

Original Research Article

Assessment of Genetic Diversity in *Nigella (Nigella sativa L.)* Collections using Principle Component Analysis

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Abstract

Seventeen land races of *Nigella* along with one released variety (Rajendra Shyama) as a check; collected at farmer's field from different parts of Bihar were evaluated in Randomized Block Design with three replications at Seed production Farm, TCA, Dholi, Bihar during Rabi 2015-16 to identify diverse *Nigella* genotypes. 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.

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

38 **Introduction:**

39 Black Cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family
40 Ranunculacea (Hammo, Y. H., 2008). It is popularly known as kalongi and an important seed
41 spice crop of India. Black cumin is grown under a wide range of environments, but flourishes
42 in cooler and dry regions (Weiss, 2002). A temperature range of 5-25⁰C with the optimum of
43 12 -14⁰C and rainfall of 400-500 mm are the most suitable to produce good crops. The crop is
44 frost sensitive at any growth stage and this limits its distribution in Europe and the highland
45 areas of the tropics. Black cumin can grow on all kinds of soils (Jansen, 1981) but, it prefers
46 loamy sand soils (Datta *et al.*, 2001). It can be grown from sea level to 2500 m of altitude
47 with a reduction in yield with increasing altitude.

48 The nigella producing countries other than India are Pakistan, Sri Lanka, Bangladesh,
49 Nepal, Egypt and Iraq. In India it is cultivated commercially in Madhya Pradesh, Bihar,
50 Punjab and Assam. It has also been noticed to occur wild in these areas. The other states
51 where its cultivation has been taken up on small scale are Uttar Pradesh, Rajasthan, Tamil
52 Nadu and West Bengal.

53 The dried seeds of nigella are the commercial product being used in food. The seeds
54 contain 0.5 to 1.4% essential oil which has demand in the pharmaceutical and perfume
55 industry (Malhotra, 2004). It has been used since antiquity for culinary, seasoning and
56 pharmacological purposes (Shah, S. H., 2008). Many medical properties have been attributed
57 to the *Nigella sativa* L. seeds and its oil, including carminatives, diuretics, antineoplastic
58 (antitumour), antifungal, anti-helminthic, while their oil has protective action against
59 histamine induced bronchospasm, cough and bronchial asthma (Worthen *et al.*, 1998; Khan *et*
60 *al.*, 2003), antidiabetics (Fararh *et al.*, 2002; El-Dakhakhny *et al.*, 2002 ; Kumari *et al.*,
61 2019), spasmolytic and bronchodilator (Boskabady *et al.*, 2004), anti-inflammatory
62 (Hajhashemi *et al.*, 2004), antibacterial (Mashhadian & Rakhshandeh, 2005), galactagogue,
63 antioxidant (Brutis & Bucar, 2000; Kanter *et al.*, 2003) and insect repellent effects (Fisher,
64 2002). Additionally, black seed is a valuable source of protein, carbohydrates, essential fatty
65 acids, vitamins as well as minerals such as calcium, potassium, iron, magnesium, selenium,
66 manganese and zinc. *Nigella sativa* L. is being considered important for both oil and
67 bioactive compounds because their constituents have unique chemical properties and may
68 augment the supply of edible oils (Ramadan & Morsel, 2003). Black cumin seed has higher
69 total phospholipid content than cotton and soybean seed oil (Gunstone *et al.*, 1986; Atta,
70 2003; Ramadan and Morsel, 2003).

71 Genetic diversity is pre-requisite for any crop improvement programme, as it helps in
72 the development of superior recombinant (Manonmani & Fazlullah Khan., 2003). Genetic
73 distance estimates for population grouping can be estimated by different methods as it is
74 crucial to understand the usable variability existing in the population panel (Nachimuthu *et*
75 *al.*, 2014). Principal Component Analysis (PCA) is a powerful tool in modern data analysis
76 because this is a well-known multivariate statistical technique which is used to identify the
77 minimum number of components, which can explain maximum variability out of the total
78 variability (Anderson, 1972 and Morrison, 1978) and also to rank genotypes on the basis of
79 PC scores. Principal components are generally estimated either from correlation matrix or

80 covariance matrix. Considering the importance of PCA this study is conducted with the
81 objective of identifying diverse *Nigella* genotypes.

