

# Original Research Article

## Principle Component Analysis for Assessment of Genetic Diversity

### in *Nigella* (*Nigella sativa* L.) Collections

Comment [AMA1]: Assessment of Genetic Diversity in *Nigella* (*Nigella sativa* L.) Collections using Principle Component Analysis

#### Abstract

Seventeen land races of *Nigella* along with one released variety (Rajendra Shyama) as a check; collected from different parts of Bihar (Table 1) were evaluated in Randomized Block Design with three replications at Seed production Farm, TCA, Dholi, Bihar during Rabi 2015-16. Principle component analysis (PCA) showed that first three PCs had >1.00 Eigen value and accounted to 84.71 % of total variation. Rotated component matrix for various traits revealed that PC1 was strongly associated with secondary branches/plant followed by yield/plant, length of fruit, fruit per plant, primary branches/plant, height of the plant, days to 50% flowering and grains/plant. The traits that mostly contributed to PC2 were grains/plant followed by height of the plant and width of fruit whereas, days to maturity followed by width of fruit, height of the plant, days to 50% flowering and length of fruit contributed mostly to the PC3. The characters that contributed most to the PC4 were height of the plant, fruit/plant and length of fruit. Therefore, intensive selection procedures can be adopted to bring about rapid improvement of above mentioned traits. The k-mean of different clusters indicated that genotype falling in cluster III possess high values for all the traits under study indicating their potentiality as a parent in hybridization programmes for further improvement of *Nigella*. Highest inter-cluster distance was noted between cluster III and V indicating the genetic diversity among genotypes of these two clusters. Therefore, genotypes from these two clusters are recommended to use in hybridization programmes for further improvement.

Comment [AMA2]: These landraces wild or domesticated.

Comment [AMA3]: These genotypes from the same province with the same environment did not show much variation.

Comment [AMA4]: This one season or evaluated during two seasons. If it is one season you cannot judge correctly. So, you must evaluated during different seasons.

Keywords: *Nigella sativa*, Principle Component Analysis, Genetic Diversity, Black Cumin

#### Introduction:

Black Cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranunculacea (Hammo, Y. H., 2008). It is popularly known as kalongi and an important seed spice crop of India. Black cumin is grown under a wide range of environments, but flourishes in cooler and dry regions (Weiss, 2002). A temperature range of 5-25°C with the optimum of 12 -14°C and rainfall of 400-500 mm are the most suitable to produce good crops. The crop is frost sensitive at any growth stage and this limits its distribution in Europe and the highland areas of the tropics. Black cumin can grow on all kinds of soils (Jansen, 1981) but, it prefers loamy sand soils (Datta *et al.*, 2001). It can be grown from sea level to 2500 m of altitude with a reduction in yield with increasing altitude.

Comment [AMA5]: Specie

The *nigella* producing countries other than India are Pakistan, Sri Lanka, Bangladesh, Nepal, Egypt and Iraq. In India it is cultivated commercially in Madhya Pradesh, Bihar, Punjab and Assam. It has also been noticed to occur wild in these areas. The other states

40 where its cultivation has been taken up on small scale are Uttar Pradesh, Rajasthan, Tamil  
41 Nadu and West Bengal.

