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GENETIC VARIATION AND PATH ANALYSIS FOR YIELD AND OTHER AGRONOMIC TRAITS IN *NIGELLA SATIVA* L. GERMPLASM

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Abstract

Thirty two genotypes of *Nigella sativa* L. were evaluated for three consecutive years which showed significant differences for all the traits indicating high level of genetic variation. Heritability in broad sense ranged from 0.28 to 0.98 and the highest heritability was calculated for days to maturity and days to flowers. Grain yield was positively associated with plant height, capsule weight, capsule length, root length, whereas negatively with capsule width and 1000-seed weight that required the use of novel breeding techniques to break this undesired linkage to improve grain yield in *N. sativa*. Path coefficient indicated that direct effects of all the traits were positive except days to first flower, days to 50% flowers, flowering duration, number of capsules, root weight and harvest index. The characters exhibiting correlation along with direct effect towards grain yield *viz.*, days to maturity, capsule weight, capsule length and root length should be given more preference while selecting high yielding *N. sativa* genotypes for future crop improvement programs.

Introduction

The genus *Nigella* contains 20 species of annual herbs indigenous to the Mediterranean and west Asia region (Weiss 2002). *N. sativa* is cultivated from Morocco to Northern India, in sub-Saharan Africa particularly Niger, and Eastern Africa (especially Ethiopia, Egypt, Tunisia),Europe including Russia, North America and Iran (Cheikh-Rouhou *et al.* 2006, Gharby *et al.* 2015). In South-East Asia, *N. sativa* seeds are used mainly for medicinal purposes. An evaluation and comparison of the chemical and morphological characteristics of different regional types of *N. sativa* is being compiled (Iqbal *et al.* 2005, Iqbal *et al.* 2013).

More than 60% people are still relaying on herbal medicines for their ailments and cures across Asian continent. It is also used as food additive, an ingredient and supplementary food etc. Generally seeds have been serving as food preservative and spice (Iqbal *et al.* 2014). It is unique underutilized crop having potential both for agriculturally important traits and industry. Its evaluation and characterization at *ex situ* could accelerate genetic resources conservation efforts and availability of unique genes to select high yielding generic lines.

N. sativa acts on the principle of immune system enhancement by natural healing process (Morsi 2000, Salem and Hussain 2000). Traditionally it was used to treat fever, headache, anxiety, diarrhea, asthma, and stroke (Mohammed *et al.* 2016). It increases body tone, stimulates menstrual periods and increases the flow of breast milk (Hussain and Hussain 2016). It contains more than 100 bioactive constituents of both herbal as well as industrial importance (Iqbal *et al.* 2014). It is reported to be rich in essential fatty acids, sterols and higher concentration of thymoquinone (Piras *et al.* 2013).

Examination of genetic variance is important for plant breeders in general and particularly in a newly introduced crop like *N. sativan* (Iqbal *et al.* 2010). Dong *et al.* (2001) advocated that new

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genotypes are important source to meet national oil and food demands as well as in the development of commercial varieties. Correlation analysis provides information about the degree of relationship between important plant traits and is also a good index to predict the yield response in relation to the change of a particular character. Arshad *et al.* (2006) have expressed apprehension about total reliance on yield component analysis. Study of direct and indirect effects of yield and its components provide the basis for its successful breeding program. In the present study, different genotypes of *N. sativa* for presenting data on genetic variation, correlation coefficient and path analysis among various yield and yield contributing traits were analyzed.

Materials and Methods

Thirty two genotypes of *Nigella sativa* germplasm were evaluated under field conditions at Plant Genetic Resources Program, National Agricultural Research Center, Islamabad, Pakistan (33.40^{o}N and 73.07^{o}E; 540m) for three consecutive years, i.e., from 20011-12, 2012-13 and 2013-14. Germplasm sowing was completed during last week of November and harvested in the end of April each year. Two rows of 4 m length for each genotype with 30 cm distance were planted in randomized complete block design (RCBD). All other cultural practices were followed throughout the crop season (Anon. 2008).

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 30 randomly sampled plants from each genotype. Harvest index was expressed as a ratio between grain yield and biological yield.

Data were analysed for analysis of variance (ANOVA), mean, coefficient of variability and heritability in broad sense by using computer software MS Excel. Genotypic, phenotypic and environmental correlation coefficients and path coefficient analyses were performed using computer software written by one of the author using the method by Dewey and Lu (1959) and Singh and Chaudhary (1979).