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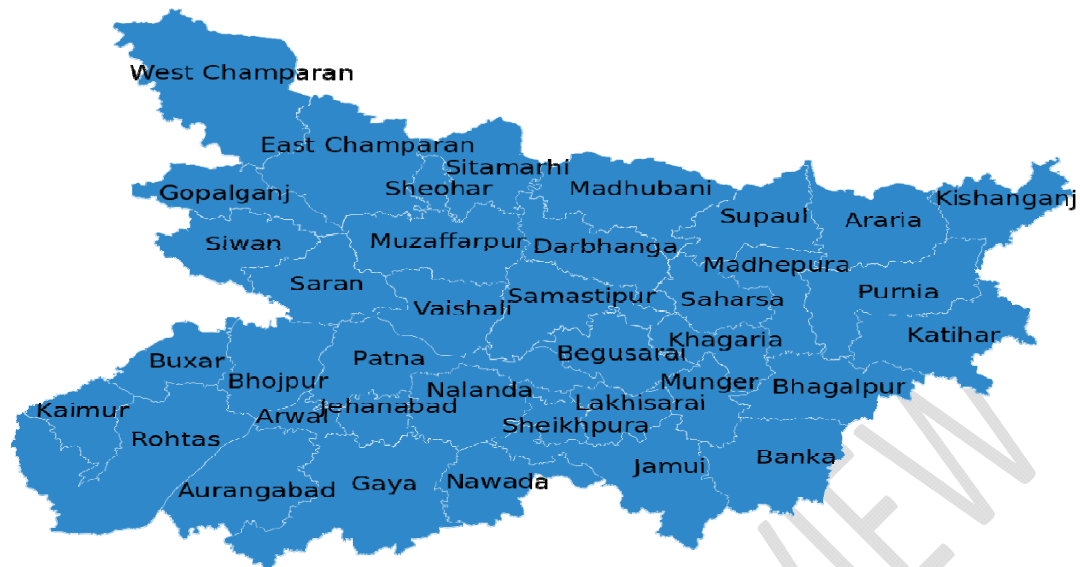
83 **Material and Methods:**

84 Seventeen land races of *Nigella* along with one released variety (Rajendra Shyama) as a check,
85 collected from different parts of Bihar (**Table 1.1**) were evaluated in Randomized Block Design
86 with three replications at Seed production Farm, TCA, Dholi, Bihar during *Rabi* 2015-16. The
87 experimental site is located at 25.59 N latitude and 85.75 E longitudes and has altitude of
88 51.20 m above mean sea level. Soil of TCA, Dholi, Bihar is mainly young alluvium and
89 calcareous. Standard agronomic practices were adopted with row to row and plant to plant
90 spacing of 25×5 cm, recommended dose of fertilizer was applied during the time of crop
91 period. Data was recorded for ten different traits *viz.*, Plant height, primary branches per plant,
92 secondary branches per plant, days to 50 per cent flowering, Fruit length, Fruit width, days to
93 maturity, fruit per plant, grains per plant and yield per plant. Five competitive plants from
94 each plot were randomly chosen for recording the data except for days to 50 per cent
95 flowering and days to maturity whose data were recorded on plot basis.

96 **Table 1.1 Name, Source and collection year of the *Nigella* genotypes**

S. No.	Name	Source	Collection Year
1)	RN-20	East Champaran	2000
2)	RN-22	Muzaffarpur	2001
3)	RN-25	Bhagalpur	2001
4)	RN-65	Nalanda	2002
5)	RN-66	Banka	2002
6)	RN-68	Jammui	2002
7)	RN-69	Munger	2003
8)	RN-70	Nawada	2003
9)	RN-71	Gopalganj	2004
10)	RN-72	Siwan	2004
11)	RN-73	Chhapra	2004
12)	RN-74	Arrah	2005
13)	RN-75	Khagaria	2005
14)	RN-76	Gopalganj	2005
15)	RN-77	Vaishali	2006
16)	RN-78	Darbhangha	2006
17)	RN-79	Sitamarhi	2007
18)	Rajendra Shyama	Released variety (Check)	2011

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Fig1: Map showing different districts of Bihar

