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MULTIVARIATE ANALYSIS OF GENETIC DIVERGENCE AMONG NIGER GENOTYPES IN RELATION TO SEED OIL QUALITY TRAITS

S. C. PULATE*, H. S. PATIL AND M. R. PATIL
Department of Agricultural Botany, College of Agriculture,
Dhule, Mahatma Phule Krishi Vidypeeth, Rahuri - 413 722 (M.S.), INDIA
e-mail: santoshpulate11@gmail.com

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***Corresponding author**

ABSTRACT
A study was conducted to assess the extent and pattern of genetic variability of niger genotypes with respect to twelve seed oil quality traits in forty niger genotypes. The analysis of variances has shown that there was significant variation among the genotypes in all traits. The high heritability (>60%) was recorded for all oil parameters except for gamma tocopherol (2.80%). The multivariate analysis has resulted in formation of eleven clusters. Out of eleven clusters formed, cluster 1 was the largest with eighteen genotypes followed by cluster 4 with six genotypes. The highest genetic distance was recorded between cluster 7 and 11 (28.19), while lowest genetic distances noticed between cluster 6 and 7 (8.24). Cluster 2 included four genotypes with highest means for oil content (39.83), protein (33.51), oleic acid (36.53), stearic acid (10.31), alpha tocopherol (94.42) and gamma tocopherol (7.50). Thus, on the basis of present finding there is a possibility of simultaneously improving the fatty acid profile, tocopherol as well as the oil content with seed yield per plant of the genotypes through inter crossing between genotypes *viz*., JNS-164, JNS-165, Utakal Niger -150, GA-10, IGPN-08-66, IGPN-2004-1, JNS-501, IGPN-8004 and BAU-10-5. Among twelve traits studied palmatic acid (28.97%) contributed highest towards the genetic divergence followed by linoleic acid (27.44%) and stearic acid (18.46%).

INTRODUCTION

Niger (*Guizotia abyssinica* (L.f.) Cass) is a little known oilseed crop. The major niger growing countries of the world are Ethiopia and India. In Ethiopia 50-60% of the edible oil requirement of the country is met by niger seed oil, whereas only about 2% of the edible oil requirement is provided by niger seed in India (Riley and Belayneh 1989 and Dutta *et al*. 1994). The oil content of niger is variously reported as 29-39% (Dutta *et al*. 1994), 30-35% (Kandel and Porter 2002) and 42-44% (Dagne and Johnsson 1997). Dutta *et al*. (1994) reported that the Ethiopian niger seed oil contains more than 70% linoleic acid, whereas, Dagne and Johnsson (1997) reported 66-69% linoleic acid. In all the works so far done on the fatty acid composition of niger, linoleic acid is unequivocally the dominant fatty acid present in niger seed oil followed by palmitic, oleic and stearic acids (Dutta *et al*. 1994 and Dagne and Johnsson 1997). The percentage of oleic acid in the Ethiopian niger seed oil was reported to be in the range of 6-11% (Dutta *et al*. 1994), 5.4-7.5% (Dagne and Jonsson 1997). It is indicated that the oil content and the fatty acid profile may vary depending on the origin of the material and the maturity level of the seeds (Riley and Belayneh 1989).

The quality of oil and its suitability for a particular purpose is it for industrial use or for human consumption depends on the proportion of the different fatty acids it contains. There are opportunities which favor cultivation of oilseeds in general in the country which ranges from import substitution of edible oils to export of high value seed and oil. Although efforts have been done to improve oil quality of niger seed using various breeding strategies, such as genetically modify the degree of unsaturation in oils through genetic engineering (Kinney 1994, Chapman *et al*.2001). Genetically engineered food crops, however, are not appealing for the most part to the public and its use for human consumption is still controversial in many countries around the world. Thus, to circumvent the ethical, public health as well as economic problems presented by the chemical and genetic modification of oils, modification of the proportion of the fatty acids towards the desired composition by plant breeding remains the best alternative to date. Therefore, much need to be done to utilize natural variation that might exist among genotypes for fatty acid profile. Assessing the genetic diversity and relation among niger genotypes based on their seed oil quality traits is thus prerequisite which may help in identifying important genotypes and selection criteria for improvement of niger seed oil. Therefore, this study was executed with the objective of assessing the extent and pattern of genetic variability of niger genotypes of diverse agro climatic region of the country.