Results and Discussion

ANOVA revealed significant differences among genotypes for all the traits under study (Table 1). The range in days to flowering and maturity will help to select early maturing lines for future use. Genotype PK-020620 took 23 days for flowering duration which was the lowest as compared to other genotypes. The highest plant height was recorded in genotype PK-020878 while PK-020545 was the lowest for genotype. Highest grain yield was produced by the genotype PK-020878 whereas the lowest grain yield was recorded in PK-020873. Highest harvest index ranged from 8.54 in PK-020609 to 35.85% in PK-020878. Further, heritability estimates in broad sense ranged from 0.28 for number of branches to 0.98 for days to maturity. High heritability (< 50%) was observed for all the traits except number of branches, number of locules, root weight, and root length that indicated the scope of selection from this material. Many researchers have reported the importance of high genetic variation and heritability for simple selection (Sanjukta and Biswas 2003). Parents diverse in nature could be selected to start hybridization as well as breeding program.

Results regarding genotypic, phenotypic and environmental coefficient of correlation showed that the genotypic correlations were higher than the phenotypic and environmental ones for most of the traits exhibiting high degrees of genetic association among traits under consideration (Table 2). The environmental correlation coefficients were not very important in most of the cases indicating low environmental influence in the experiment. Grain yield/plant was significantly

Table 1. Mean, ANOVA, genetic traits and standard error for yield and its components in 32 genotypes of *N. sativa* germplasm.