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

105 **Results and Discussion:**

106 RN-20 has highest mean value for primary branch /plant (12.00), secondary branch /
 107 plant (36.67), grain yield (129.33) and yield / plant (16.23g).RN-78 show highest mean value
 108 for fruit length (2.25 cm) and fruit/plant (49.67). RN-71 has highest mean value for fruit
 109 width (1.86 cm). RN-77 has least mean value for plant height (52.67) while the check variety
 110 Rajendra Shyama has least mean value for days to flowering (67.33) and days to maturity
 111 (136.67).The analysis of variance indicated that mean squares for **genotypes** were highly
 112 significant for all the traits under study. **For establishing genetic relationship among the**
 113 **genotypes and their genetic discrimination Principal Component analysis was performed.**
 114 Association between traits emphasised by this method may correspond to genetic linkage
 115 between loci controlling the traits or a pleotropic effect (Iezzoni and Pritts, 1991)

116 The genetic variation present in breeding population was divided into four principal
 117 components (PCs) which explained 91.69% of total variation (**Table 3**). According to Brejda
 118 *et al.*, 2000, the PC with Eigen values >1 and which explained at least 5% of the variation in
 119 the data will be considered as principal components. The first three PCs had >1.00 Eigen
 120 value and accounted to 84.71 % of total variation. It indicates that the identified characters
 121 within these components exhibited immense influence on the phenotype of the genotypes.
 122 The first principal component (PC₁) explained 55.47% of the total variation. The second
 123 principal component (PC₂) explained 17.14% variation individually and 72.62% cumulative

124 variation. The third principal component (PC₃) explained 12.08% variation individually and
125 84.71% cumulatively.

126 Rotated component matrix for various traits revealed that PC₁ was strongly associated
127 with secondary branches/plant followed by yield/plant, length of fruit, fruit per plant, primary
128 branches/plant, height of the plant, days to 50% flowering and grains/plant. The traits that
129 mostly contributed to PC₂ were grains/plant followed by height of the plant and width of fruit
130 whereas, days to maturity followed by width of fruit, height of the plant, days to 50%
131 flowering and length of fruit contributed mostly to the PC₃. The characters that contributed
132 most to the PC₄ were height of the plant, fruit/plant and length of fruit. Therefore, intensive
133 selection procedures can be adopted to bring about rapid improvement of above mentioned
134 traits.

135 **Cluster analysis:**

136 Cluster analysis helps in selection of suitable genotype(s) or parent to use in
137 hybridization programme for the manipulation of desirable traits. Choice of proper parent(s)
138 plays a vital role for a successful plant breeding programme. Parents having more genetic
139 distance believed to create higher variations by generating higher recombination frequency,
140 which increase the genetic gain in selection (Fehr, 1987). The grouping of collected lines was
141 done by K-mean clustering pattern. The distribution of 18 lines along with check into five
142 clusters and their cluster means are presented in **Table 5** and **6** respectively. Cluster III and
143 IV comprised of only one line forming the smallest cluster followed by cluster V which was
144 comprised of only two lines. Cluster I and II comprised of nine and five nigella lines
145 respectively. The check Rajendra Shyama was clustered in Cluster V. The k-mean of
146 different clusters indicated that genotype falling in cluster III possess high values for all the
147 traits under study. The genotypes in cluster I have minimum values for height of the plant,
148 primary branches/plant, secondary branches/plant, days to 50% flowering, length of fruit,
149 days to maturity and yield/plant while, genotypes in cluster II have minimum values for
150 width of fruit, fruits/plant and grains/plant. It indicates that representative lines can be chosen
151 from particular diverse groups based on their cluster mean and can be involved in
152 improvement programmes for Nigella improvement.

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

156 Intra and inter cluster distance was carried out on the basis of **yield and its component**
157 **traits** between eighteen genotypes (Table 8.). In general, inter-cluster were greater than the
158 intra-cluster distances indicating the considerable amount of genetic diversity among the
159 genotypes studied. The average intra-cluster distance between genotypes was maximum
160 (1.48) for the cluster II followed by Cluster I (0.57). Cluster III, IV and V did not show intra-
161 cluster distance indicating their genetic **similarity**. The highest inter-cluster distance was
162 noted between cluster III and V (22.32) followed by cluster III and IV (19.54). The least
163 inter-cluster distance was observed for cluster I and II (1.69) indicating their genetic
164 **similarity**.

165 3-D Plot diagram was constructed on the first three principle components (**Fig. 2**).
166 Researchers use 3-D plot in principle component analysis to visually assess which
167 components explain most of the variability in the data. In 3-D diagram, Rajendra shyama and
168 RN-77 were plotted at distant end whereas; RN-78 and RN-20 were plotted at other end of 3-
169 D plot indicating their effectiveness in breeding programme for improvement of Nigella.

