African Journal of Biotechnology Vol. 10(66), pp. 14757-14766, 26 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2219
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Study of nutritional characteristics, mineral nutrients and agro-biodiversity in black cumin (*Nigella sativa* L.) genotypes from Pakistan

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Accepted 3 June, 2011

Nigella sativa **(L.) a member of the family Ranunculaceae is being used for edible and medicinal purposes in several countries of the world. This study revealed the analysis of genetic diversity of thirty six (36) genotypes of *N. sativa* based on yield traits, nutritional characteristics, and mineral nutrients. Two genotypes PK - 020561 and PK - 020646 differed significantly from other 34 genotypes with respect to grain yield (P = 0.0007), biomass (P = 0.049), oil content (P = 0.001), nitrogen (P = 0.0117), calcium (P = 0.0164) and boron (P = 0.0004). These results indicated the scope of these traits for selection of genotypes. Eigen values were greater than 1 indicating the significance of principal component analysis. Five and six clusters were observed for nutritional characteristics and mineral nutrients respectively. Based on principal component analysis, yield traits contributed 86% of the variability pertained to nutritional characteristics, whereas four factors contributed 68% of the genotypes variability based on mineral nutrients. The genotype PK - 020646 produced the highest biomass, harvest index, grain yield, oil content, protein, moisture, fibre, N, Pb, Co and Mn, whereas PK - 020561 produced the highest Ca, Mg, Pb, Zn, Mn, Na and P contents. UPGMA cluster analysis based on nutritional characteristics revealed five distinct clusters with genotypes which were markedly different. Thus, all clusters were cluster 5 and cluster 1, dominating other clusters. Based on thirteen (13) mineral components, all genotypes formed six clusters, with clusters 4 and 5 having 11 and 10 genotypes. Thus, Euclidean distance among genetically distinct clusters supports different patterns of yield and mineral nutrients. The overall grouping of clusters corresponded with principal component analysis confirming the genetic variability among genotypes. These genotypes are suggested for their further application in the genetic improvement of *N. sativa* L, for their use as quality food to substitute Nigella cultivars currently being used.**

Key words: Biodiversity, black seed, cluster analyses, kalonji, physico-chemical traits, principal components' analysis.

INTRODUCTION

Among the medicinal plants in use from prehistoric times, black cumin (*Nigella sativa* L.) is being used for healthcare. It is commonly known as black seeds, fennel flower, Roman coriander and Kalonji (Weiss, 2002).

Different bioactive compounds have been reported in various spices and these are well known for their beneficial effects on health (Hussain et al., 2006). These contain essential macro and micronutrients which play vital roles as structural and functional components of metalloproteins and enzymes in the living cells (Ansari et al., 2004). *N. sativa* L. contains more than 100 valuable bioactive compounds (Duke, 1992). *N. sativa* is used for edible and medicinal purposes in India, Pakistan, Saudi

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Arabia, Syria, Iran, Egypt and many other countries (Fillippo et al., 2002). Its constituents have unique chemical properties and may augment the supply of edible oils (Ramadan and Morsel, 2003; D'Antuono, 2002).

The whole seed or its extract has antitumor (Worthen et al., 1998; Khan et al., 2003), antidiabetic (Fararh et al., 2002; El-Dakhakhny et al., 2002), spasmolytic and bronchodilator (Boskabady et al., 2004), anti-inflammatory (Hajhashemi et al., 2004), antibacterial (Mashhadian and Rakhshandeh, 2005), galactogogue, antioxidant (Brutis and Bucar, 2000; Kanter et al., 2003) and insect repellent (Fisher, 2002) properties. It is also used as seasoning and flavoring supplement of food, bread, pickles and especially bakery products (Ramadan and Morsel, 2002). In spite of its several applications, its productivity is low mainly due to the application of low yielding genotypes, poor crop management practices, high weed incidence, disease, insect damage, inadequate planting density, and lack of nutritional processing techniques. Chemical studies on *N. sativa* L. have been reported at species level, whereas investigation on nutritional and mineral constituents at intra-specific level is scarce in literature (Palevitch, 2004). In this study, we analyzed thirty four (34) genotypes of *N. sativa* L. in comparison with two check genotypes (PK - 020561 and PK - 020646) for genetic diversity based yield traits, mass of nutritional and mineral constituents, so as to identify source material for crop improvement program for their potential applications in health and food sectors.

MATERIALS AND METHODS

Agro-botanical traits

Thirty six (36) genotypes of *N. sativa* L. including two genotypes (PK - 020663, PK - 020783) as checks were evaluated under field conditions for three consecutive years at Plant Genetic Resources Program, Institute of Agri-Biotechnology and Genetic Resources, National Agricultural Research Center, Islamabad, Pakistan (33.40°N and 73.07°E; 540 m). The sowing in each year was conducted in the last week of November and the crop was harvested at the end of April. Data for grain yield, biomass and harvest index (a ratio between grain yield and biomass and expressed in percent) were recorded by sampling 30 plants at random from each genotype in triplicates.