Genotypes	D1F	D50%F	DM	FD	PH	BY	NB	CWt	NC	CL	CW	NL	RW	RL	1000SW	GY	HI
PK-020545	135	150	177	41	35.2	11.25	8	3.18	14	12.82	9.8	5	.35	10.42	3.68	1.44	12.78
PK-020561	135	148	180	45	44	12	6	2.41	10	14.38	10.08	6	.34	7.98	3.17	1.47	12.07
PK-020567	138	145	183	45	70.5	30.5	8	6.81	29	15.47	10.23	6	.8	7.68	3.11	4.59	23.64
PK-020576	141	150	181	40	71	26.75	8	7.52	43	13.32	10.08	5	1.33	7.3	3.42	6.08	24.51
PK-020585	160	169	186	25	65.7	20	7	5.28	23	13.57	9.48	5	.71	9.52	3.68	4.54	32.28
PK-020592	124	141	176	52	64.7	32.25	8	9.85	40	19.63	10	6	.93	11.42	3.29	7.86	34.06
PK-020609	138	149	179	41	61.7	25	7	9.66	62	13.27	10.32	6	1.31	12.48	2.31	8.94	35.85
PK-020620	160	169	183	23	61.2	27	6	8.09	32	14.38	10.02	5	.52	9.42	3.24	6.7	29.06
PK-020631	132	144	183	51	46	35.75	6	6.62	27	13.93	9.65	5	.35	8.68	3.29	4.57	24.55
PK-020646	139	149	179	40	56.5	27.25	7	8.4	44	13.3	9.4	5	1.25	11.35	3.52	6.76	35.53
PK-020654	171	176	195	24	55	19.75	7	8.37	52	12.48	10	6	1.46	10.7	3.79	4.38	24.4
PK-020662	131	143	178	46	52.2	14.75	6	7.1	38	13.02	9.82	6	.87	9.82	2.57	3.81	25.15
PK-020663	136	146	180	44	64.7	17.5	7	9.14	44	13.52	9.95	6	1.43	8.68	2.06	5.36	31.41
PK-020699	151	158	185	34	69	16.5	7	7.9	45	12.08	9	5	2.3	10.58	2.78	4.18	26.1
PK-020720	165	172	197	32	64.2	25.25	7	12.32	65	12.4	9.27	5	1.45	10.63	2.79	8.39	32.86
PK-020729	172	178	198	25	52	20.75	6	10.9	52	12.25	9.7	5	1.08	11.52	2.66	6.48	31.46
PK-020742	134	146	178	44	57	16	7	8.83	54	13.2	9.6	5	1.33	11.05	2.95	3.16	21.21
PK-020749	136	146	180	44	71.2	18.75	6	9.83	50	12.73	8.92	5	.64	10.13	2.77	5.5	29.03
PK-020766	139	147	183	43	62.7	22.25	8	7.51	51	11.18	9.18	6	.94	8.27	2.77	4.07	19.78
PK-020780	162	169	199	37	61.7	16	7	7.25	50	11.52	9.38	6	1.12	8.77	3.84	3.96	24.62
PK-020781	129	139	178	49	61.2	14.75	8	6.91	43	11.85	9.52	6	.9	8.82	6.59	3.28	22.74
PK-020783	143	150	182	38	70	17.75	7	8.57	54	12.63	9.18	6	1.43	11.32	2.24	4.67	26.8
PK-020867	143	150	180	36	72.7	12	6	6.4	36	12.8	9.48	5	1.55	8.65	5.82	3.59	29.27
PK-020868	126	138	179	53	61.2	18.75	8	5.94	34	12.6	9.75	6	.55	10.38	6.34	4.83	28.41
PK-020871	172	180	204	31	70.5	16	8	7.63	42	13	9.27	6	.76	8.68	1.99	3.95	24.3
PK-020872	138	146	179	40	62.7	16.5	6	9.9	45	13.3	9.52	6	.91	9.57	2.52	4.42	26.64
PK-020873	166	171	203	36	64	7.75	5	3.87	25	12.1	9.98	5	.69	10.7	2.66	2	27.9
PK-020874	153	163	186	32	76.2	21.25	10	8.15	58	11.92	9.58	6	1.45	8.73	2.14	4.38	21.03
PK-020875	139	148	178	39	57	19	8	8.28	51	12.52	9.9	6	2.72	9.13	2.43	4.48	24.84
PK-020876	165	170	201	35	49.2	14.25	8	5.26	32	12.48	9.82	6	1	10.42	6.83	3.51	23.96
PK-020877	164	173	203	39	65.2	18.25	7	5.47	32	12.88	9.92	5	1.25	10.3	2.8	2.9	21.24
PK-020878	125	143	179	54	78.2	6.25	6	15.16	10	26.05	3.95	5	1.37	11.1	.71	12.06	8.54
MS (VAR/TR)	935.69**	679.03**	322.97**	278.46**	366.26**	183.57**	3.52**	25.62**	796.36**	29.57**	4.59**	.95**	1.08**	6.52**	7.40**	19.45**	160.88**
MS(REPLICATES)	32.92 *	19.66 ns	17.83**	19.93 ns	21.30 ns	58.57*	1.729 ns	8.67*	277.50*	0.234 ns	0.49 ns	0.09 ns	0.92*	2.56 ns	0.21 ns	3.94*	5.51 ns
MS (ERROR)	11.384	8.091	1.597	12.346	47.864	17.683	1.374	2.606	74.99	2.409	.415	0.185	0.25	1.314	0.226	1.284	11.438
ST.ERROR	1.687	1.422	.632	1.757	3.459	2.103	.586	.807	4.33	.776	.322	.215	.25	.573	.238	.567	1.691
CD1	4.724	3.983	1.769	4.92	9.686	5.887	1.641	2.26	12.124	2.173	.902	.602	.7	1.605	.666	1.587	4.735
CD2	6.244	5.264	2.338	6.502	12.802	7.782	2.169	2.987	16.025	2.872	1.192	.796	.926	2.121	.88	2.097	6.258
$\sigma^2\delta$	231.077	167.735	80.343	66.528	79.6	41.473	.535	5.755	180.342	6.791	1.044	.191	.208	1.302	1.795	4.541	37.36
$\sigma^2\rho$	242.462	175.827	81.94	78.874	127.464	59.157	1.91	8.361	255.333	9.2	1.459	.377	.459	2.615	2.021	5.826	48.799
GCOV	10.411	8.325	4.829	20.56	14.454	33.36	9.922	30.889	33.01	19.278	10.764	7.587	41.611	11.62	40.911	43.624	23.969
PCOV	10.664	8.524	4.877	22.386	18.29	39.842	18.738	37.231	39.278	22.438	12.725	10.642	61.739	16.47	43.412	49.409	27.394
COH	.953	.954	.981	.843	.624	.701	.28	.688	.706	.738	.716	.508	.454	.498	.888	.78	.766

D1F = Days to first flower, D50% F= Days to 50% flowers, DM = Days to maturity, FD = Flowering duration, PH = Plant height, BY= Biological yield, NB = Number of branches, CWt = Capsule weight, NC= Number of capsules, CL = Capsule length, CW = Capsule weight, NL = Number of locules, RW= Root weight, RL = Root length, 1000-seed weight, GY= Grain yield, HI = Harvest index; $\sigma^2\delta$ = Symbols for genotypic variances and $\sigma^2\rho$ = phenotypic variances; COH = Co-heritability in broad sense.