42 The dried seeds of nigella are the commercial product being used in food. The seeds  
43 contain 0.5 to 1.4% essential oil which has demand in the pharmaceutical and perfume  
44 industry (Malhotra, 2004). It has been used since antiquity for culinary, seasoning and  
45 pharmacological purposes (Shah, S. H., 2008). Many medical properties have been attributed  
46 to the *Nigella sativa* L. seeds and its oil, including carminatives, diuretics, antineoplastic  
47 (antitumour), antifungal, anti-helminthic, while their oil has protective action against  
48 histamine induced bronchospasm, cough and bronchial asthma (Worthen *et al.*, 1998; Khan  
49 *et al.*, 2003), antidiabetics (Fararh *et al.*, 2002; El-Dakhakhny *et al.*, 2002), spasmolytic and  
50 bronchodilator (Boskabady *et al.*, 2004), anti-inflammatory (Hajhashemi *et al.*, 2004),  
51 antibacterial (Mashhadian & Rakhshandeh, 2005), galactagogue, antioxidant (Brutis &  
52 Bucar, 2000; Kanter *et al.*, 2003) and insect repellent effects (Fisher, 2002). Additionally,  
53 black seed is a valuable source of protein, carbohydrates, essential fatty acids, vitamins as  
54 well as minerals such as calcium, potassium, iron, magnesium, selenium, manganese and  
55 zinc. *Nigella sativa* L. is being considered important for both oil and bioactive compounds  
56 because their constituents have unique chemical properties and may augment the supply of  
57 edible oils (Ramadan & Morsel, 2003). Black cumin seed has higher total phospholipid  
58 content than cotton and soybean seed oil (Gunstone *et al.*, 1986; Atta, 2003; Ramadan and  
59 Morsel, 2003).

60 Genetic diversity is pre-requisite for any crop improvement programme, as it helps in  
61 the development of superior recombinant (Manonmani & Fazlullah Khan., 2003). Genetic  
62 distance estimates for population grouping can be estimated by different methods as it is  
63 crucial to understand the usable variability existing in the population panel (Nachimuthu  
64 *et al.*, 2014). ~~One of the approaches is to apply multivariate analysis.~~ Principal Component  
65 Analysis (PCA) is a powerful tool in modern data analysis because this is a well-known  
66 multivariate statistical technique which is used to identify the minimum number of  
67 components, which can explain maximum variability out of the total variability (Anderson,  
68 1972 and Morrison, 1978) and also to rank genotypes on the basis of PC scores. Principal  
69 components are generally estimated either from correlation matrix or covariance matrix.  
70 Considering the importance of PCA this study is conducted on diverse *Nigella* genotypes for  
71 identification of traits responsible for yield differences.

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#### 74 **Material and Methods:**

75 Seventeen land races of *Nigella* along with one released variety (Rajendra Shyama) as a check,  
76 collected from different parts of Bihar (Table 1) were evaluated in Randomized Block Design  
77 with three replications at Seed production Farm, TCA, Dholi, Bihar during *Rabi* 2015-16. The  
78 experimental site is located at 25.59 N latitude and 85.75 E longitudes and has altitude of  
79 51.20 m above mean sea level. Soil of TCA, Dholi, Bihar is mainly young alluvium and  
80 calcareous. Standard agronomic practices were adopted with row to row and plant to plant  
81 spacing of 25×5 cm, recommended dose of fertilizer was applied during the time of crop  
82 period. Data was recorded for ten different traits *viz.*, height of the plant, primary branches  
83 per plant, secondary branches per plant, days to 50 per cent flowering, length of fruit, width

Comment [AMA6]: Black Cumin is cross pollination. These genotypes are selfed seeds or not.

Comment [AMA7]: You can use Bihar map to show different collection location.

Comment [AMA8]: Plant height

Comment [AMA9]: Fruit length

84 of fruit, days to maturity, fruit per plant, grains per plant and yield per plant. Five competitive  
 85 plants from each plot were randomly chosen for recording of data except for days to 50 per  
 86 cent flowering and days to maturity whose data were recorded on plot basis.

87 **Table 1. Name, Source and collection year of the Nigella genotypes**

S. No.	Name	Source	Collection Year
1)	RN-20	East Champaran, Bihar	2000
2)	RN-22	Muzaffarpur, Bihar	2001
3)	RN-25	Bhagalpur, Bihar	2001
4)	RN-65	Nalanda, Bihar	2002
5)	RN-66	Banka, Bihar	2002
6)	RN-68	Jammui, Bihar	2002
7)	RN-69	Munger, Bihar	2003
8)	RN-70	Nawada, Bihar	2003
9)	RN-71	Gopalganj, Bihar	2004
10)	RN-72	Siwan, Bihar	2004
11)	RN-73	Chhapra, Bihar	2004
12)	RN-74	Arrah, Bihar	2005
13)	RN-75	Khagaria, Bihar	2005
14)	RN-76	Gopalganj, Bihar	2005
15)	RN-77	Vaishali, Bihar	2006
16)	RN-78	Darbhanga, Bihar	2006
17)	RN-79	Sitamarhi, Bihar	2007
18)	Rajendra Shyama	Released variety (Check)	