Nutritional characteristics

Nutritional characteristics were analyzed in the Norwegian Accreditation ISO certified 17025 Lab, at Grain Quality Testing Laboratory, National Agricultural Research Center, Islamabad. The reagents and solvents used in this study were of the highest purity and HPLC grade (Sigma Chemical Company, St. Louis, MO, USA). Oil, protein, nitrogen, carbohydrates, ash, moisture and fiber contents were studied following the standard methods of AOAC (2005). Oil percentage was estimated by NMR and calibrated both at point one and point two by comparing with mustard oil standards. Moisture content was determined by oven drying 2 g test sample at 102°C; ash content by igniting 5 g test sample in a muffle furnace at 550°C, until light gray ash resulted and crude fibre by acid-base reflux method. For protein determination, N_2 content were multiplied by 6.25 using modified semi micro-Kjeldahl method, by using cupric sulphate kjeltabs instead of mercuric oxide (Searle, 1974; Lynch et al., 1997). The apparatus used was Kjel Auto Model DTP - 3 (Mitamura Riken Kogyo Inc. Tokyo, Japan).

Conditions of the equipment standardized for *N. sativa* L. included protein factor 6.250, blank 0.000, factor 0.900, normality 0.100, heater 60%, distillation 240 (S), NaOH 4 (S), and H_2O_2 (S). Oil contents (fat) were determined by Soxtec system HT 1043 extraction unit (Tecator, made in Sweden), following extraction with three solvent systems [(n-Hexane, chloroform: methanol (2:1) (Ramadan and Morsel, 2003)] and chloroform : methanol (1:3) for 2 h each. One gram (1 g) seed samples were grinded with the help of pestle and mortar, then wrapped in a tissue paper and placed inside thimbles. Boiling was performed for the first 45 min and then samples were rinsed for 45 min. Oil was extracted in beakers and placed in desiccators for evaporation of extra solvent. The experiments were repeated three times to minimize error. Results were presented as mean ± standard error.

Mineral nutrients

For quantification of mineral nutrients, sample solutions were prepared following the standard procedure recommended by the Royal Committee of Experts for the digestion of plant materials (SCA, 1987). Salt standards and reagent blanks were prepared to calibrate and check the reliability of analytical methods. Moreover, standards were repeated after every batch of 36 samples during operation to minimize error. Seed sample of 0.50 g was crushed, grinded and powdered in a mortar and pestle and added into 50 ml conical flask. Digestion was performed in the presence of nitric-perchloric acid mixture (HNO_3:$HClO_4$ at 2:1 ratio). Digest was stored in sterilized bottles and used for the determination of Fe, Cu, Zn, Mg, Co, and Mn, on flame atomic absorption spectroscopy (Varian AA240 FS Fast sequential atomic absorption spectrophotometer) following specific instrumental conditions as described earlier (Wright and Stuczynski, 1996).

Wavelength and particular flame type were as follows: Fe (248.3, air), Ca (422.7, N_2O), Cu (324.7, air), Mg (285.2, air), Pb (217.0, air), Zn (213.0, air), Co (240.7, air), Mn (279.5, air) and Na (589.0, air). Due to the instrumental sensitivity, the flame position was also changed to detect the particular element (nutrient) even at the lowest concentration. The phosphorus (P) was determined by adding ammonium vanadate (2.5 g), ammonium molybdate (45 g) and HNO_3, (500 ml), by developing vanadomolybdo-phosphoric acid yellow color (Cottenie, 1980), and K by emission spectroscopy (Wright and Stuczynski, 1996).

Dry ashing

Seeds were dry-ashed for boron determination (Gaines and Mitchell, 1979) and B concentrations were determined colorimetrically using azomethine - H method (Bingham, 1982).

Statistical analysis

The data were analyzed for mean, variance, coefficient of variability, principal component analysis and correlation using computer software MS Excel and numerical taxonomic techniques following the methods of cluster and principal component analyses (Sneath and Sokal, 1973) with the help of computer software 'Statistica' version 6.0 and 'SPSS' version 10.01 for windows XP Professional. For cluster analysis, a dendrogram was constructed

Table 1. Descriptive statistics for agro-botanical traits, nutritional characteristics and mineral nutrients in *N. sativa* L. germplasm consisted of 36 genotypes.