correlated with plant height, capsule weight, capsule length, root length, whereas it was negatively with capsule width and 1000-seed weight. At the genotypic and phenotypic levels, grain yield exhibited the correlation coefficient with capsule weight, capsule length and capsule width while association of capsule width with yield was negative. Kizil and Toncer (2005) reported significant correlation between seed yield and number of capsules per plant, number of seeds per capsule, 1000 seed weight and seed yield per plant in *N. sativa*. The significant combinations in correlation comprehend that choice for such traits could be made for further improvement to develop high yielding generic lines. Moreover, Biabani *et al.* (2011) reported that at phenotypic level, seed yield per plant had significant positive association with pods per plant, harvest index, biological yield per plant and primary branches per plant. Further, positive association of seed yield per plant with primary branches per plant, biological yield per plant, pods per plant and harvest index were also observed.

Table 2. Coefficient of correlation for genotypic, phenotypic and environmental matrix for yield with various yield contributing agronomic traits in 32 genotypes of *N. sativa* germplasm.

Variables	Genotypic correlation (Gr)	Phenotypic correlation (Pr)	Environmental correlation (Er)
Days to first flower (D1F)	–0.17	–0.13	0.20
Days to 50% flowers (D50%F)	–0.12	–0.09	0.11
Days to maturity (DM)	–0.18	–0.15	0.20
Flowering duration (FD)	0.12	0.07	–0.13
Plant height (PH)	0.45 *	0.35 *	0.13
Biological yield, (BY)	0.29	0.37 *	0.62 **
Number of branches (NB)	–0.26	0.01	0.34
Capsule weight (CWt)	0.85 **	0.84 **	0.83 **
Number of capsules (NC)	0.16	0.29	0.68 **
Capsule length (CL)	0.69 **	0.51 **	–0.07
Capsule width (CW)	–0.58 **	–0.46 **	–0.09
Number of locules (NL)	–0.20	–0.16	–0.12
Root weight (RW)	0.22	0.21	0.22
Root length (RL)	0.45 **	0.28	0.02
1000-seed weight (100 SW)	–0.37 *	–0.32	–0.11
Harvest index (HI)	0.26	0.28	0.36 *

*Significant and **highly significant at 0.05% probability level.

Genotypic correlation coefficients were partitioned into direct and indirect effects contributing towards grain yield (Table 3). The direct effects of all the traits were positive except days to first flower, days to 50% flowers, flowering duration, number of capsules, root weight and harvest index. The highest positive direct effect was exhibited by days to maturity (7.18) and it was followed by capsule weight (0.74), capsule length (0.39) and root length (0.34). It was also observed that direct contribution was also exhibited via biological yield by most of the yield components and hence these two traits (days to maturity and capsule weight) may be given more emphasis while selecting high yielding *N. sativa* genotypes for future use. Very high direct effect of days to maturity contributed grain yield throw increase in numbers of days to maturity.

Table 3. Direct (parenthisis) and indirect effect matrix, dependent variable is grain yield. The last column shows genotypic correlations of independent variables with grain yield.

