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

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SPS, Avinash Kumar, Banshidhar and Ashutosh Kumar planned and conducted the experiment. Author SKS analyzed the data while collection of review, preparation of first hand manuscript and correction of the manuscript was carried out by authors PPS, KK, UKS, VKC and RK. All authors read and approved the final manuscript.

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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

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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.

1. INTRODUCTION

Black Cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranunculacea [1]. 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 [2]. 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 [3] but, it prefers loamy sand soils [4]. It can be grown from sea level to 2500 m of altitude with a reduction in yield with increasing altitude.

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

The dried seeds of nigella are the commercial product being used in food. The seeds contain 0.5 to 1.4% essential oil which has demand in the pharmaceutical and perfume industry [5]. It has been used since antiquity for culinary, seasoning and pharmacological purposes [6]. Many medical properties have been attributed to the *Nigella sativa* L. seeds and its oil, including carminatives, diuretics, antineoplastic (antitumour),antifungal, anti-helminthic, while their oil has protective action against histamine induced bronchospasm, cough and bronchal asthma [7,8], antidiabetics [9,10], spasmolytic and bronchodilator [11], anti-inflammatory [12], antibacterial [13], galactogogue, antioxidant [14,15] and insect repellent effects [16]. Additionally, black seed is a valuable source of protein, carbohydrates, essential fatty acids, vitamins as well as minerals such as calcium, potassium, iron, magnesium, selenium, manganese and zinc. *Nigella sativa* L. is being considered important for both oil and bioactive compounds because their constituents have unique chemical properties and may augment the supply of edible oils [17]. Black cumin seed has higher total phospholipid content than cotton and soybean seed oil [18].

Genetic diversity is pre-requisite for any crop improvement programme, as it helps in the development of superior recombinant [19]. Genetic distance estimates for population grouping can be estimated by different methods as it is crucial to understand the usable variability existing in the population panel [20]. Principal Component Analysis (PCA) is a powerful tool in modern data analysis because this is a well-known multivariate statistical technique which is used to identify the minimum number of components, which can explain maximum variability out of the total variability [21,22] and also to rank genotypes on the basis of PC scores. Principal components are generally estimated either from correlation matrix or covariance matrix. Considering the importance of

PCA this study is conducted with the objective of identifying diverse Nigella genotypes.

2. MATERIALS AND METHODS

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. The experimental site is located at 25.59 N latitude and 85.75 E longitudes and has altitude of 51.20 m above mean sea level. Soil of TCA, Dholi, Bihar is mainly young alluvium and calcareous. Standard agronomic practices were adopted with row to row and plant to plant spacing of 25×5 cm, recommended dose of fertilizer was applied during the time of crop period. Data was recorded for ten different traits *viz.,* Plant height, primary branches per plant, secondary branches per plant, days to 50 per cent flowering, Fruit length, Fruit width, days to maturity, fruit per plant, grains per plant and yield per plant. Five competitive plants from each plot were randomly chosen for recording the data except for days to 50 per cent flowering and days to maturity whose data were recorded on plot basis.

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

3. RESULTS AND DISCUSSION

RN-20 has highest mean value for primary branch /plant (12.00), secondary branch / plant (36.67), grain yield (129.33) and yield / plant (16.23g).RN-78 show highest mean value for fruit length (2.25 cm) and fruit/plant (49.67). RN-71 has highest mean value for fruit width (1.86 cm). RN-77 has least mean value for plant height (52.67) while the check variety Rajendra Shyama has least mean value for days to flowering (67.33) and days to maturity (136.67).The analysis of variance indicated that mean squares for genotypes were highly significant for all the traits under study. For establishing genetic relationship among the genotypes and their genetic discrimination Principal Component analysis was performed. Association between traits emphasised by this method may correspond to genetic linkage between loci controlling the traits or a pleotropic effect [23].

