020592 and Pk-020872. On the other hand, shortest distance of 8.35 between Pk-020871 and Pk-020874 followed by 9.74 between Pk-020609 and Pk-020878 were recorded. Accession Pk-020592 was placed at the genetic distances of 82.89 with Pk-020662, likewise Pk-020585 was placed at 81.88 with Pk-020592. Accessions presented close to each other could be used to make crosses with accessions found on longest distances to obtain desirable ones.

During 2009 and 2011, eight clusters were formed, while during 2010 four clusters as shown in Table 10.

Table 7: Variation within clusters for morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2009

Frequency	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6	Cluster-7	Cluster-8
	4	4	4	4	5	3	4	4
Traits	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ
Days to first flower	158.77±1.12	155.27±1.28	135.75±1.67	143.02±2.02	143.67±1	146.36±2.69	148.58±0.24	137.44±2.05
Days to 50% flowers	166.94±0.89	162.35±0.88	144.49±1.68	152.16±0.86	153.96±1.73	157.70±2.23	158.75±0.2	148.72±1.51
Days to maturity	192.83±1.44	190.67±0.95	180.08±1.33	185.33±1.16	183.53±1.13	180.89±0.24	190.58±1.18	180.50±0.61
Flowering duration	34.07±2.32	35.40±0.84	44.34±2.99	42.31±1.79	39.87±0.84	34.53±2.76	42.00±1.08	43.06±1.87
Plant height	68.28±2.75	62.64±2.39	66.31±1.38	63.10±3.43	54.00±4.43	64.37±2.03	62.44±2.21	55.20±0.34
Biomass	15.20±1.84	20.34±1.65	15.77±0.62	18.22±1.54	24.28±1.38	27.04±2.24	14.50±4.41	20.03±1.09
Number of branches	7.55±0.47	7.03±0.65	7.48±0.28	7.09±0.36	6.83±0.48	7.45±0.48	7.62±1.24	7.80±1.11
Capsule weight	7.45±0.98	10.00±1.15	6.96±0.5	8.36±0.33	7.29±0.76	8.98±1.08	8.54±0.69	4.98±0.51
Number of capsule	40.75±2.37	54.01±2.98	42.51±4.65	51.48±2.74	38.95±4.01	42.50±3.64	31.76±4.73	24.31±2.29
Capsule length	12.35±0.36	12.79±0.48	12.47±0.32	12.16±0.43	14.01±1.87	14.49±0.64	15.98±0.43	14.00±0.5
Capsule width	9.56±0.21	9.52±0.31	9.49±0.19	9.27±0.16	9.75±0.18	9.97±0.17	8.40±0.78	10.04±0.05
Number of locules	5.90±0.09	5.66±0.33	6.19±0.14	5.83±0.04	5.66±0.16	5.72±0.06	5.61±0.12	5.52±0.16
Root weight	0.93±0.17	1.59±0.28	1.11±0.36	1.01±0.16	0.94±0.1	0.89±0.1	1.58±0.49	0.71±0.1
Root length	9.84±0.95	9.91±0.35	9.80±0.74	9.56±0.16	10.02±0.8	11.07±0.38	10.24±0.58	8.33±0.64
Seed weight	2.33±0.07	2.57±0.15	5.25±0.32	3.08±0.17	3.22±0.31	3.20±0.08	3.19±0.29	3.35±0.18
Grain yield	3.82±0.88	5.97±1.07	4.09±0.29	4.17±0.21	4.94±0.47	7.62±1.24	5.74±0.67	3.39±0.09
Harvest index	25.75±2.08	29.67±3.13	26.80±2	23.66±1.02	27.98±1.55	33.66±0.9	19.64±1.44	18.25±0.32

Table 8: Variation within clusters for morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2010

	Cluster-1	Cluster-2	Cluster-3	Cluster-4
Frequency	2	4	6	20
Traits	Mean±σ	Mean±σ	Mean±σ	Mean±σ
Days to first flower	145.75±5.66	172.52±7.24	176.40±5.5	151.75±6.3
Days to 50% flowers	161.75±1.41	187.21±8.59	188.56±5.96	167.54±6.17
Days to maturity	197.38±3.36	210.67±6	212.50±3.21	201.58±7.03
Flowering duration	51.63±9.02	38.15±1.68	36.10±2.31	49.83±7.07
Plant height	90.45±2.83	95.57±2.21	82.47±3.1	80.34±7.83
Biomass	80.69±20.35	68.18±9.41	35.13±6.67	44.17±9.73
Number of branches	23.13±0.88	18.87±2.17	14.76±2.29	17.82±2.39
Capsule weight	25.76±3.1	21.30±2.95	12.47±3.56	14.20±2.26
Number of capsule	151.38±21.39	116.04±18.08	66.32±16.56	78.39±17.79
Capsule length	14.70±0.1	15.80±0.23	15.96±0.79	14.62±0.57
Capsule width	11.99±0.25	11.87±0.14	12.08±0.5	11.50±0.32
Number of locules	6.38±0.04	6.99±0.21	6.60±0.18	6.51±0.26
Root weight	4.33±1.89	2.28±0.45	1.67±0.35	2.51±0.55
Root length	16.00±2.05	10.64±0.58	9.74±0.72	12.82±1.64
Seed weight	2.33±0.11	2.38±0.15	2.58±0.22	3.16±1.01
Grain yield	14.34±1.88	12.08±2.49	7.80±2.49	8.43±1.32
Harvest index	20.16±0.66	19.46±3.32	22.50±3.95	20.13±2.54