Trait	Unit	Mean ± SE	RSD	Range	CV	Check - 1*	Check- 2**	
Agro-botanical traits								
Grain yield	(g)	5.75 ± 0.44	2.67	1.47 – 11.24	46.42	9.53 ± 0.55	11.06 ± 0.26	a
Biomass	(g)	23.98 ± 1.49	8.96	7.96 – 46.34	37.37	12.40 ± 3.74	16.54 ± 3.45	b
Harvest Index	(%)	24.52 ± 0.83	4.97	13.28 – 31.33	20.26	19.68 ± 4.44	21.13 ± 4.94	c
Nutritional characteristics								
Oil contents	(%)	31.16 ± 0.23	1.37	28.40 – 34.33	4.38	31.39 ± 0.16	33.95 ± 3.06	
Protein	(%)	22.10 ± 0.25	1.53	19.21 – 26.28	6.91	21.05 ± 4.32	21.75 ± 4.16	
Carbohydrates	(%)	25.47 ± 0.62	3.70	14.71 – 30.80	14.52	27.15 ± 3.25	28.02 ± 4.51	
Ash	(%)	5.07 ± 0.07	0.44	3.52 – 6.06	8.67	5.52 ± 0.37	5.77 ± 0.68	
Moisture	(%)	5.89 ± 0.05	0.30	5.42 – 6.74	5.10	5.79 ± 0.23	6.01 ± 0.24	
Fiber	(%)	10.52 ± 0.39	2.34	6.03 – 15.08	22.24	8.88 ± 0.63	8.57 ± 0.36	
Hexane	(%)	25.92 ± 0.79	4.75	18 – 35	18.34	26 ± 1.32	18 ± 2.31	d
C:M (2:1)	(%)	31.72 ± 0.68	4.06	22 – 37	12.80	30 ± 1.36	35 ± 1.13	
C:M (1:3)	(%)	27.00 ± 0.66	3.94	18 – 34	14.57	19 ± 2.21	26 ± 1.99	e
Mineral nutrients								
Nitrogen (N)	(%)	3.57 ± 0.09	8.96	1.67 – 5.56	15.83	3.94 ± 0.01	3.54 ± 0.08	f
Iron (Fe)	mg kg^{-1}	0.26 ± 0.02	4.11	0.10 – 0.74	39.51	0.27 ± 0.35	0.22 ± 0.14	
Calcium (Ca)	mg kg^{-1}	9.13 ± 0.16	9.86	7.38 – 10.83	10.39	8.11 ± 0.14	9.03 ± 0.25	g
Copper (Cu)	mg kg^{-1}	0.03 ± 0.00	0.05	0.02 – 0.03	14.77	0.02 ± 0.62	0.03 ± 0.31	
Magnesium (Mg)	mg kg^{-1}	10.20 ± 0.09	2.66	9.40 – 11.56	5.11	9.46 ± 0.55	10.01 ± 0.16	
Lead (Pb)	mg kg^{-1}	0.06 ± 0.00	0.58	0.02 – 0.09	31.38	0.08 ± 0.12	0.02 ± 0.75	
Zinc (Zn)	mg kg^{-1}	0.05 ± 0.00	0.57	0.01 – 0.09	34.51	0.04 ± 0.07	0.04 ± 0.23	
Cobalt (Co)	mg kg^{-1}	0.03 ± 0.00	0.61	0.00 – 0.06	45.68	0.04 ± 0.65	0.02 ± 0.11	
Manganese (Mn)	mg kg^{-1}	0.05 ± 0.00	0.31	0.03 – 0.07	25.49	0.05 ± 0.54	0.06 ± 0.06	
Sodium (Na)	mg kg^{-1}	0.35 ± 0.02	4.70	0.17 – 0.68	36.64	0.32 ± 0.09	0.33 ± 0.14	
Phosphorus (P)	(%)	0.57 ± 0.01	0.19	0.50 – 0.66	5.83	0.51 ± 0.15	0.50 ± 0.08	
Boron (B)	mg kg^{-1}	23.68 ± 0.99	150.47	12.78 – 39.58	25.21	26.09 ± 0.14	30.88 ± 0.30	h
Potassium (K)	(%)	0.83 ± 0.01	0.85	0.63 – 0.99	10.14	0.77 ± 0.14	0.75 ± 0.21	

Agro-botanical traits were average of 30 samples over three years. Nutritional characteristics and mineral nutrients data were averages of triplicate determinations. SE- standard error, RSD-relative standard deviation and CV- coefficient of variability. Symbol (g) represent gram and (%) percentage. *Check - 1 (PK - 020663), **Check - 2 (PK - 020783). Probability (P) values for a, b, c, d, e, f, g and h are 0.007, 0.049, 0.169, 0.024, 0.006, 0.011, 0.016 and 0.0004, respectively.

by using the un-weighted group method with arithmetic average (UPGMA) means of each character were standardized prior to analysis to avoid the effect of scale difference.