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171 **Conclusion:**

172 Based on above discussion, PCA analysis revealed the possibility for improvement of Nigella
173 through various agro-morphological traits. In 3-D diagram (Fig.2) Rajendra shyama and RN-
174 77 were found most divergent mutant lines with RN-78 and RN-20 which can be utilized
175 effectively in breeding programme for improvement of Nigella. RN-20 seems to be a
176 promising line for higher grain yield (129.33) with high mean value for yield atributing traits
177 like primary branch /plant (12.00), secondary branch / plant (36.67) and yield / plant
178 (16.23g).The highest inter-cluster distance was noted between cluster III and V (22.32)
179 indicating the genetic diversity among genotypes of these two clusters. Therefore, genotypes
180 from these two clusters were recommended for use in hybridization programmes for further
181 improvement.

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 251 **component analysis of morpho-physiological traits in mutants lines of rice**
 252 **under submerged condition. The Pharma Innovation Journal 2019; 8(4): 402-**
 253 **407**

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 258 Table 1.2. Mean performance of genotypes for studied traits
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S. No.	Genotypes	Plant height	Pri. branches/plant	Sec. branches/plant	Days to flowering	Fruit length(cm)	Fruit width(cm)	Days to maturity	Fruit/plant	Grains/plant	Yield/plant (Gm)
1	RN-20	83.67	12.00	36.67	68.67	2.18	1.73	138.33	76.00	129.33	16.23
2	RN-22	69.00	8.67	26.67	69.67	1.96	1.58	137.67	61.67	87.67	9.13
3	RN-25	67.00	9.67	29.00	74.33	2.09	1.43	138.67	69.67	62.00	8.82
4	RN-65	69.00	8.67	27.00	76.67	1.95	1.48	137.67	62.67	67.00	8.56
5	RN-66	71.67	9.33	30.67	73.00	2.14	1.58	138.00	69.67	92.67	10.51
6	RN-68	66.00	8.00	20.00	78.67	2.00	1.93	138.67	66.00	91.00	9.85
7	RN-69	65.00	10.67	29.67	77.33	1.99	1.53	139.67	68.00	83.00	10.70
8	RN-70	87.00	9.00	31.67	76.33	2.15	1.84	140.00	71.67	94.33	8.76
9	RN-71	72.00	9.00	24.67	74.67	1.99	1.88	139.67	58.00	102.00	9.68
10	RN-72	60.67	10.00	25.67	73.00	1.99	1.63	137.67	58.00	92.33	8.80
11	RN-73	68.67	9.00	25.33	77.67	2.07	1.63	139.33	61.67	58.67	9.43
12	RN-74	67.67	10.67	34.67	78.33	2.00	1.45	141.67	63.67	79.00	10.75
13	RN-75	65.67	8.00	20.00	77.00	1.90	1.50	140.00	71.67	92.00	8.24
14	RN-76	67.67	8.00	27.00	77.33	2.00	1.55	139.33	67.67	65.00	11.46
15	RN-77	52.67	8.67	24.00	79.33	1.95	1.50	140.00	62.67	63.67	8.09
16	RN-78	73.00	12.00	55.67	78.00	2.25	1.58	141.00	99.67	78.00	12.39
17	RN-79	74.67	10.00	26.00	72.00	2.06	1.45	138.67	65.67	70.00	10.30
18	Rajendra	55.00	7.67	19.00	67.33	1.73	1.32	136.67	55.67	55.00	7.59

	Shyama										
	Mean	68.67	9.39	28.52	74.96	2.02	1.59	139.04	67.20	81.26	9.96
	C.V.	8.66	13.68	14.32	4.29	6.34	10.62	0.88	12.70	8.35	12.23
	F ratio	5.87	3.05	12.29	3.82	2.53	2.82	3.28	3.89	22.63	8.11
	F Prob.	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	S.E.	3.43	0.74	2.36	1.86	0.07	0.10	0.71	4.93	3.92	0.70
	CD. 5%	9.87	2.13	6.77	5.34	0.21	0.28	2.03	14.16	11.27	2.02
	CD. 1%	13.25	2.86	9.10	7.17	0.29	0.38	2.72	19.01	15.12	2.71

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Table 2. Analysis of variance for various yield traits of Nigella

Traits	SOV	Mean squares (MS)		
		Replications	Genotypes	Error
	d.f	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

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* and ** Significant at 0.05 and 0.01 probability levels, respectively.