MATERIAL AND METHOD

The material used for the present investigation comprised of forty genotypes, out of them thirty genotypes were collected from the Project Co-ordinating Unit, AICRP on Sesame and Niger, Jabalpur (M.P.) and ten from AICRP on niger at Zonal Agriculture Research Station, Igatpuri. The experiment was carried out using Randomized Block Design with two replication at Botany Section, College of Agriculture, Dhule (Maharashtra) during *kharif* 2011. Each genotype was grown in a double row of 4.5 m length with a spacing 30 cm between rows and 10 cm within a row. All recommended agronomic

practices were followed to raise good crop. Ten plants were tagged at random in each replication for find out seed yield/ plant (g).

Data were collected on seed oil quality traits, oil determined using Nuclear Magnetic Resonance Spectrometry (NMRS). Protein, crude fiber, fatty acid composition and tocopherol of the seed were determined by using near infrared reflectance spectrometry (NIRS). Major fatty acid compositions such as oleic, stearic, palmatic, linoleic, omega 3, and alpha, beta, gamma, tocopherol were consider in study. Each was measured as percentage of total fatty acid and tocopherol were performed on 25 g of samples using NIR software package (Spectra Analyzer) in 1445-2348 nm spectral ranges.

Analysis of variance was performed following the standard procedures described by Panse and Sukhatme (1995). The phenotypic and genotypic coefficients of variability were computed according to the method suggested by Burton (1952). Heritability (broad sense) and genetic advance were estimated as per Johnson *et al*. (1955a). Multivariate analysis such as clusters and principal components analysis of the genotypes values computed as per Tocher's method (Rao,1952). Genetic distance between clusters was calculated using the generalized Mahalanobis D^2 statistics.

RESULT AND DISCUSSION

The analysis of variance has shown that there was significant variation among the genotypes in all traits. This indicates that existence of considerable genetic variability for selection and breeding. Means and range of quality traits of the genotypes are presented in Table 1. The estimates of genetic variability (table 1) exhibited the highest GCV for beta tocopherol (26.48) and seed yield/plant (26.70). The GCV was medium for alpha tocopherol followed by stearic, palmatic and oleic acid. The differences between GCV and PCV were very low for all characters, was mostly due to genetic factors (Yadav *et al*. 2012). The presence of high genetic variability is an indication of good scope for their improvement through hybridization followed by selection.

High heritability recorded almost all traits except gamma tocopherol. High heritability coupled with high genetic advance as per cent of mean was recorded for alpha, beta tocopherol, seed yield per plant, palmatic, stearic and oleic acid revealing the influence of additive gene action for these traits. Hence the improvement of these traits can made through direct phenotypic selection. High heritability coupled with low genetic advance as per cent mean recorded for oil content, crude fiber, linoleic acid, omega3 and gamma tocopherol, indicating the effect of non additive gene action in crop improvement like heterosis breeding may be beneficial.