The genetic variation present in breeding population was divided into four principal components (PCs) which explained 91.69% of total variation (Table 4). According to Brejda et al. [24], the PC with Eigen values >1 and which explained at least 5% of the variation in the data will be considered as principal components. The

Table 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	Darbhanga	2006
17)	RN-79	Sitamarhi	2007
18)	Rajendra Shyama	Released variety (Check)	2011

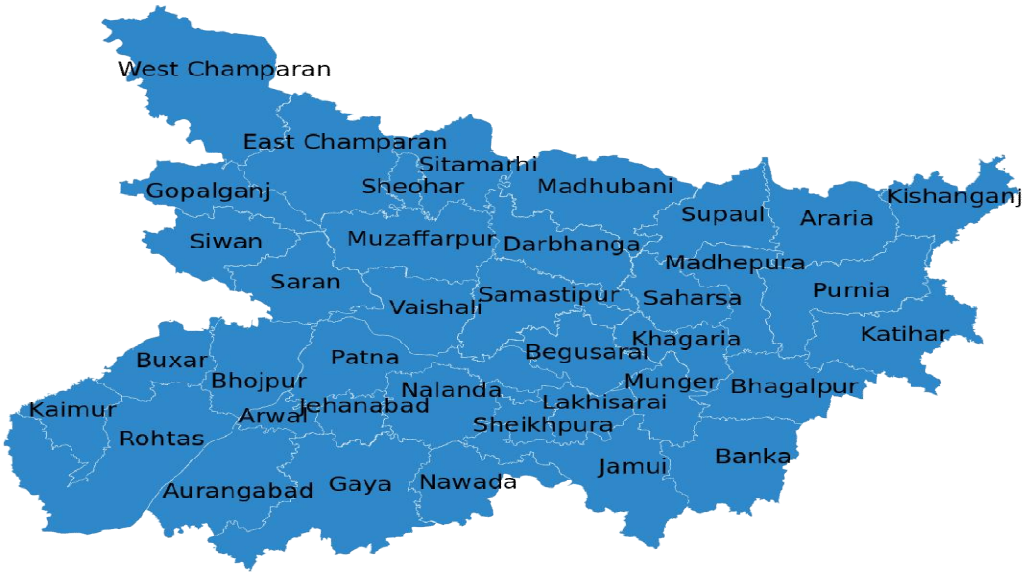

Fig. 1. Map showing different districts of Bihar

first three PCs had >1.00 Eigen value and accounted to 84.71 % of total variation. It indicates that the identified characters within these components exhibited immense influence on the phenotype of the genotypes. The first principal component (PC_1) explained 55.47% of the total variation. The second principal component (PC_2) explained 17.14% variation individually and 72.62% cumulative variation. The third principal component (PC_3) explained 12.08% variation individually and 84.71% cumulatively.

Rotated component matrix for various traits revealed that PC_1 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 PC_2 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 PC_3. The characters that contributed most to the PC_4 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.

3.1 Cluster Analysis

Cluster analysis helps in selection of suitable genotype(s) or parent to use in hybridization programme for the manipulation of desirable traits. Choice of proper parent(s) plays a vital role for a successful plant breeding programme. Parents having more genetic distance believed to create higher variations by generating higher recombination frequency, which increase the genetic gain in selection [25]. The grouping of collected lines was done by K-mean clustering pattern. The distribution of 18 lines along with check into five clusters and their cluster means are presented in Tables 5 and 7 respectively. Cluster III and IV comprised of only one line forming the smallest cluster followed by cluster V which was comprised of only two lines. Cluster I and II comprised of nine and five nigella lines respectively. The check Rajendra Shyama was clustered in Cluster V [26]. The k-mean of different clusters indicated that genotype falling in cluster III possess high values for all the traits under study. The genotypes in cluster I have minimum values for height of the plant, primary branches/plant, secondary branches/plant, days to 50% flowering, length of fruit, days to maturity and yield/plant while, genotypes in cluster II have minimum values for width of fruit, fruits/plant and grains/plant. It indicates that representative lines can be chosen from particular diverse groups based on their cluster mean and can be involved in improvement programmes for Nigella improvement.

The character contribution of various clusters towards the genetic diversity by Tochers clustering method indicated that Grains per plant and Yield per plant were the major contributors towards total divergence (Table 8).