Table 9: Variation within clusters for morpho-physiological traits in 32 accessions of *Nigella sativa* L. germplasm for 2011

	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6	Cluster-7	Cluster-8
Frequency	4	4	4	4	3	4	4	5
Traits	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean±σ	Mean± σ
D1F	148.92±1.93	142.92±1.56	136.50±1.95	143.25±1.34	147.28±1.28	155.92±1.45	150.33±1.36	142.43±1.45
D50%F	167.75±1.87	166.33±2.45	157.25±2.27	173.67±1.55	164.39±1.18	166.67±1.36	168.25±2.25	169.37±4.65
DM	197.92±1.74	196.83±1.74	197.08±1.9	198.33±2.55	191.47±0.71	197.42±2.66	194.04±1.31	187.12±1.8
FD	49.00±0.54	53.92±0.31	60.58±0.31	55.08±1.25	44.19±1.00	41.50±1.27	43.71±0.22	44.68±1.10
PH	111.27±0.70	100.07±1.08	90.03±1.47	89.28±1.06	80.81±0.45	80.84±1.08	90.71±1.12	80.95±1.20
BM	16.57±0.90	17.29±1.05	18.52±1.01	16.13±0.63	20.70±4.92	17.47±0.09	15.55±0.27	14.71±0.58
NB	8.90±0.25	9.03±0.49	9.58±0.42	9.88±0.89	9.54±0.43	8.56±0.22	10.03±0.86	7.65±0.43
CWt	3.47±0.45	4.08±0.32	3.91±0.40	3.31±0.47	4.05±0.41	2.76±0.17	2.99±0.49	2.59±0.16
NC	20.69±2.16	23.03±1.79	26.60±2.17	27.00±3.59	30.77±3.3	19.92±0.75	15.60±3.49	14.85±1.12
CL	13.56±0.45	13.48±10	12.48±0.30	11.65±0.13	11.90±0.36	12.88±0.25	12.47±0.57	12.20±0.35
CW	10.20±0.45	10.14±0.31	10.01±0.21	9.31±0.18	9.91±0.31	10.05±0.21	9.83±0.42	10.07±0.37
NL	5.46±0.11	5.87±0.20	5.71±0.19	5.38±0.14	5.56±0.1	5.70±0.12	5.46±0.22	5.56±0.13
RW	2.68±0.36	2.35±0.06	2.28±0.22	3.58±0.15	2.75±0.1	2.68±0.34	2.63±0.27	2.38±0.41
RL	14.87±0.29	12.96±0.69	13.93±0.36	16.14±0.63	14.67±0.53	15.93±0.36	14.41±0.77	15.14±1.04
1000SW	3.35±0.48	3.02±0.17	2.24±0.10	2.85±0.03	3.17±0.25	2.83±0.07	2.60±0.22	3.00±0.33
TY	2.58±0.18	2.55±0.15	2.51±0.10	2.46±0.28	2.73±0.3	2.18±0.03	2.34±0.31	2.00±0.18
HI	15.56±0.28	15.22±1.73	14.40±0.37	17.68±1.50	15.20±2.14	12.47±0.34	16.78±2.85	17.40±2.68

σ, variance

Table 10: Cluster analysis for morpho-physiological traits for three years 2009, 2010 and 2011

Clusters	2009	2010	2011
Cluster-1	Days to first flowers, days to 50% flowers, days to maturity, plant height	Flowering duration, biomass, number of branches, capsule weight, Number of capsules, root weight, root length, grain yield	Plant height, capsule length, capsule width, 1000 seed weight
Cluster-2	Capsule weight, number of capsules, root weight, root length	Plant height, number of locules	Capsule weight, number of locules
Cluster-3	Flowering duration, number of locules, 1000 seed weight	Days to first flowers, days to 50%flowers, days to maturity, capsule length, capsule width, harvest index	Flowering duration
Cluster-4		1000 seed weight	Days to 50%flowers, days to maturity, root weight, root length, harvest index
Cluster-5			Biomass, number of capsules, grain yield
Cluster-6	Biomass, grain yield, harvest index		Days to first flowers
Cluster-7	Capsule length		Number of branches
Cluster-8	Number of branches, capsule width		Days to 50% flowers, capsule width, root length, harvest index

Morpho-physiological traits such as days to first flower, days to 50% flowers, days to maturity, plant height, contributed for cluster 1 during 2009. While during 2010, flowering duration, biomass, number of branches, capsule weight, number of capsules, root weight, root length, and grain yield contributed for cluster 1. Whereas during 2011, plant height, capsule length, 1000 seed weight contributed for cluster 1. Thousand (1000) seed weight was contributed by cluster1 in 2009, while in cluster 4 during 2010 and in cluster 1 during 2011. Grain yield was present in cluster 6 in 2009 and in cluster1 in 2010 and in cluster 5 during 2011. Selection can be made on the basis of the respective traits for their consistent performance.