RESULTS

Evaluation for agro-botanical traits, nutritional characteristics and mineral nutrients

Summary of statistical analysis for agro-botanical traits (grain yield/plant, biomass/ plant, and harvest index), nutritional characteristics (oil, protein, carbohydrates, ash, moisture and fiber contents) and mineral nutrients (Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N) is presented in Table 1. Though, harvest index was non-significantly different, check 1 and check 2 differed significantly with respect to grain yield (P = 0.0007), biomass (P = 0.049), oil content (P < 0.001), oil extracted with hexane (P = 0.024), or chloroform and methanol (1:3) (P = 0.0065) respectively. Among mineral nutrients, all genotypes different significantly with respect to nitrogen (P = 0.0117), calcium (P = 0.0164), and boron (P = 0.0004). All other agrobotanical traits, nutritional factors and mineral nutrients (Table 1) did not differ significantly. Variance was high for carbohydrates, fibre and boron, whereas for other nutritional characteristics and mineral nutrients low variation was observed. Mineral nutrients showed quantitative differences: Mg and Ca, being the highest as compared to other nutrients. Among other mineral nutrients, Fe, Na, P, N, B and K, were high in

Table 2. Correlation of grain yield, oil contents and protein with other agro-botanical traits, nutritional characteristics and mineral nutrients in *N. sativa* L. germplasm.

Trait	Grain yield	Oil content	Protein
Agro-botanical plant traits			
Biomass / plant	0.89**	0.03	0.10
Harvest Index (%)	0.62**	0.26	0.03
Nutritional characteristics			
Oil content	0.20	-	- 0.30
Protein	0.08	- 0.30*	-
Carbohydrates	- 0.23	- 0.47**	- 0.37*
Ash	- 0.23	0.10	0.15
Moisture	0.49**	- 0.06	0.57**
Fiber	0.36*	0.45**	- 0.05
Mineral nutrients			
Nitrogen (N)	0.06	- 0.08	0.44**
Iron (Fe)	- 0.06	0.35*	- 0.25
Calcium (Ca)	0.20	- 0.24	- 0.11
Cupper (Cu)	0.10	0.07	- 0.11
Magnesium (Mg)	0.19	0.08	- 0.34*
Lead (Pb)	0.12	0.30*	- 0.14
Zinc (Zn)	0.11	- 0.30*	0.16
Cobalt (Co)	- 0.01	0.32*	- 0.04
Manganese (Mn)	- 0.04	- 0.17	0.23
Sodium (Na)	0.04	- 036*	0.03
Phosphorus (P)	- 0.32*	- 0.50**	- 0.16
Boron (B)	0.15	0.64**	- 0.44**
Potassium (K)	- 0.07	-0.34*	- 0.13

*, ** Significant at 0.05 and 0.01% probability.

quantity, whereas micronutrients Cu, Pb, Zn, Co, and Mn, were the lowest.

Variation observed among the genotypes could be exploited through simple selection as CV, for most of the characteristics were in appropriate range and indicated high acceptance of results reported in this study. Low variance for both the checks for most of the characters indicated negligible environmental influence and confirmed the validity of the results. The results regarding correlation of grain yield, oil and protein contents with other characteristics presented in Table 2 revealed significant association of grain yield with biomass / plant, harvest index, moisture, and fibre content. Oil contents were positively associated with fibre, Fe, Pb, Co and B, whereas negatively linked with protein, carbohydrates, Zn, Na, P and K. Protein exhibited positive association with moisture and N, whereas negative with carbohydrates, Mg and B. The characters indicating significant association with grain yield, oil and protein contents could be utilized through direct selection without loosing genes of economic importance.

Agro-biodiversity

First three factors with Eigen values greater than unity contributed 86% of the variability amongst thirty six (36) genotypes evaluated for six nutritional characteristics (Table 3). Principal component analysis indicated that characters that contributed more positively to PC_1, were oil and fibre contents. For PC_2, protein and moisture contributed more positively whereas ash contributed maximum to PC_3. Four factors exhibited Eigen value > 1, which contributed 62% of the variability amongst genotypes evaluated for 13 mineral nutrients (Table 4). Characters that contributed more positively to PC_1 were Cu, Mg, Mn, Na, P and K, whereas Ca, Pb and Co, was important for PC_2. The PC_3 was contributed through Fe and B, whereas Zn contributed more for PC_4. Based on six nutritional characteristics, cluster diagram was

Table 3. Principal component analysis for 6 nutritional characteristics in 36 genotypes of *N. sativa* L. germplasm.

Variable	PC_1	PC_2	PC_3
Eigen value	2.41	1.67	1.08
Cumulative %	40.18	68.04	86.09
Eigen vector			
Oil contents	0.51	- 0.66	- 0.13
Protein	0.36	0.83	0.24
Carbohydrates	- 0.96	- 0.03	- 0.06
Ash	0.27	- 0.13	0.93
Moisture	0.54	0.66	- 0.34
Fiber	0.85	- 0.29	- 0.18

Table 4. Principal component analysis for 13 mineral nutrients in 36 genotypes of *N. sativa* L. germplasm.