In any crop improvement venture, genetically distant parents are needed for crossing programs. This is to create the required genetic diversity between genotypes in terms of gene frequencies which may result of heterotic group and transgressive sergeants. Clustering produced clear grouping of the forty genotypes into eleven clusters (Table 2). Yadav *et al*. (2012) reported that high divergence between the clusters of 35 genotypes which were grouped in to three clusters. Similarly, Belete *et al*. (2011) reported that the existence of genetic diversity among 36 mustard genotypes which were grouped into seven clusters for seed oil quality traits. C1 was the largest with eighteen genotypes followed by C4 with six genotypes, C5 with five genotypes, C2 with four genotypes and C3, C6, C7, C8, C9, C10, and C11 monogenotypic. The individuals within any one cluster are more closely related than are individuals in different clusters. Among eleven clusters studied important traits contributing to the divergence was palmatic acid (28.97%) followed by linoleic acid (27.44%), stearic acid (18.46%), whereas alpha tocopherol do not showed contribution towards the divergence (Table 6). The pair wise generalized D^2 distance among the clusters is presented in Table 3. Genetic distances among most clusters were significant. The highest genetic distance was recorded between C7 and C11 (28.19) followed by C7 and C9 (26.56), C2 and C8 (26.13), C6 and C11 (25.97), C8 and C10 (25.48), C10 and C11 (25.03), C3 and C10 (24.56), C5 and C10 (24.47) were highly significant from which parents may be selected for crossing in order to obtain genetic recombination and transgressive segregants in the subsequent generation. However, it is also valuable considering genotypes with in cluster with respect to a trait of interest as suggested by Chahal and Gosal (2002) and Keneni *et al*. (2005). Genetic distances between C6 and C7 (8.24) followed by C9 and C11 (10.85), C1 and C3 (12.33) and C1 and C6 (12.34) were non significant, indicating close relationship among the genotypes (Belete *et al*. 2011).

Pattern of distribution of genotypes in different clusters showed no correspondence between genetic diversity and geographic origin. The grouping of genotypes of the same origin in the same cluster may be result of their similar genetic background (JNS-508, JNS-255, JNS-253 and JNS-502 were same origin group in C1). On the other hand, there are also genotypes with same geographical origin but grouped in different clusters (JNS-502 in C1, JNS-164, JNS-165 in C2, JNC-1 in C6, JNS-505 in C8, JNS-204 in C9, and JNS-501in C11 were same origin

Table 1: Distribution of forty niger genotypes in different clusters

Cluster Number	Total no. of genotypes in each cluster	Genotypes included in the cluster
1	18	DNC-21, JNS-508, DNC-08-9, NSH-5637, NSH-5631, DNC-08-5, JN-08-04, NSH-5638, JNS-255, JNS-253, NSH-5633, NSH-5636, JNS-502, IGPN-9001, IGP-76, Birsa Niger-,1 BAU-10-2, NSH-5632
2	4	JNS-164, JNS-165, Utakal Niger -150, GA-10
3	1	JNS-503
4	6	IGPN-08-16, NSH-5644, IGPN-08-66, Birsa Niger-2, BAU-10-5, IGPN-2004-1
5	5	Birsa Niger-3 DNC-08-2 JNC-6 IGPN-8007 BAU-09-2
6	1	JNC-1
7	1	IGPN-8004
8	1	JNS-505
9	1	JNS-204
10	1	DNC-22
11	1	JNS-501

Table 2: Range and estimates of genetic parameters for different twelve quality traits of niger

Sr. No.	Characters	Range	Mean	GCV	PCV	h^2 (BS)	GA	GA as % Mean
1	Seed Yield/Plant (g)	1.74-6.04	03.33	24.70	29.22	71.50	1.43	43.03
2	Oil Content	37.12-40.49	38.24	01.92	02.46	60.60	1.18	03.07
3	Protein	26.41-35.26	31.04	05.76	06.84	71.00	3.11	10.01
4	Crude Fiber	19.11-25.14	22.84	05.18	05.57	86.40	2.26	09.92
5	Oleic acid	24.80-39.44	30.08	10.15	12.07	70.70	5.29	17.57
6	Stearic acid	6.00-10.70	8.04	13.27	13.43	97.60	2.17	27.01
7	Palmatic acid	7.43-12.66	9.59	10.93	11.03	98.10	2.14	22.31
8	Linoleic acid	55.32-60.44	58.07	02.01	02.39	71.10	2.03	03.49
9	Omega 3	42.62-51.16	46.35	04.70	05.05	86.90	4.18	09.03
10	Alpha Tocopherol	2.99-8.21	05.71	19.69	20.28	94.30	2.25	39.38
11	Beta Tocopherol	1.18-4.12	02.77	26.48	26.98	96.30	1.48	53.51
12	Gamma Tocopherol	49.61-93.36	89.25	01.74	10.41	02.80	0.54	00.60