Comment [AMA10]: Fruit width

Comment [AMA11]: The

Comment [AMA12]: You can describe the characterization of these genotypes.

Comment [AMA13]: All of them from Bihar and you explain this in materials. So, their no need to repeated it again with each genotype.

Comment [AMA14]: Year of released

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89 The data were subjected to pooled analysis for genetic divergence by using statistical  
 90 package WINDOSTAT version 9.2 (INDOSTAT service, Hyderabad). Intra and inter-cluster  
 91 Euclidean distances generated were used to describe the relationship among the genotypes.  
 92 Cluster analysis was carried out to construct the dendrogram, depicting the relationship  
 93 among genotypes based on the genetic distances.

Comment [AMA15]: Where is the dendrogram in the manuscript.  
K means did not give dendrogram?

## 94 Results and Discussion:

95 The analysis of variance indicated that mean squares for genotype were highly  
 96 significant for all the traits under study. For establishing genetic relationship among the traits  
 97 and their genetic discrimination Principal Component analysis was performed. Association  
 98 between traits emphasised by this method may correspond to genetic linkage between loci  
 99 controlling the traits or a pleotropic effect (Iezzoni and Pritts, 1991)

Comment [AMA16]: genotypes

Comment [AMA17]: Genotypes here the studied relationship between genotypes not traits.

100 The genetic variation present in breeding population was divided into four principal  
 101 components (PCs) which explained 91.69% of total variation (Table 3). According to Brejda  
 102 *et al.*, 2000, the PC with Eigen values >1 and which explained at least 5% of the variation in  
 103 the data will be considered as principal components. The first three PCs had >1.00 Eigen  
 104 value and accounted to 84.71 % of total variation. It indicates that the identified characters  
 105 within these components exhibited immense influence on the phenotype of the genotypes.

106 The first principal component (PC<sub>1</sub>) explained 55.47% of the total variation. The second  
107 principal component (PC<sub>2</sub>) explained 17.14% variation individually and 72.62% cumulative  
108 variation. The third principal component (PC<sub>3</sub>) explained 12.08% variation individually and  
109 84.71% cumulatively.

110 Rotated component matrix for various traits revealed that PC<sub>1</sub> was strongly associated  
111 with secondary branches/plant followed by yield/plant, length of fruit, fruit per plant, primary  
112 branches/plant, height of the plant, days to 50% flowering and grains/plant. The traits that  
113 mostly contributed to PC<sub>2</sub> were grains/plant followed by height of the plant and width of fruit  
114 whereas, days to maturity followed by width of fruit, height of the plant, days to 50%  
115 flowering and length of fruit contributed mostly to the PC<sub>3</sub>. The characters that contributed  
116 most to the PC<sub>4</sub> were height of the plant, fruit/plant and length of fruit. Therefore, intensive  
117 selection procedures can be adopted to bring about rapid improvement of above mentioned  
118 traits.