Discussion

The grouping of accessions by multivariate analysis in our study is of practical value to medicinal plant breeders particularly working on *N. sativa*. Accessions can be chosen from a particular group for hybridization and breeding program. Based on current findings, the greater part of genetic variation was accounted by days to maturity, branches plant^{-1}, capsules plant^{-1}, biomass and grain yield in PCA for quantitative traits. Multivariate analysis provided a method of evaluation and identification of land races that could be further characterized by DNA molecular markers (Iqbal *et al*., 2011).

Abdi (2003) considered multivariate analysis appropriate for choosing parents for hybridization. Similarly in our study the accessions possessing the genes of interest for traits of economic importance were identified and hence are recommended to be utilized directly or included in hybridization program for varietal development. During 2009, six principal components were formed that contributed 87.85% of variability while during 2010 three PCs were formed and contributed 79.75% of variability. For 2011, five PCs were formed and contributed 79.42% of variability. The PCs showed variable contribution of traits in each year, this may apprehended to the non-uniform climatic conditions over the years. High fluctuation of the seasonal rain, temperature, evaporation rate and humidity influenced the seed and oil yield in the reported years.

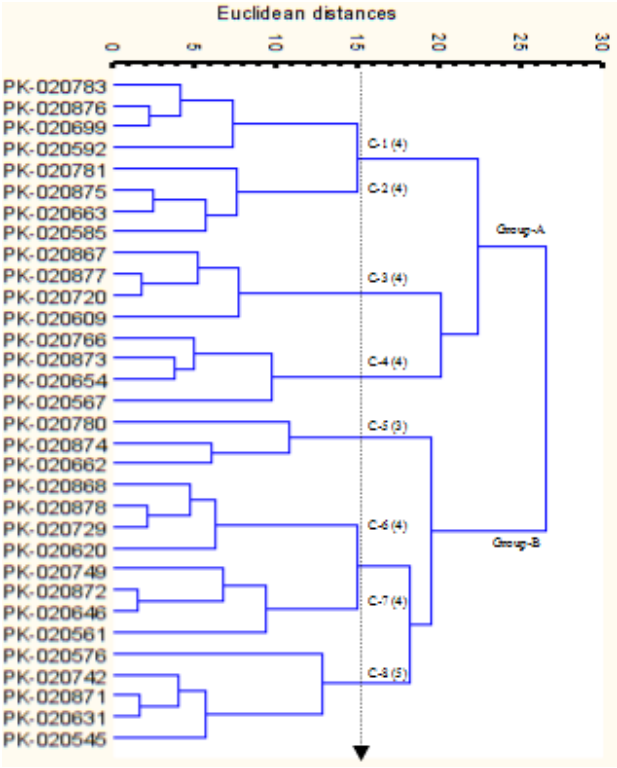

Fig. 3: Cluster pattern for morpho-physiological traits constructed by UPGMA in 32 genotypes of *Nigella sativa* L. germplasm for 2011

Cluster analyses proved their validity to establish phenotypic diversity within accessions in case of present study that is also supported by Iqbal *et al.* (2010), and the statistics on the basis of quantitative characters revealed more reliability than biochemical markers, which are specific for particular conditions. Germplasm evaluation for quantitative traits may help to work out the relative importance of various traits within each cluster. Moreover, results have more biological significance because chosen traits are directly related to agronomic adaptability of the germplasm such as flowering, maturity, yield and yield contributing characters (Iqbal *et al*., 2009). Determination of germplasm diversity and genetic relationships among

breeding materials is valuable aid in crop improvement strategies. A benefit of the cluster analysis on the basis of quantitative traits for selection of phenotypically distinct germplasm with more breeding values has been reported previously by Evgenidis *et al.* (2011). Though cluster analysis grouped together accessions with greater morphological similarity in the present study, the cluster did not necessarily include all the accessions from the same geographical sites. No geographic relationship was found in any case.

Cluster analysis conducted for morpho-physiological traits for three years illustrated that various clusters were based on the performance of individual plant traits. The importance of grain yield or traits related to high yield potential was noticed within all clusters. This may be due to the selection of high yielding genotypes by the plant breeders and it is therefore suggested to broaden the genetic base of cultivated *N. sativa* involving diverse accessions in breeding program. On the other hand, results of physiological traits were not consistent for all the clusters performance during three years. This may be due to the environmental factors, which may have influenced growth in a particular environment. It may also be due to geographical and climatic differences where *Nigella* seeds had been grown (Atta, 2009).

Consequently principle component analysis also provided help for identification of superior accessions based on multiple traits performance. It is suggested that selection of superior accessions may be carried out for more than one trait (Iqbal *et al*., 2010). This technique will broaden the genetic base of *N. sativa* germplasm.

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