Variable	PC_1	PC_2	PC_3	PC_4
Eigenvalue	3.06	2.24	1.61	1.10
Cumulative %	23.57	40.85	53.28	61.77
Eigen vector				
Nitrogen (N)	- 0.22	- 0.16	- 0.62	- 0.06
Iron (Fe)	- 0.25	- 0.06	0.61	0.28
Calcium (Ca)	0.41	0.42	- 0.35	- 0.04
Copper (Cu)	0.48	0.36	- 0.07	0.45
Magnesium (Mg)	0.61	0.49	0.36	0.13
Lead (Pb)	- 0.26	0.55	0.31	0.40
Zinc (Zn)	0.38	- 0.37	- 0.26	0.65
Cobalt (Co)	- 0.52	0.62	- 0.22	- 0.03
Manganese (Mn)	0.36	- 0.78	0.16	0.22
Sodium (Na)	0.43	0.30	- 0.39	- 0.30
Phosphorus (P)	0.80	0.23	- 0.03	- 0.18
Boron (B)	- 0.50	0.27	0.59	0.20
Potassium (K)	0.68	- 0.07	0.13	- 0.09

constructed using Wards methods for Euclidian distances that were divided into five clusters at 50% genetic distance (Figure 1, upper). Cluster 1 had nine genotypes, clusters 2 and 3 two in each case, cluster 4 contained eight and cluster 5 fifteen genotypes.

On the basis of average performance, cluster 2 containing two genotypes (PK - 020561 and PK - 020646) produced the highest values for biomass, harvest index, grain yield, oil contents, protein, moisture, fibre, N, Pb, Co and Mn, whereas PK - 020561 and PK - 020646 members of cluster 3 produced the highest Ca, Mg, Pb, Zn, Mn, Na and P, (Table 5). For mineral nutrients, cluster diagram was constructed by UPGMA that revealed six clusters (Figure 1, lower). Clusters 1, 2, 3, 4, 5 and 6 consisted of two, five, seven, eleven and ten genotypes, whereas one genotype (PK - 020749) formed cluster 5. On the basis of average performance, cluster 1 consisting of two genotypes (PK - 020545 and PK - 020561) produced the highest values for harvest index, fibre, Ca, Cu, Mg, Pb, Co, Na and P, whereas cluster 3 consisting of seven genotypes (PK - 020567, PK - 020766, PK - 020631, PK - 020620, PK - 020646, PK - 020663 and PK - 020783) gave the highest performance for biomass, grain yield, moisture, Cu, Zn and K, (Table 6). Similarly, cluster 5 consisting of one genotype (PK - 020749) gave the best performance for oil contents,