### 119 Cluster analysis:

120 Cluster analysis helps in selection of suitable genotype(s) or parent to use in  
121 hybridization programme for the manipulation of desirable traits. Choice of proper parent(s)  
122 plays a vital role for a successful plant breeding programme. Parents having more genetic  
123 distance believed to create higher variations by generating higher recombination frequency,  
124 which increase the genetic gain in selection. The grouping of the mutant lines was done by K-  
125 mean clustering pattern. The distribution of 18 lines along with check into five clusters and  
126 their cluster means are presented in Table 5 and 6 respectively. Cluster III and IV comprised  
127 of only one line forming the smallest cluster followed by cluster V which was comprised of  
128 only two lines. Cluster I and II comprised of nine and five nigella lines respectively. The  
129 check Rajendra Shyama was clustered in Cluster V. The k-mean of different clusters  
130 indicated that genotype falling in cluster III possess high values for all the traits under study.  
131 The genotypes in cluster I have minimum values for height of the plant, primary  
132 branches/plant, secondary branches/plant, days to 50% flowering, length of fruit, days to  
133 maturity and yield/plant while, genotypes in cluster II have minimum values for width of  
134 fruit, fruits/plant and grains/plant. It indicates that representative lines can be chosen from  
135 particular diverse groups based on their cluster mean and can be involved in improvement  
136 programmes for Nigella improvement.

137 The character contribution of various clusters towards the genetic diversity by  
138 Tochers clustering method indicated that Grains per plant and Yield per plant were the major  
139 contributors towards total divergence (Table 7).

140 Intra and inter cluster distance was carried out on the basis of ten morphological traits  
141 between eighteen genotypes (Table 8.). In general, inter-cluster were greater than the intra-  
142 cluster distances indicating the considerable amount of genetic diversity among the genotypes  
143 studied. The average intra-cluster distance between genotypes was maximum (1.48) for the  
144 cluster II followed by Cluster I (0.57). Cluster III, IV and V did not show intra-cluster  
145 distance indicating their genetic relatedness. The highest inter-cluster distance was noted

Comment [AMA18]: Reference like Fehr, 1987 or Bernado, 1992

Comment [AMA19]: These studied lines are mutant. If it is you should write it in materials.

Comment [AMA20]: Yield and its components and maturity traits

Comment [AMA21]: Similarity

146 between cluster III and V (22.32) followed by cluster III and IV (19.54). The least inter-  
147 cluster distance was observed for cluster I and II (1.69) indicating their genetic relatedness.

Comment [AMA22]: Similarity

148 3-D Plot diagram was constructed on the first three principle components (Fig. 1).  
149 Researchers use 3-D plot in principle component analysis to visually assess which  
150 components explain most of the variability in the data. In 3-D diagram, Rajendra shyama and  
151 RN-77 were plotted at distant end whereas; RN-78 and RN-20 were plotted at other end of 3-  
152 D plot indicating their effectiveness in breeding programme for improvement of Nigella.

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#### 154 **Conclusion:**

155 Based on above discussion, PCA analysis revealed the possibility for improvement of Nigella  
156 through various agro-morphological traits. In 3-D diagram (Fig.1) Rajendra shyama and RN-  
157 77 were found most divergent mutant lines with RN-78 and RN-20 which can be utilized  
158 effectively in breeding programme for improvement of Nigella. The genotype (RN-20) from  
159 cluster III have higher mean value for all the traits indicating their potentiality as a parent in  
160 hybridization programmes for further improvement of Nigella. The highest inter-cluster  
161 distance was noted between cluster III and V (22.32) indicating the genetic diversity among  
162 genotypes of these two clusters. Therefore, genotypes from these two clusters were  
163 recommended for use in hybridization programmes for further improvement.

Comment [AMA23]: You should make mean performance for all studied genotypes.

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256 **Table 2. ANOVA for various characters of Nigella**

Traits	SOV	Mean squares (MS)		
	d.f	Replications	Genotypes	Error
		2	17	34
Height of the plant		13.55	207.76**	35.37
Primary branches per plant		0.36	5.02**	3.04
Secondary branches per plant		0.96	204.87**	16.66
Days to 50 per cent flowering		1.40	39.48**	10.34
length of fruit		0.003	0.04*	0.016
Width of fruit		0.011	0.080**	0.028
Days to maturity		0.46	4.89**	1.49
Fruit per plant		15.79	283.29**	72.79
Grains per plant		19.79	1042.92**	46.09
Yield per plant		0.28	12.03**	1.48

Comment [AMA24]: Analysis of variance for various yield traits of Nigella

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Comment [AMA25]: Under this table you must note that \* and \*\* Significant at 0.05 and 0.01 probability levels, respectively.