GCV: genetic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2 (BS): heritability in broad sense, GA: genetic advance

Table 3: Pair wise generalized squared distance (D^2) among forty genotypes of niger in eleven clusters based on their quality traits

Cluster Distances	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	C 11
C 1	9.23	17.73	12.33	15.01	13.9	12.34	15.07	15.66	14.7	15.77	18.02
C 2		7.62	20.47	24.13	18.29	21.91	23.27	26.13	20.57	20.41	21.22
C 3			0	16.69	8.65	14.08	12.17	13.33	20.14	24.56	18.46
C 4				12.22	19.44	19.29	21.75	13.86	15.42	21.41	17.98
C 5					10.23	16.8	15.8	16.22	19.81	24.47	17.45
C 6						0	8.24	20.9	22.64	18.2	25.97
C 7							0	22.53	26.56	23.16	28.19
C 8								0	14.58	25.48	12.71
C 9									0	16.48	10.85
C 10										0	25.03
C 11											0

Underline figures indicate intra cluster D^2 values, C-Cluster

Table 4: Intra class average values for twelve quality traits of the eleven clusters of niger genotypes

Cluster	Fatty acid composition									Tocopherol		
	SYP	OC	PC	CF	OA	SA	PA	LA	O3	α	β	γ
C 1	3.52	37.96	30.38	22.62	29.84	7.84	9.70	57.97	46.57	89.85	2.84	5.01
C 2	1.77	39.83	33.51	23.08	36.53	10.31	11.49	58.89	48.83	92.42	3.24	7.50
C 3	3.66	37.17	29.70	24.20	26.32	8.01	8.65	57.00	42.62	89.15	3.12	6.08
C 4	3.10	39.10	33.27	24.24	27.99	6.97	8.93	59.51	44.68	90.70	2.04	6.23
C 5	2.97	37.59	29.3	22.16	27.14	8.61	8.88	56.6	44.92	91.07	3.23	6.53
C 6	4.05	38.23	28.82	22.85	31.65	6.73	10.07	58.74	48.54	49.61	4.21	4.31
C 7	3.68	37.33	29.62	23.29	30.46	6.62	9.92	55.46	45.83	88.35	4.15	4.94
C 8	6.04	38.05	33.47	23.47	27.33	7.85	7.43	58.15	44.06	90.02	1.75	5.53
C 9	3.61	37.63	32.02	20.93	35.01	8.56	9.28	59.40	46.70	88.49	1.35	5.64
C 10	4.93	38.32	30.75	20.83	35.02	7.39	11.60	57.82	51.16	87.85	2.26	4.65
C 11	3.30	37.60	30.29	22.23	30.67	9.41	8.16	57.15	48.67	89.99	1.32	7.02

SYP: seed yield/plant, OC: oil content, PC: protein, CF: crude fiber, OA: oleic acid, SA: stearic acid, PA: palmatic acid, LA: linoleic acid, O3: omega 3, α: alpha tocopherol, β : beta tocopherol, γ : gamma tocopherol

Table 5: Component scores of the first five principal components of forty genotypes of niger

Sr. No.	Traits	Score1	2	3	4	5
1	Seed yield/plant	0.087	0.023	0.045	0.178	0.019
2	Oil content	-0.042	-0.085	-0.023	-0.271	-0.077
3	Protein	0.030	-0.092	-0.045	-0.182	0.184
4	Crude Fiber	0.282	0.147	0.074	-0.639	0.089
5	Oleic Acid	-0.140	-0.153	0.024	-0.097	-0.236
6	Stearic Acid	-0.496	-0.185	-0.617	0.061	0.115
7	Palmatic Acid	-0.620	-0.320	0.511	-0.349	0.206
8	Linoleic Acid	0.419	-0.685	0.122	-0.042	-0.269
9	Omega 3	-0.187	-0.210	-0.061	0.062	-0.692
10	Alpha Tocopherol	0.065	0.013	-0.002	-0.141	-0.005
11	Beta Tocopherol	-0.208	0.534	0.255	-0.087	-0.508
12	Gamma Tocopherol	0.038	0.059	-0.513	-0.534	-0.177
13	Eigene Value (Root)	1533.643	1327.775	1097.429	466.152	103.059
14	Variance %	32.596	28.220	23.325	9.908	2.190
15	Cumulative %	32.596	60.816	84.141	94.048	96.239