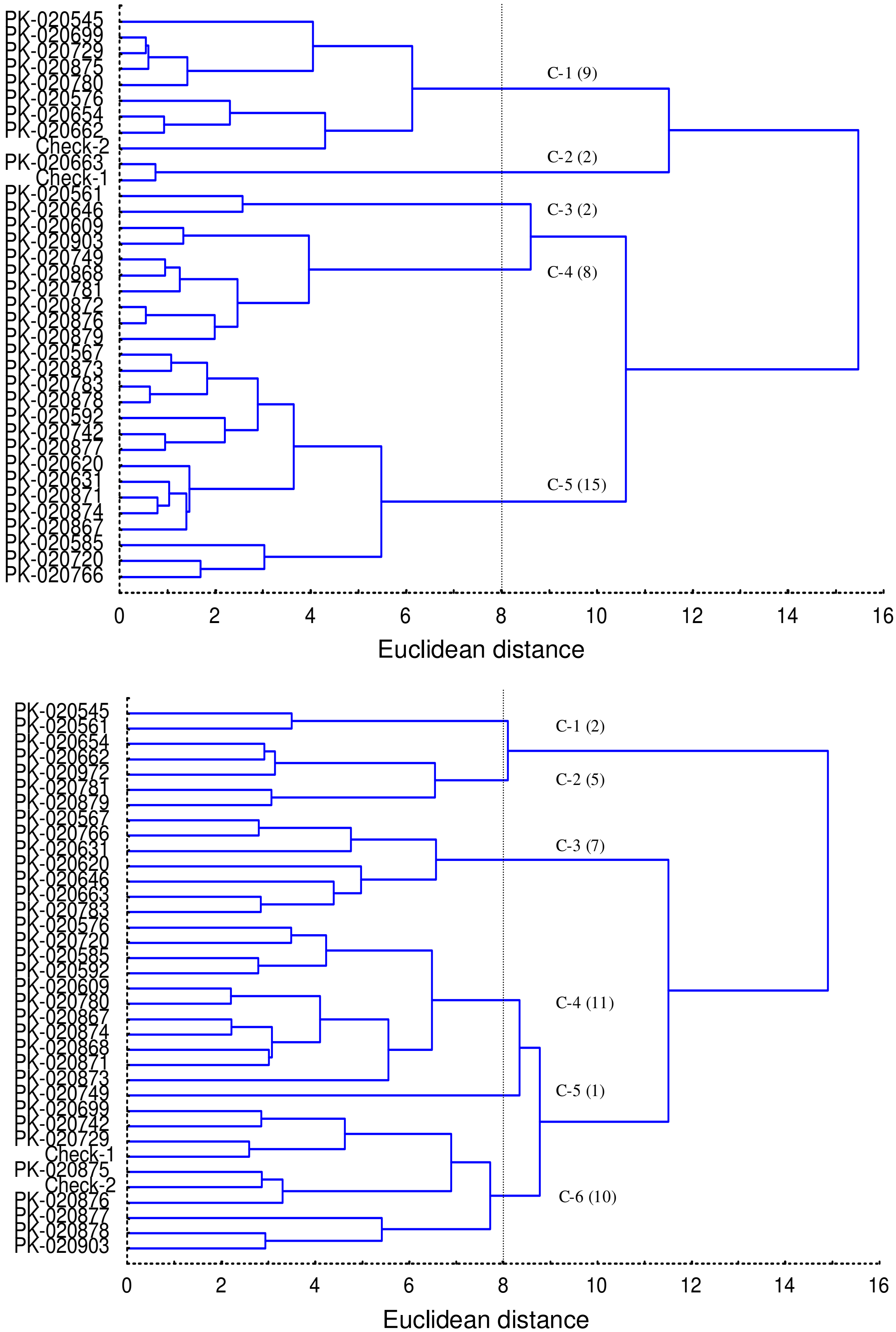

Figure 1. Cluster diagrams based on six nutritional characteristics (upper) and thirteen mineral nutrients (lower) in 36 genotypes of black cumin (*N. sativa* L.).

Table 5. Average performance of 5 clusters obtained by UPGMA for nutritional characteristics in *N. sativa* L. germplasm. Clusters, frequency and Mean ± Standard deviation presented for 36 genotypes.

Trait	Cluster - 1	Cluster - 2	Cluster - 3	Cluster - 4	Cluster - 5	
Frequency	9	2	2	8	15	
Agro-botanical traits						
Biomass	26.98 ± 8.31	28.72 ± 1.47	22.45 ± 13.52	24.36 ± 9.04	21.54 ± 9.56	
Harvest Index	25.80 ± 4.75	31.33 ± 0.00	28.10 ± 0.34	22.91 ± 4.23	23.23 ± 5.20	
Grain yield	6.68 ± 2.38	8.87 ± 0.16	6.29 ± 3.75	5.79 ± 2.93	4.67 ± 2.46	
Nutritional characteristics						
Oil contents	31.67 ± 1.32	33.38 ± 0.00	29.34 ± 0.47	32.01 ± 0.86	30.35 ± 0.90	
Protein	21.74 ± 1.08	24.95 ± 0.44	21.70 ± 0.89	20.66 ± 0.88	22.76 ± 1.34	a
Carbohydrates	22.94 ± 2.03	14.71 ± 0.00	27.95 ± 2.12	28.35 ± 1.05	26.55 ± 1.85	b
Ash	5.24 ± 0.37	5.02 ± 0.14	3.73 ± 0.30	4.95 ± 0.30	5.22 ± 0.21	c
Moisture	5.73 ± 0.11	6.70 ± 0.06	6.14 ± 0.28	5.73 ± 0.20	5.93 ± 0.24	d
Fiber	12.69 ± 1.81	14.71 ± 0.52	11.14 ± 2.52	9.14 ± 0.68	9.32 ± 1.71	e

Probability (P) values for a, b, c, d, and e, are 0.0031, 0.0001, 0.0003, 0.0006, and 0.0087 respectively. Genotypes in cluster 1 (PK - 020545, PK - 020699, PK - 020729, PK - 020875, PK - 020780, PK - 020576, PK - 020654, PK - 020662 and Check - 2), cluster 2 (PK - 020663, and Check - 1), cluster 3 (PK - 020561 and PK - 020646), cluster 4 (PK - 020609, PK - 020903, PK - 020749, PK - 020868, PK - 020781, PK - 020872, PK - 020876 and PK -020879), cluster 5 (PK - 020567, PK - 020873, PK - 020783, PK - 020878, PK - 020592, PK - 020742, PK - 020877, PK - 020620, PK - 020631, PK - 020871, PK - 020874, PK - 020867, PK - 020585, PK - 020720, and PK - 020766)

Table 6. Average performance of 6 clusters obtained by UPGMA for mineral nutrients in *N. sativa* L. germplasm. Clusters, frequency and Mean ± Standard deviation presented in 36 genotypes.