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259 **Table 3. Eigen values and variability explained by each principal components (PCs)**

	PC1	PC2	PC3	PC4
Eigene Value (Root)	5.547	1.714	1.208	0.698
% Var. Exp.	55.47	17.14	12.08	6.98
Cum. Var. Exp.	55.47	72.62	84.71	91.69

260

261 **Table 4. Rotated component matrix for various traits**

Trait	PC1	PC2	PC3	PC4
Height of the Plant (cm)	0.27047	0.40103	0.29174	0.40761
Primary Branches/Plant	0.35236	0.02250		
Secondary Branches/Plant	0.41041			
Days to 50% Flowering	0.25405		0.26469	0.02384
Length of Fruit (cm)	0.38104	0.07956	0.18150	0.32709
Width of Fruit (cm)		0.31455	0.33182	
Days to Maturity			0.67470	
Fruit/Plant	0.37405		0.04416	0.33129
Grains/Plant	0.17236	0.51885	0.39816	
Yield/ Plant (in G)	0.39107	0.06985		

Comment [AMA26]: Traits

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263 Height of the Plant (cm)

264 Primary Branches/Plant

265 Secondary Branches/Plant

266 Days to 50% Flowering

267 Length of Fruit (cm)

268 Width of Fruit (cm)

269 Days to Maturity

270 Fruit/Plant

271 Grains/Plant

272 Yield/ Plant (in G)

Comment [AMA27]: g

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276 **Table 5. Distribution of Nigella genotypes in various clusters**

277	Group K	No. of genotypes	Genotypes within clusters
278		Within clusters	
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280	I	9	RN-25, RN-65, RN-66, RN-69, RN-70, RN-73, RN-74
281			RN-76 & RN-79
282	II	5	RN-22, RN-68, RN-71, RN-72, RN-75
283	III	1	RN-20
284	IV	1	RN-78
285	V	2	RN-77 & Rajendra Shyama
286			

287 **Table 6. Mean characteristics (K-Mean) on various traits for each cluster in Nigella genotypes**

Cluster	Height of the plant	Primary branches/ plant	Secondary branches/ plant	Days to 50% flowering	Length of fruit	Width of fruit	Days to maturity	Fruits/ plant	Grains/ plant	Yield/plant
I	37.852	5.148	16.056	42.481	1.131	0.917	77.444	38.500	47.444	5.471
II	40.200	5.567	16.100	44.633	1.190	0.875	83.267	37.900	39.167	5.544
III	76.333	10.333	31.667	69.167	2.072	1.655	138.000	68.833	108.50	12.677
IV	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
V	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Comment [AMA28]: Plant height

Comment [AMA29]: Fruit length

Comment [AMA30]: Fruit width

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289 **Table 7. Contribution percentage of traits towards genetic divergence**

S. No.	Source	Times Ranked 1st	Contribution (%)
1.	Height of the plant	10	6.54%
2.	Primary branches per plant	0	0.00%
3.	Secondary branches per plant	2	1.31%
4.	Days to 50 per cent flowering	6	3.92%
5.	length of fruit	2	1.31%
6.	Width of fruit	4	2.61%
7.	Days to maturity	4	2.61%
8.	Fruit per plant	1	0.65%
9.	Grains per plant	57	37.25%
10.	Yield per plant	67	43.79%

