

## EVALUATION OF *NIGELLA SATIVA* L., FOR GENETIC VARIATION AND *EX-SITU* CONSERVATION\*

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### Abstract

*Nigella sativa* L., has been widely used for various purposes in Pakistan but its production in the country has not been reported. Therefore 31 genotypes collected and acquired from various sources were evaluated to investigate the potential of this crop in Pakistan. The experiment was planted in RCBD with three replications under field conditions during winter 2002-2003. High genetic variation was recorded for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, seed weight and harvest index. The best genotypes identified from the germplasm could be tested under diverse environment to select the best ones. Three accessions (MP00023, MP00111 and MP00120) were better for more than one character and hence are expected to be a potential for improving *N. sativa* L. in future. Negative association of seed weight with yield and its components is suggested to be broken through mutation, selective diallel or back cross method. Based on genetic diversity, selected accessions from various clusters could be used in hybrid program to develop superior cultivars.

### Introduction

Medicinal plants are in use by the human being for long times, one of these is black cumin (*Nigella sativa* L.) that has been used for healthcare since civilization. It is also known as black seeds, fennel flower, Roman coriander and locally as Kalonji. It is the member of the *Ranunculaceae* or buttercup family. It is an annual herbaceous plant, believed to be indigenous to the Mediterranean region but has been cultivated into other parts of the world including Asia and Africa (Weiss, 2002). It is used for edible and medicinal purposes in India, Pakistan, Saudi Arabia, Syria, Iran, Egypt and many other countries. Due to increased demand of medicinal plants, it is a potential plant species for crop diversification to reduce risks of crop failure and improve crop productivity and income generation especially in smaller land holdings (Ahmad *et al.*, 2004).

It helps in the regulation of immune system and is excellent for treating chronic allergies, improve digestion and lowers blood sugar levels (Ali & Blunden, 2003). It is used to dispel the worms and parasites from intestinal tract and is useful in soothing bronchitis and coughs (Chakravarty, 1993), increase body tone, stimulates menstrual periods, increases the flow of breast milk (Anon., 2002), provides quick energy, increases sperm count, calms the nervous system, encourages hair growth and retards hair fallout, prevents skin wrinkling (Saxena & Vyas, 1986; Akgul, 1989 and Aqel, 1993). Amino acid analysis of seeds protein hydrolysate showed the presence of 15 amino acids of which amazingly includes all 9 essential amino acids (Al-Awadi, 1998). Although *N. sativa* has been widely used for various purposes in Pakistan but its production in the country has not been reported. Hence the germplasm collected and acquired from various sources was evaluated to study the potential of this crop in Pakistan.

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\*The study is a part of Ph.D. by the principal author

The main objective of this study was to explore the yield potential of this medicinal herb for cultivation on large scale at framers field and to assess the genetic diversity present in the available germplasm to identify the potential genotypes for exploration of this important medicinal herb.

#### Materials and Method

Thirty one accessions of *Nigella sativa* were collected from various sources from all the country during last three years. Germplasm was planted in randomized complete block design (RCBD) with three replications under field conditions at Plant Genetic Resources Program, National Agricultural Research Center, Islamabad during winter 2002-2003. Land was prepared and mixed with farm yard manure (FYM). All other cultural practices were followed throughout the crop season (Ahmad & Ghafoor, 2003). The data regarding plant height, number of branches, number of capsules, biomass and grain yield were recorded on 10 plants sampled at random within each replication. Days to first flower, days to 50% flowers and days to maturity were observed on line basis and represents single value for each genotype within each replication. The capsule traits (capsule length, capsule width and capsule weight) were recorded on 10 capsules sampled randomly within each accession then subjected to statistical analysis for simple variance, range and coefficient of variance by MS Excel for Windows XP Professional. Diversity was estimated by constructing tree diagram with the help of computer software STATISTICA version 6.0 for windows and agronomic performance was calculated for each cluster to investigate the contribution of various traits for determining genetic diversity.