Trait	Cluster-1	Cluster -2	Cluster - 3	Cluster - 4	Cluster - 5	Cluster - 6
Frequency	2	5	7	11	1	10
Mineral nutrients						
Nitrogen (N)	3.22 ± 0.21	3.32 ± 0.20	3.59 ± 0.22	3.45 ± 0.63	3.27 ± 0.00	3.93 ± 0.72
Iron (Fe)	0.31 ± 0.06	0.28 ± 0.04	0.20 ± 0.08	0.26 ± 0.06	0.74 ± 0.00	0.25 ± 0.05
Calcium (Ca)	9.89 ± 0.81	9.42 ± 1.11	9.85 ± 0.50	8.69 ± 0.88	8.52 ± 0.00	8.86 ± 0.96
Copper (Cu)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Magnesium (Mg)	11.20 ± 0.50	10.02 ± 0.39	10.69 ± 0.47	9.97 ± 0.35	10.51 ± 0.00	9.96 ± 0.32
Lead (Pb)	0.09 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.06 ± 0.03
Zinc (Zn)	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.02
Cobalt (Co)	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	0.03 ± 0.01
Manganese (Mn)	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.05 ± 0.01
Sodium (Na)	0.61 ± 0.09	0.23 ± 0.06	0.40 ± 0.16	0.38 ± 0.09	0.35 ± 0.00	0.29 ± 0.08
Phosphorus (P)	0.60 ± 0.02	0.57 ± 0.02	0.59 ± 0.04	0.58 ± 0.03	0.59 ± 0.00	0.54 ± 0.03
Boron (B)	26.45 ± 1.76	27.30 ± 4.64	18.87 ± 3.71	22.04 ± 6.73	33.54 ± 0.00	25.50 ± 4.94
Potassium (K)	0.83 ± 0.09	0.84 ± 0.12	0.87 ± 0.09	0.85 ± 0.07	0.82 ± 0.00	0.77 ± 0.06

Cluster-1 (PK - 020545 and PK - 020561), cluster-2 (PK - 020654, PK - 020662, PK - 020972, PK - 020781 and PK - 020879), cluster-3 (PK - 020567, PK - 020766, PK - 020631, PK - 020620, PK - 020646, PK - 020663 and PK - 020783), cluster-4 (PK - 020576, PK - 020720, PK - 020585, PK - 020592, PK - 020609, PK - 020780, PK - 020867, PK - 020874, PK - 020868, PK - 020871 and PK - 020873), cluster-5 (PK - 020749), cluster-6 (PK - 020699, PK - 020742, PK - 020729, Check-1, PK - 020875, Check-2, PK - 020876, PK - 020877, PK - 020878 and PK - 020903).

performance, specific genotypes could be selected for exploitation under various agro-ecological zones of the country to select the best ones for mass cultivation.

DISCUSSION

Evaluation for agro-botanical traits, nutritional characteristics and mineral nutrients

Agro-botanical traits exhibited high level of genetic diversity that can help to identify and select promising genotypes with respect to their performance based on individual plant traits. Based on evaluation, genotypes with desirable traits can be exploited for higher grain and oil yield (Tuncturket et al., 2005). High level of genetic diversity observed in almost all the characters studied, especially in oil contents could be used to develop *N. sativa* L. cultivars, through simple selection or hybridization to develop open pollinated cultivars (Suchand et al., 2003). Oil extraction by chloroform: methanol (2:1) yielded higher oil contents as compared to the extraction by n-Hexane and chloroform: methanol (1:3) and this type of findings has been reported previously (Ramadan and Morsel, 2002) as well. Oil ranging from 28.40 to 34.33% is the main source of volatile and non-volatile oils (Ashraf et al., 2006), and in high concentrations may be used as biofuel; an alternative source of energy. High protein contents (22.41%) can be suggested as a supplementary meal for ruminant animals after oil extraction (El-Ayek et al., 1998). Due to high fibre contents, *N. sativa* could be used as a supplementary dietary fibre in various herbal and pharmaceutical preparations. Carbohydrates in *N. sativa* L. contain a non-starch component that helps in smooth digestion and along with high fibre it is highly recommended in stomach problems.

High diversity for mineral nutrients in *N. sativa* L. germplasm increases its value as a supplementary food / feed for essential nutrients (Al-Bataina et al., 2003). Lead (Pb) in traces is reported to be helpful for enzymatic functions. It also gave high variation in the material evaluated, although, Al-Jassir (1992) has reported most of the mineral nutrients except Pb in *N. sativa* L., thus, we are reporting its presence in *N. sativa* for the first time. The variation for nutritional characteristics and mineral nutrients reported in the germplasm provides baseline information for selection and development of better cultivars that has not been reported before. Although, previous studies indicated the presence of most of the minerals, little information is available at intra specific level in *N. sativa* L. (Takruri and Dameh, 1998). In general, the order of concentration of different nutrients (Mg > Ca > N > K > P > Na > Fe > Pb > Zn > Mn > Cu and Co > B) is quite in agreement with previous reports (Ansari et al., 2004). Influence of biomass and harvest index for determining grain yield can be exploited through simple selection or involving selected genotypes in hybridization (Sharma et al., 2000).