Table 6: Per cent contributions of traits into total divergence

Sr. No.	Traits	Times Ranked 1st	Contribution %
1	Seed Yield/Plant (g)	1	00.13%
2	Oil Content	3	00.39%
3	Protein	2	00.26%
4	Crude Fiber	57	07.31%
5	Oleic Acid	4	00.52%
6	Stearic Acid	144	18.46%
7	Palmatic Acid	226	28.97%
8	Linoleic Acid	214	27.44%
9	Omega 3	5	00.64%
10	Alpha Tocopherol	0	00.00%
11	Beta Tocopherol	60	07.69%
12	Gamma Tocopherol	64	08.20%

but group in different cluster) in which differential selection criteria, genetic drift and adaptation to different agro-climatic conditions might be the cause. Paradoxically, genotypes with different geographical origin were grouped in same cluster (JNS-165 and Utakal Niger -150 were group in C2 and Birsa Niger-3, DNC-08-2, JNC-6, IGPN-8007 and BAU-09-2 were differen origin but grouped in cluster 4) in which case synchronization of selection differential for different traits in different areas might have been occurred. The aforementioned phenomena have also reported by Rawanappa and Sheriff (1994) and Patil (2007).

The existence of diversity among the genotypes was also assessed by the considerable amount of variation in intra class average for different quality traits (Table 4). High oil content with protein, oleic, stearic, alpha and gamma tocopherol but low in seed yield per plant were showed by C2. Other hand C8 had high seed yield per plant with low oleic acid and palmatic acid content. The highest linoleic acid content in their seed was revealed in C4, followed by C2 and C6, lowest showed by C7. The genotype in C7 was recorded highest omega 3 content. The genotypes in C4 were highest crude fiber content.

In order to assess the patterns of variations, Principal Component Analysis (PCA) was done by considering twelve quality traits simultaneously. Principal Component Analysis (PCA) (Table 5) showed that 96.23 % of variation was contributed by first five principle components. 32.59 % of the variation was depicted by first principal component in which seed yield per plant, protein, crude fiber, linoleic acid and alpha and gamma tocopherol positive contributors. In this principle components oil, oleic acid, stearic, palmatic, omega 3, beta tocopherol had negative weight. The present findings are corroborative with findings of Belete *et al*. (2011). Additional variation of 28.22 % was revealed by the second principal components which accounted positively mainly for seed yield/plant, crude fiber and tocopherol (alpha, beta and gamma), linoleic acid (-0.68) had the highest negative weight in this principal component. Another additional variation of 23.32%, 9.90% and 2.19% were shown by the third, fourth, and fifth principal component, respectively. Palmatic acid (0.511) content in the third, seed yield per plant (0.178) in fourth and palmatic acid (0.20) and protein (0.186) in fifth principal components were the major positive contributors.

Clustering of genotypes into groups was mainly attributed by cumulative effects of individual traits. In general, this study indicate that there is a possibility of simultaneously improving the fatty acid profile, tocopherol as well as the oil content with seed yield per plant of the genotypes through further breeding endeavor such as inter crossing between genotypes *viz.*, JNS-164, JNS-165, Utakal Niger -150, GA-10, IGPN-08-66, IGPN-2004-1, JNS-501, IGPN-8004 and BAU-10-5 were selected as per the inter-cluster distance, cluster mean and per se performance of genotypes and divergent cluster combination observed in the present study. The present investigation also revealed that diverse geographic origins of the genotypes could not necessarily be an index of variation and the factors other than geographic diversity such as genetic drift, selection pressure and environment may be responsible for discrepancy of genotypes.

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