#### Results

High genetic variability was recorded for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, 1000 - seed weight and harvest index, whereas for other characters low to medium variability was observed (Table 1). High CV for biomass, grain yield and 1000 seed weight indicated the diverse nature of germplasm for these characters. Acceptance range of CV for most of the characters enhanced the validity of experiment. Some of these accessions might be mixture because this germplasm was mostly collected from market sources and better plants could be selected and bulked for developing superior cultivars. To develop optimum production package for a new crop like *Nigella sativa* L., evaluation of diverse germplasm is helpful not only to identify the superior genotypes but also for the use in agronomic experiments. The best genotypes identified from the germplasm and presented in the Table 2 could be tested under diverse environments to select the best ones for future cultivation. Some of these accessions are better for more than one character *i.e.*, MP00023 (days to maturity, biomass, number of capsule and yield), MP00111 (plant height, biomass and yield) and MP00120 (yield and harvest index), hence these accessions are expected to be a potential source for improving *N. sativa* L., in future.

The results presented in Table 3 revealed varying degrees of association among fourteen characters. Seed yield exhibited positive correlation with biomass, number of capsules, capsule length, capsule width, capsule weight and harvest index. It was observed that short duration plants with high number of capsules could be selected for future potential cultivars of *N. sativa* L. As 1000-seed weight is an important yield contributing trait in most of the crops, therefore selection could be made on the basis of higher seed weight in *N. sativa* L. Harvest index has been reported important for almost all the grain crops, hence genotypes with high harvest index and better for capsule traits are suggested to test under a wide range of agro-climatic conditions that is expected to give rise suitable cultivars for general production. The phenogram based on fourteen

characters for 31 genotypes presented in Figure 1 revealed five clusters at 50% linkage distance. The average values for the accessions in five different clusters are given in the Table 4 that exhibited the performance of individual cluster that could be used for developing high yielding cultivars for better seed size because bold seed varieties are more acceptable to producers and consumers. The accessions of cluster 1 were short stature, early maturing but low yielding whereas the accessions of cluster 3 and 4 were high yielding and late in maturity with intermediate height. Therefore the selected accessions from these clusters could be used in hybrid program to develop superior cultivars. Similarly the accessions of cluster 5 were having better seed weight that is also suggested to use in breeding programs.

**Table 1. Statistics of 31 genotypes of *Nigella sativa* L., germplasm.**

Traits	Unit	Mean $\pm$ SE	$\sigma$	$\sigma^2$ (% of mean)	Range	CV %
Plant height	Cm	61.8 $\pm$ 1.88	10.49	178.1	35.1 77.1	16.96
Days to first flower	Days	146 $\pm$ 2.54	14.14	136.9	125 171	9.68
Days to 50% flowers	Days	154 $\pm$ 2.04	11.35	83.7	138 175	7.34
Days to maturity	Days	188 $\pm$ 1.83	10.17	55.0	177 210	5.39
Biomass	Gram	22.67 $\pm$ 1.48	8.23	298.8	7.96 44.75	36.31
Number of branches	Number	7.34 $\pm$ 0.16	0.89	10.8	5.40 9.90	12.08
Number of capsule	Number	41.40 $\pm$ 2.36	13.12	415.8	10.60 63.50	31.69
Capsule length	Cm	13.12 $\pm$ 0.29	1.64	20.5	11.18 20.63	12.47
Capsule width	Millimeter	9.67 $\pm$ 0.06	0.35	1.3	8.91 10.35	3.63
Capsule weight	Gram	7.48 $\pm$ 0.38	2.13	60.7	2.48 12.00	28.41
Number of locules	Number	28.86 $\pm$ 0.39	2.17	16.3	25.1 33.2	7.51
Yield	Gram	5.36 $\pm$ 0.44	2.45	112.0	1.47 10.64	45.68
1000 Seed weight	Gram	3.41 $\pm$ 0.28	1.57	72.3	1.98 8.07	45.99
Harvest index	Percent	24.23 $\pm$ 0.92	5.10	107.3	5.10 13.28	21.07