Comment [AMA31]: Traits

Comment [AMA32]: 1<sup>st</sup>

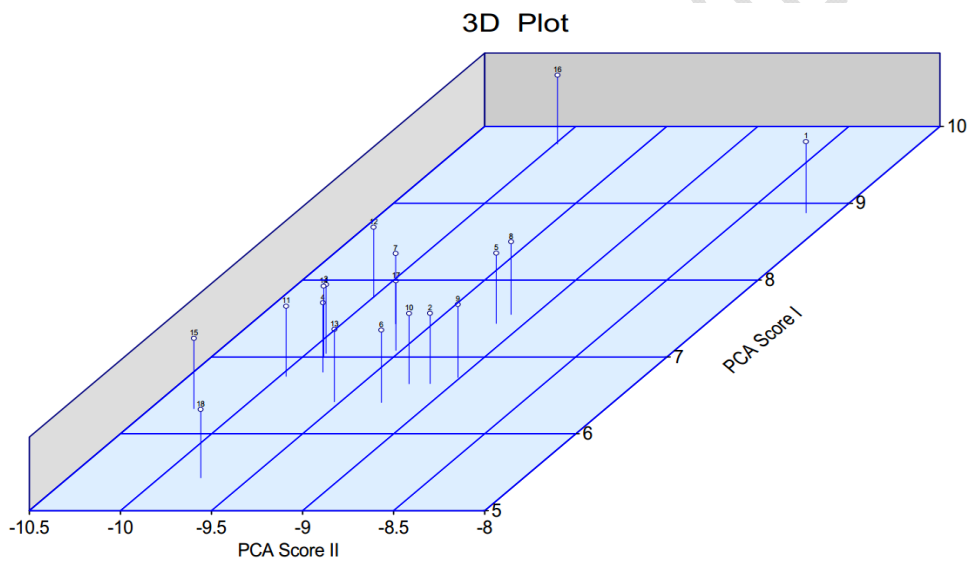
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291 **Table 8. Estimates of Intra (diagonal) and Inter-cluster distances in 18 genotypes of**  
 292 **Nigella**

Cluster	I	II	III	IV	V
I	<b>0.57</b>	1.69	2.64	10.94	11.29
II		<b>1.48</b>	4.53	8.06	11.32
III			<b>0.00</b>	19.54	22.32
IV				<b>0.00</b>	5.63
V					<b>0.00</b>

Comment [AMA33]: Nigella genotypes

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302 Fig. 1: 3-D distribution of the diverse genotype based on principal components.

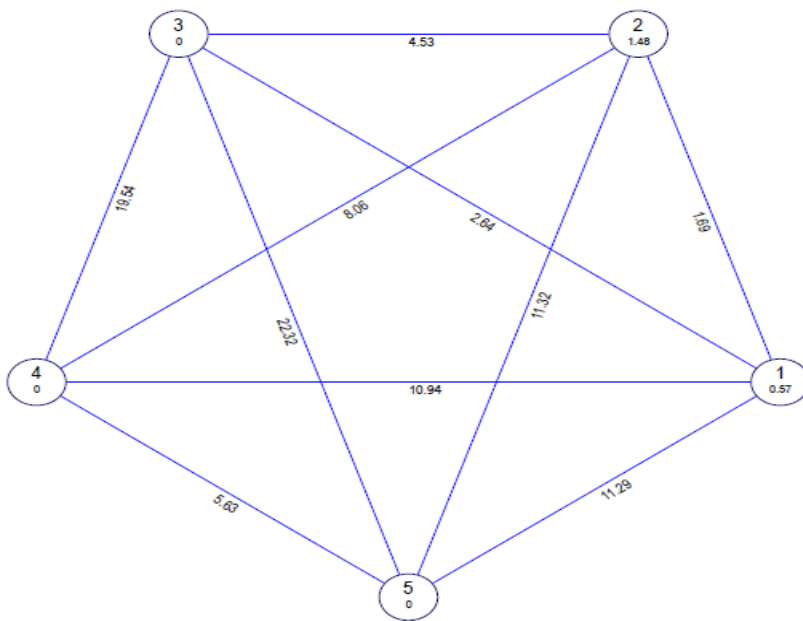


Fig.2: Mahalanobis Euclidean Distance (Not to the Scale) by Tocher Method

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