Variables	D1F	D50	DM	FD	PH	BY	NB	CWt	NC	CL	CW	NL	RW	RL	1000SW	HI	GY
D1F	**(-10.89)**	-1.43	6.45	6.03	0.01	-0.03	-0.01	-0.08	-0.05	-0.16	0.01	0.00	-0.02	0.02	-0.00	-0.02	-0.17
D50%F	-10.76	**(-1.45)**	6.40	5.92	0.00	-0.03	-0.01	-0.06	-0.03	-0.12	0.01	-0.00	-0.01	0.04	-0.00	-0.01	-0.12
DM	-9.79	-1.29	**(7.18)**	3.97	0.01	-0.04	-0.02	-0.09	-0.02	-0.12	0.01	0.00	0.00	0.02	0.00	-0.01	-0.18
FD	9. 56	1.25	-4.15	**(-6.87)**	-0.00	0.00	0.01	0.05	0.06	0.17	-0.02	0.00	0.04	-0.02	0.00	0.02	0.12
PH	-0.39	-0.01	0.36	0.10	**(0.16)**	0	0.02	0.38	-0.07	0.11	-0.03	0.01	-0.09	-0.06	-0.02	-0.01	0.45
BY	1.07	0.14	-1.12	-0.09	0	**(0.28)**	0.04	0.08	-0.06	-0.02	0.03	-0.00	0.02	-0.03	-0.00	-0.05	0.29
NB	1.01	0.13	-0.69	-0.45	0.02	0.06	**(0.17)**	-0.19	-0.08	-0.10	0.02	0.02	-0.08	-0.12	0.01	0.01	-0.26
CWt	1.20	0.12	-0.87	-0.47	0.08	0.03	-0.04	**(0.74)**	-0.09	0.23	-0.05	-0.00	-0.09	0.15	-0.03	-0.02	0.85
NC	-2.22	-0.19	0.70	1.87	0.05	0.07	0.06	0.31	**(-0.23)**	-0.20	0.02	0.01	-0.12	0.08	-0.01	-0.05	0.16
CL	4.58	0.45	-2.16	-3.09	0.04	-0.01	-0.04	0.39	0.12	**(0.39)**	-0.05	-0.01	0.01	0.08	-0.02	0.03	0.69
CW	-2.36	-0.22	0.74	1.97	-0.07	0.12	0.05	-0.45	-0.07	-0.30	**(0.06)**	0.01	0.04	-0.08	0.02	-0.03	-0.58
NL	-0.15	0.07	0.55	-0.44	0.02	-0.03	0.09	-0.08	-0.07	-0.08	0.02	**(0.04)**	-0.03	-0.11	0.00	-0.00	-0.20
RW	-1.13	-0.07	-0.03	1.39	0.07	-0.03	0.07	0.32	-0.13	-0.03	-0.01	0.01	**(-0.21)**	0.04	-0.01	-0.01	0.22
RL	-0.75	-0.16	0.48	0.38	0-.03	-0.03	-0.06	0.31	-0.06	0.09	-0.01	-0.01	-0.03	**(0.34)**	-0.01	-0.03	0.45
1000SW	0.18	0.09	0.07	-0.30	-0.05	-0.01	0.04	-0.36	0.03	-0.14	0.02	0.00	0.05	-0.05	**(0.06)**	-0.01	-0.37
HI	-2.20	-0.22	0.50	2.07	.03	0.15	-0.01	0.15	-0.13	-0.13	0.03	0.00	-0.03	0.13	0.01	**(-0.08)**	0.26

D1F = Days to first flower, D50% F = Days to 50% Flowers, DM = Days to maturity, FD = Flowering duration, PH = Plant height, BY = Biological yield, NB = Number of branches, CWt = capsule weight, NC = Number of capsules, CL = Capsule length, CW = Capsule weight, NL = Number of locules, RW = Root weight, RL = Root length, 1000-seed weight, GY = grain yield and HI = Harvest index

The highest direct effect observed for days to maturity, number of branches, capsule length and root length, respectively, showed that early maturing lines with highest number of branches may contribute more towards grain yield and biological yield. According to Sanjukta and Biswas (2003), seed weight and number of capsules per plant were the major components of seed yield. The number of primary branches may exhibit the greatest direct effect on seed yield under suitable growing conditions in *N. sativa*. The promising results may be due to genetic variation (plant cultivar), seed maturity, or accuracy of detection, and also high number of experimental material than those reported earlier. In another study, the path coefficient analysis revealed that biological yield per plant and harvest index exhibited positive and high direct effects on seed yield per plant. Therefore, these characters could be considered as the best selection parameters for the improvement of seed yield per plant, as observed in chickpea by Bala *et al.* (2015). The path coefficient analysis revealed that at phenotypic level, the high positive direct effects on seed yield could be contributed by biological yield per plant and harvest index (Zali *et al.* 2011).Likewise Prakash and Vanniarajan (2015), studies exhibited that singleear head weight had maximum direct effects on grain yield/plant followed by straw yield/plant, ear head length and plant height.

Present results revealed that *N. sativa* L. contains enormous potential; its diversity could be explored more by cultivating promising lines on large scale, in addition to marginal lands. Additionally it exhibited great potential to provide raw material for herbal and pharmaceutical industry. Alternatively, it is assumed that large scale cultivation and market price can be a good source of income for farmers. Highest grain yield can be obtained by employing improved cultivars, proper sowing time, optimal environmental conditions and capital intensive high input cultivation methods.

In this study for the first time a diverse collection of *N. sativa* was evaluated and characterized for agro-morphic traits and their correlation coefficient for three years. Genotypic, phenotypic and environmental matrices revealed many significant combinations. While tracing/conducting path coefficient analysis it is worth to mention that direct effects of number of traits were positive and could comprehend the need for developing desired genotypes rich in grain yield. Further, no proper reports are available for germplasm evaluation on this crop by using current statistical technique. Genetic characterization for these traits would be beneficial for starting breeding programs.

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