SE, Standard error,  $\sigma$ , Standard deviation,  $\sigma^2$ , variance, CV, coefficient of variability

**Table 2. Superior genotypes of *Nigella sativa* L., germplasm identified for specific traits.**

Traits	Units	Identified genotypes
Plant height	> 70 cm	MP00041 (75.22), MP00053 (77.1), MP00111 (73.94), MP00262 (70.24), MP00359 (72.57), MP00360 (71.33), MP00365 (73.03)
Days to first flower	< 130 days	MP00076 (125.7), MP00087 (128.6), MP00269 (127.3)
Days to maturity	< 180 days	MP00023 (177), MP00065 (178.6), MP00216 (177), MP00269 (178.6), MP00354 (178.6)
Biomass	> 30 g	MP00023 (32.07), MP00065 (36.10), MP00102 (32.01), MP00111 (32.80), MP000269 (44.75), MP00340 (31.07)
Number of capsules	> 50	MP00032 (56.3), MP00023 (61.7), MP00041 (50.40), MP00171 (63.5), MP00202 (50.20), MP00223 (53.70), MP00262 (50.60), MP00340 (52.90), MP00355 (55.6), MP00364 (58)
Capsule weight	> 10 g	MP00171 (12.00), MP00340 (10.96)
Yield	> 8.00 g	MP00023 (8.82), MP00065 (9.56), MP00102 (8.73), MP00111 (8.24), MP00119 (8.15), MP00120 (8.75), MP00171 (8.12), MP00269 (10.63)
1000 Seed weight	> 5.00 g	MP00017 (5.99), MP00053 (8.07), MP00087 (6.61), MP00076 (7.85)
Harvest index	> 30 %	MP00001 (30.49), MP00119 (30.98), MP00120 (31.32),

The values in parenthesis are for specific characters based on averages of 30 plants

Table 3. Correlation coefficient in various agro-physiological and quantitative traits in *Nigella sativa* L., germplasm.

Traits	PH	DIF	D50%F	DM	BM	NB	NC	CL	CWd	CWt	NL	Yld	1000SW
DIF	0.589**												
D50%F	0.540**	0.975***											
DM	0.215**	0.361**	0.311**										
BM	-0.054	-0.196*	-0.106*	-0.331**									
NB	0.121*	-0.086	-0.139*	0.181*	0.080								
NC	0.299**	0.134*	0.077	0.410**	0.289**	0.184*							
CL	-0.185*	-0.379**	-0.281**	-0.338**	0.551**	0.027	-0.246**						
CWd	-0.056	-0.110*	-0.056	-0.205**	0.172*	-0.041	-0.317**	0.378**					
CWt	0.174*	0.025	0.031	0.264**	0.538**	0.001	0.853***	0.095	-0.272**				
NL	0.362**	0.024	-0.082	0.193*	-0.185*	0.378**	0.388**	-0.131*	-0.046	0.128*			
Yld	0.068	-0.213**	-0.119*	-0.343**	0.858***	-0.003	0.203**	0.556**	0.147*	0.495**	-0.199*		
1000SW	0.053	-0.120*	-0.124*	-0.067	-0.277**	0.100*	-0.264**	-0.087	0.025	-0.335**	0.078	-0.153*	
HI	0.369**	-0.074	-0.034	-0.099	0.144*	-0.108*	0.008	0.303**	0.037	0.178*	-0.016	0.601***	0.184*

\*Significant, \*\*High significant, \*\*\*Highly significant at  $p \leq 0.05$ , PH, Plant height, DIF, Days to first flower, D50% F, Days to 50% flowers, DM, Days to maturity, NB, number of branches, NC, number of capsules, CL, Capsule length, CWd, Capsule width, CWt, Capsule weight, NL, number of locules, Yld, Grain yield, 1000SW, 1000 seed weight, HI, Harvest Index.