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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

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Table 4. Rotated component matrix for various traits

	Traits	PC1	PC2	PC3	PC4
274					
275	Height of the Plant (cm)	0.27047	0.40103	0.29174	0.40761
276	Primary Branches/Plant	0.35236	0.02250		
277	Secondary Branches/Plant	0.41041			
278	Days to 50% Flowering	0.25405		0.26469	0.02384
279	Length of Fruit (cm)	0.38104	0.07956	0.18150	0.32709
280	Width of Fruit (cm)		0.31455	0.33182	
281	Days to Maturity			0.67470	
282	Fruit/Plant	0.37405		0.04416	0.33129
283	Grains/Plant	0.17236	0.51885	0.39816	
284	Yield/ Plant (in g)	0.39107	0.06985		

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

289	Group K	No. of genotypes	Genotypes within clusters
290		Within clusters	
291			
292	I	9	RN-25, RN-65, RN-66, RN-69, RN-70, RN-73, RN-74
293			RN-76 & RN-79
294	II	5	RN-22, RN-68, RN-71, RN-72, RN-75
295	III	1	RN-20
296	IV	1	RN-78
297	V	2	RN-77 & Rajendra Shyama

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

Cluster	Plant height	Primary branches/ plant	Secondary branches/ plant	Days to 50% flowering	Fruit length	Fruit width	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

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

S. No.	Traits	Times Ranked 1 st	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%

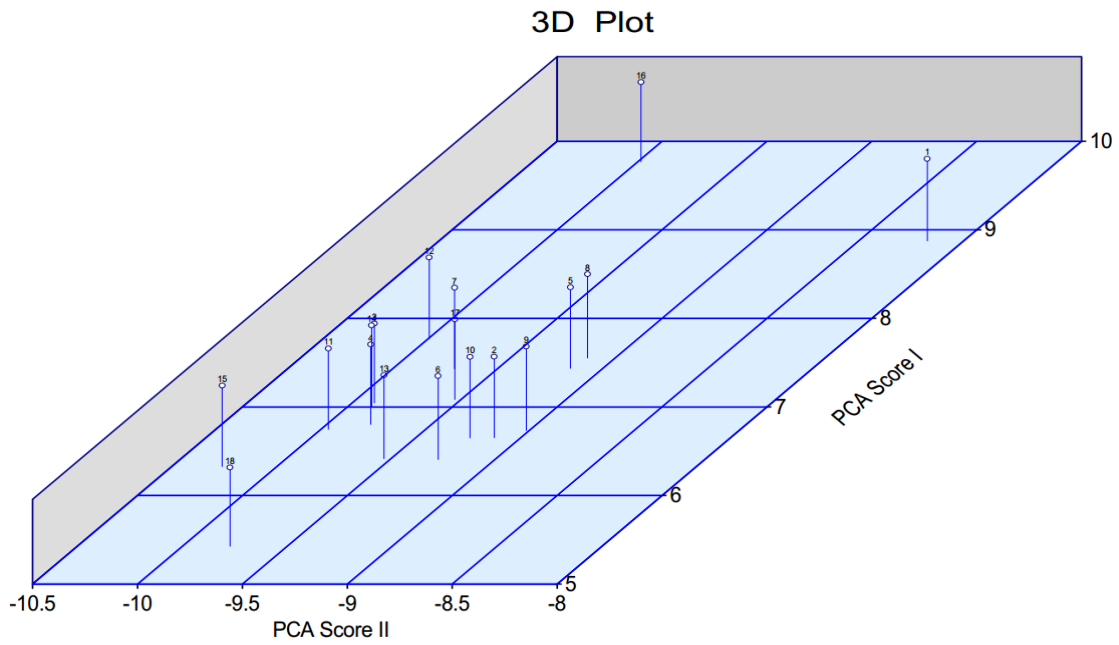
302

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

Cluster	I	II	III	IV	V
305 I	0.57	1.69	2.64	10.94	11.29
306 II		1.48	4.53	8.06	11.32
307 III			0.00	19.54	22.32
308 IV				0.00	5.63
309 V					0.00