Table 2. Mean performance of genotypes for studied traits

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 Shyama	55.00	7.67	19.00	67.33	1.73	1.32	136.67	55.67	55.00	7.59
	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
	C.D. 5%	9.87	2.13	6.77	5.34	0.21	0.28	2.03	14.16	11.27	2.02
	C.D. 1%	13.25	2.86	9.10	7.17	0.29	0.38	2.72	19.01	15.12	2.71

Table 3. 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

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

Table 4. 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

Table 5. Rotated component matrix for various traits

Traits	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		

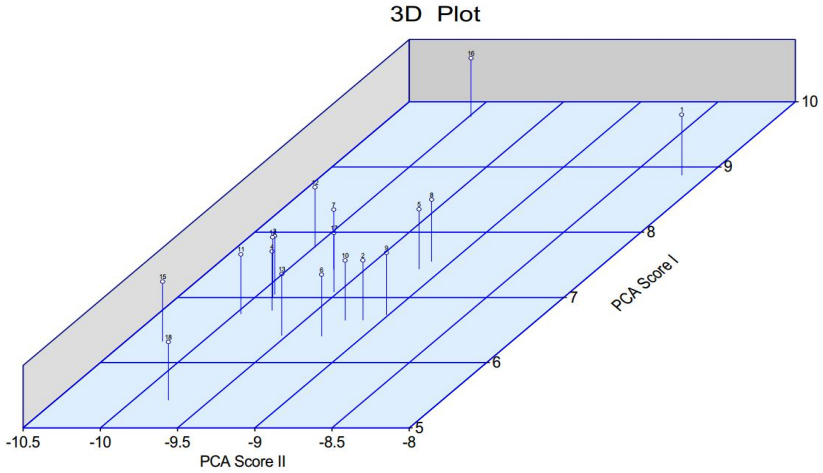

Fig. 2. 3-D distribution of the diverse genotype based on principal components

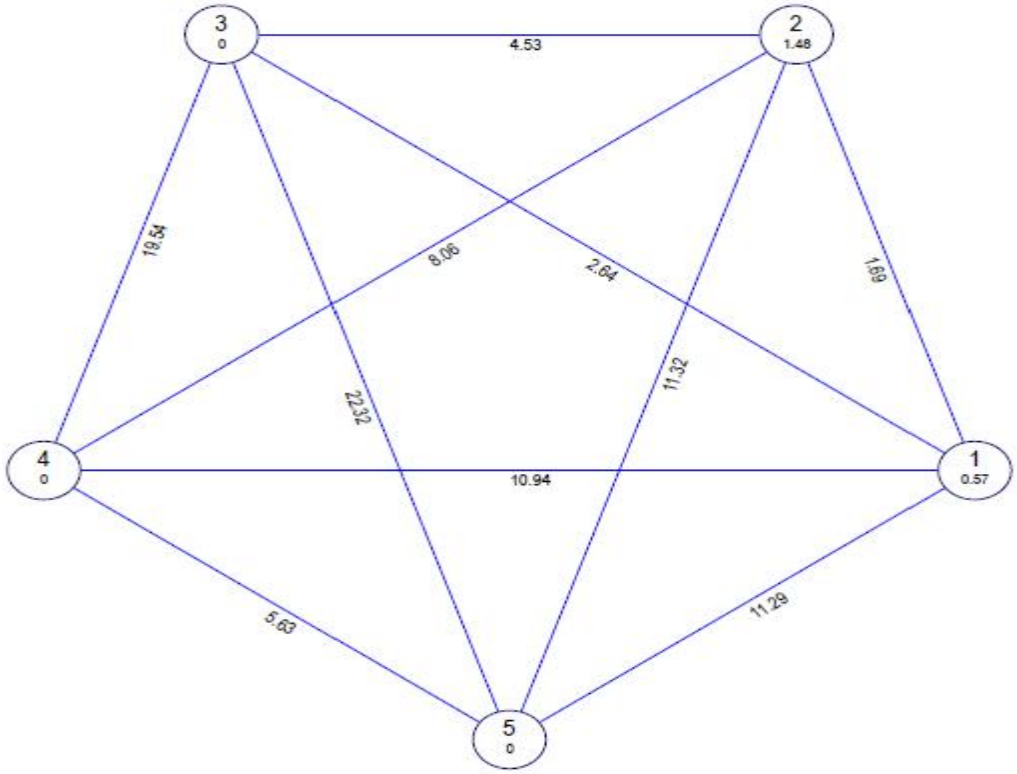

Fig. 3. Mahalonobis [27] eucladean distance (Not to the Scale) by tocher method

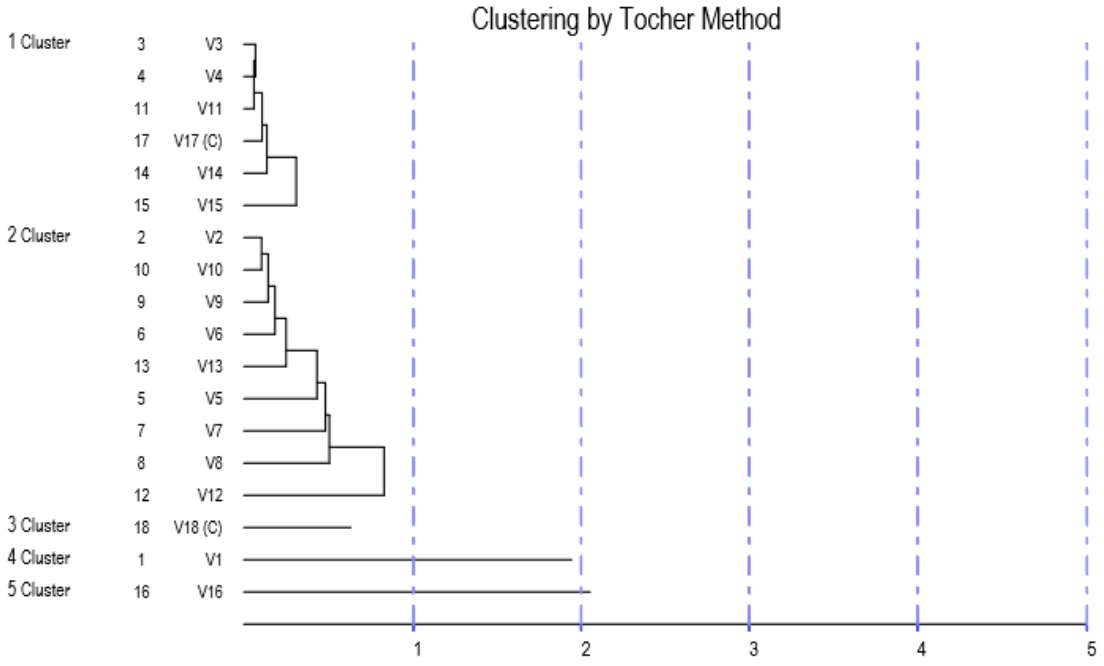

Fig. 4. Dendrogram of the diverse genotype of Nigella

Table 6. Distribution of Nigella genotypes in various clusters

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

Table 7. 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

Table 8. Contribution percentage of traits towards genetic divergence

S. No.	Traits	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%

Table 9. Estimates of Intra (diagonal) and Inter-cluster distances in 18 Nigella genotypes

Cluster	I	II	III	IV	V
I	**0.57**	1.69	2.64	10.94	11.29
II		**1.48**	4.53	8.06	11.32
III			**0.00**	19.54	22.32
IV				**0.00**	5.63
V					**0.00**

Intra and inter cluster distance was carried out on the basis of yield and its component traits between eighteen genotypes (Table 9). In general, inter-cluster were greater than the intra-cluster distances indicating the considerable amount of genetic diversity among the genotypes studied. The average intra-cluster distance between genotypes was maximum (1.48) for the cluster II followed by Cluster I (0.57). Cluster III, IV and V did not show intra-cluster distance indicating their genetic similarity. The highest inter-cluster distance was noted between cluster III and V (22.32) followed by cluster III and IV (19.54). The least inter-cluster distance was observed for cluster I and II (1.69) indicating their genetic similarity.

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

4. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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