Significantly, positive association in most of the traits with oil and yield has been reported by Gurumoorthi et al. (2003) and Toncer and Kizil (2004), but in our findings biomass and harvest index were the most important for enhancing grain yield, whereas Pb, Co and B exhibited positive indication in improving oil and N for protein. The genotypes with higher oil content > 32.0% (PK - 020699, PK - 020875, PK - 020879, PK - 020749, PK - 020729, PK - 020781, PK - 020868, PK - 020663, Check - 1, PK - 020545) and grain yield > 8.0 g / plant (PK - 020699, PK - 020662, PK - 020654, PK - 020663, PK - 020567, PK - 020646, Check-1, PK - 020609, PK - 020879, PK - 020783, PK - 020903) are suggested to test under diverse agro-ecological zones to identify the best genotypes with maximum yield and oil potential to fulfill the needs of the local herbal market. Similarly, for high protein contents (> 25.0%) ,two genotypes (PK - 020585 and PK - 020663) could be used as breeding material, among these Pk-020663 was common for all the three sets of evaluation, hence, was identified as source of genotype.

Agro-biodiversity

Diversity of crop species is useful for analyzing and monitoring germplasm during the maintenance phase and predicting potential genetic gain in breeding programmes (Sultana et al., 2007). Investigation on nutritional characteristics and mineral nutrients have been reported in *N. sativa* L., but the work is mostly at species level and little information is available on the extent of diversity for these important traits (Palevitch, 2004; Cracker, 1986; Canter et al., 2005). In this study, we used data on nutritional characteristics and mineral nutrients for investigation of intra-specific variation and is a unique systematic report on *N. sativa* L. The differences reported in this study are useful to identify promising genotypes for yield potential and quality and are pre-requisites for genetic improvement and this aspect has not yet been initiated on this crop.

In general, agro-botanical traits, nutritional characteristics and mineral nutrients are additive in nature and are suggested to get them exploited through successive selection. It is important for a researcher to find out the best suited genotypes for direct use or to incorporate them in breeding programme for exploitation of genes of economic importance. Further, it is suggested to initiate genomic research to identify the molecular basis of differences among various genotypes for different characters. The best identified genotypes for particular trait/s could be used for developing one genotype with multiple economic traits. Although, *N. sativa* L. is a new crop in Pakistan but has high acceptance from farmers and market enterprisers due to high economic return, medicinal application. Hence, there is a need to develop suitable genotypes with higher levels of nutritional and mineral contents. Due to high quality oil and other nutritional qualities, *N. sativa* L. has been referred to as the health food that enhances the scope of its improvement to meet the increasing demands of the nutritionists, industrialists, pharmacists and herbalists worldwide.

Conclusion

Genotype PK - 020663 exhibited better values for all the three sets of evaluation (agro-botanical traits, nutritional characteristics and mineral nutrients), hence was identified as source genotype. Two genotypes PK - 020561 and PK - 020646 supported the highest Ca, Mg, Pb, Zn, Mn, Na and P, contents and were the best for future utilization in genetic improvement of *N. sativa* L. Diverse germplasm is vital for any crop improvement programme and we have preserved the evaluated genotypes under the same accession numbers in the genebank at 15°C with < 40% relative humidity for 2006. These are available for research and development work to the researchers. To our knowledge, we report Pb for the first time from *N. sativa* L. germplasm and Pb in traces is important for metabolic functions. Variation for nutritional and mineral nutrients mentioned in this study provides baseline data for selection and develop better cultivars. Different genotypes were grouped into various clusters by using principal component analyses which could be utilized through direct selection. The identified genotypes of *N. sativa* L. could be a good raw material for herbal, pharmaceutical, neutraceutical and cosmetic industry and thus may enhance farmers' productivity.

ACKNOWLEDGEMENTS

Financial support for this study was provided by the Agricultural Linkages Program of Pakistan Agricultural Research Council. Authors are grateful to Western Regional Plant Introduction Station, Maryland, Washington, USA for providing seeds of two accessions. Authors thank Ms Shaheena Tariq (Cereal Chemist) and Zaheer Ahmad (Oilseeds Program), Ms Syeda Raza and Amer Mumtaz (Grain Quality Testing Laboratory, ISO 17025), Ejaz Rafiq (Land Resources Program) and Tanveer Ahmad (General Biochemistry Laboratory) for their technical assistance and cooperation for nutritional and mineral analysis.

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