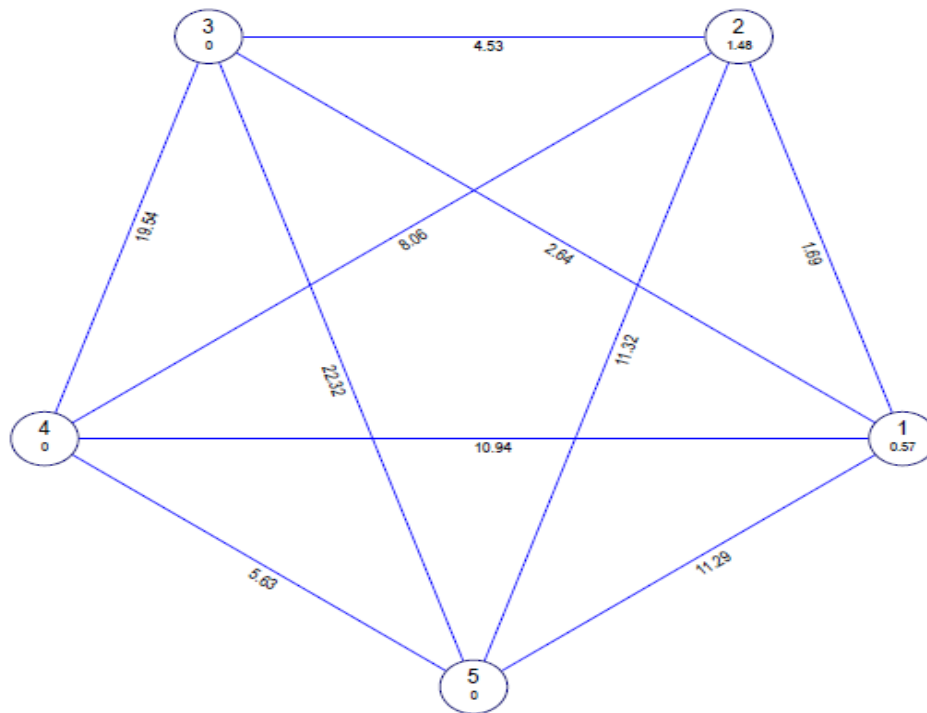
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313 Fig. 2: 3-D distribution of the diverse genotype based on principal components.

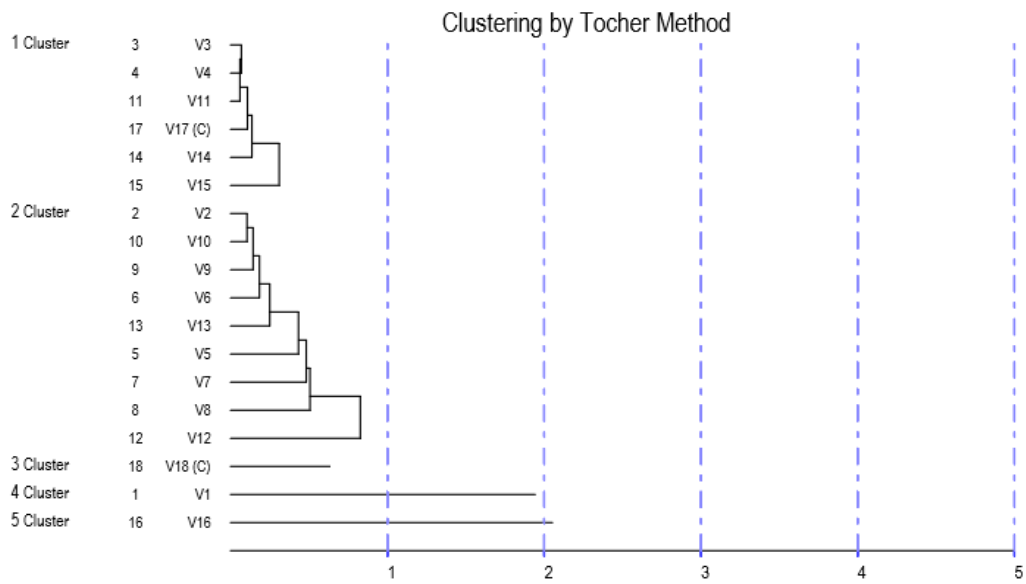


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316 Fig.3: Mahalanobis Euclidean Distance (Not to the Scale) by Tocher Method

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Fig.4: Dendrogram of the diverse genotype of Nigella

UNDER PEER REVIEW