**Table 4.** Average performance of 5 clusters based on UPGMA in *Nigella sativa* L., germplasm.

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
Traits/Frequency	2	5	15	3	6
Plant height	38.79±5.12	57.69±6.11	60.92±8.71	67.08±4.68	72.80±3.30
Days to first flower	137.50±1.41	138.26±8.27	137.83±6.14	167.60±4.65	165.27±4.03
Days to 50% flower	149.25±1.77	147.42±6.05	148.30±5.36	172.57±3.40	169.50±3.99
Days to maturity	178.50±2.12	205.24±4.69	181.55±2.90	196.93±4.64	192.37±6.64
Biomass	14.71±7.29	18.44±2.92	26.50±8.27	29.26±1.57	15.97±5.40
Number of Branches	7.45±0.78	7.90±1.30	7.23±0.81	7.03±0.32	7.25±0.98
Number of Capsules	12.45±2.62	49.78±6.43	41.95±9.70	57.57±5.41	34.60±10.22
Capsule length	13.52±1.06	12.60±0.75	13.64±2.14	12.34±0.07	12.51±0.73
Capsule width	9.96±0.13	9.62±0.14	9.66±0.44	9.67±0.33	9.62±0.31
Capsule weight	2.83±0.50	8.27±1.10	7.87±1.23	10.61±1.60	5.85±1.43
Number of locules	26.90±2.55	29.64±1.75	28.91±2.00	28.37±2.30	28.99±2.96
Yield	2.11±0.91	3.92±0.65	6.62±2.44	6.39±1.75	4.01±1.94
1000 Seed weight	3.35±0.33	3.32±1.86	3.35±1.55	3.07±0.62	3.83±2.18
Harvest index	15.06±2.51	22.55±3.12	25.78±5.22	21.84±5.39	26.02±2.93

This paper need little corrections and accepted for publications

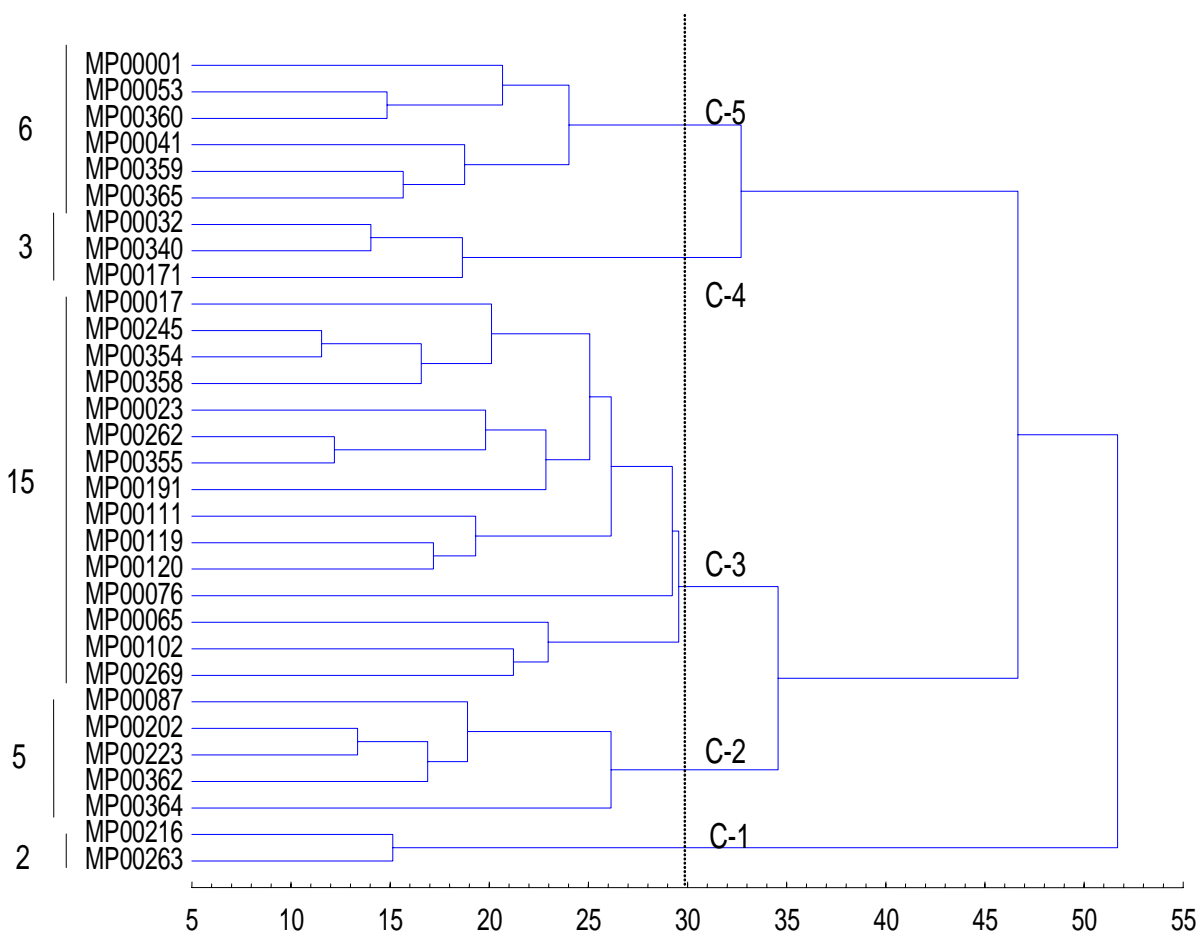


Fig. 1. Phenogram for 31 genotypes of *Nigella sativa* L., germplasm based on 14 agro-physiological and quantitative traits.

## Discussion

High variance observed in the present study is suggested to be exploited for developing superior cultivars either through simple selection or using the in breeding program (Ghafoor *et al.*, 2005, Tayyaba *et al.*, 2005). High CV for biomass, grain yield and 1000 seed weight indicated the diverse nature of germplasm for these characters. Acceptance range of CV for most of the characters enhanced the validity of experiment. Based on breeding nature, some of these accessions might be mixture because this germplasm was mostly collected from market sources and better plants could be selected and bulked for developing superior cultivars. *Nigella sativa* L., is a new crop in the country and the first step for successful introduction is evaluation of diverse germplasm that not only helps to identify the superior genotypes, but selected genotypes could also be used in agronomic experiments (Shadia *et al.*, 1998). The 1000-seed weight is an important yield contributing traits in most of the crops as reported by Ghafoor & Arshad (2008) and Iqbal *et al.*, (2003a), but negative association of 1000-seed weight with yield and its components is suggested to break through mutation, selective diallel or back cross method. (Quite amazing statement)?

Selection from diverse germplasm facilitates rapid development and Fu *et al.*, (2003) and Mahto & Singh (1996) have identified superior cultivars in *Linum usitatissimum* whereas Iqbal *et al.*, (2003b) identified superior cultivars in cowpea and Ragazinskiene *et al.*, (2002) reported these types of results in *N. sativa* L. The genotypes (MP00023, MP00111 and MP00120) can be exploited for their potential and use in future hybridization and breeding programs. The material evaluated in the present studies, the germplasm was collected from various markets and the seed is probably imported from Iran, Afghanistan or India. The high yielding identified genotypes are suggested to evaluate under diverse agro-ecological zones to identify the potential area and cultivars to reduce import of this important medicinal herb. All of these accessions have been multiplied and conservation in the genebank and seed is available for research and development to the researchers. *Nigella sativa* L. has been identified as a potential underutilized crop in Pakistan, hence selected lines that exhibited superiority for more than one trait could be used for cultivation under diverse agro-ecological climates.

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