Quantification and Diversity in the Black Seeds (*Nigella sativa* L.) Gene Stock of Pakistan for their Composition of Mineral Nutrients

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Summary: *Nigella sativa* (L.) a member of family *Ranunculaceae* is an annual herbaceous plant indigenous to the Mediterranean region that contains more than 100 nutrients and had been used for edible and medicinal purposes in major parts of the world since long. Present study is on the analysis of thirty four accessions with two check genotypes for genetic diversity based on thirteen mineral nutrients. High variation for Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N indicated the scope of sample selection for these characters. Coefficient of correlation studies revealed that Cu had significantly positive correlation with Ca, whereas Mg was significantly correlated with Ca and Cu. Linkages of desirable traits are suggested to be broken through novel techniques for maximum exploitation of genomic diversity for valuable phyto-chemicals. Based on principal component analysis, first four factors contributed 62 percent of the variability amongst genotypes for mineral nutrients. Eigen value > 1 exhibited 23.57 % of variation for component 1, 17.28 % for component 2 and 12.43 % of variation for component 3, respectively. Moreover, it also reflects the potential of improvement, through building broad based gene pool by acquiring more samples from diverse geographical areas. These principal components could be selected individually for the improvement of specific mineral nutrients for multipurpose use and applications. Six clusters were observed for 36 genotypes based on mineral nutrients. The genotypes Pk-020877, Pk-020749, Pk-020876, Pk-020545, Pk-020561, Pk-020781 & Pk-020729, Pk-020620, Pk-020561, Pk-020631, Pk-020879, Pk-020868 produced the highest N (5.56), Fe (0.74), Ca (10.83), Mg (11.56), Pb (0.09), Zn (0.09), Na (0.68), P (0.66), B (39.58), and K (0.99), whereas Pk-020873 produced lowest N (1.67), Pk-020766 Fe (0.10), Pk-020576 Ca (7.38), Pk-020585 Mg (9.40), check-2 Pb (0.02), Pk-020872 Zn (0.01), Pk-020781 & Pk-020877 Na (0.17), check-2 P (0.50), Pk-020585 B (13.67) and Pk-020699 for K (0.63) produced the lowest mineral contents, hence these genotypes are suggested to be utilized in various combinations for genetic improvement of *Nigella sativa* L.

Introduction

Plant genus *Nigella* contains about 20 species of annual herbs indigenous to the Mediterranean and West Asia [1]. The *N. sativa* L. is native to the Mediterranean region from West Asia through Northern India, and has long been domesticated. With the name "black seeds" it is mentioned in ancient Greek, Roman and Hebrew texts as a condiment and component of herbal medicines [2]. In South East Asia, *Nigella* seeds are used mainly for medicinal purposes. A report on the chemical and morphological characteristics of different regional types of *N. sativa* has been compiled [1].

Recently, many medicinal properties have been attributed to the black seeds and its oil, including carminatives, diuretics, and for delayed menses and lactation, antineoplastic (antitumour), antifungal, anti-helminthic, while the oil has protective action against histamine induced bronchospasm, cough and bronchal asthma [3-5]. The black seeds are extensively used in respiratory infections, abdominal pain, gastrointestinal diseases, and many more. The whole seed or their extracts have antitumor [6, 7], antidiabetics [8], spasmolytic and bronchodilator [9], anti-inflammatory [10], antibacterial [11], galactogogue and antioxidant [12, 13] and insect repellent effects [14].

In this study *Nigella sativa* L. was selected on the basis of high chemical constituent's diversity.

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More than 100 chemical constituents have been identified up till now [15]. Black seed is rich in nutritional elements. Monosaccharides in the form of glucose, rhamnose, xylose, and arabinose are found in the black seed. It also contains a non-starch polysaccharide component which is a useful source of dietary fiber. It is rich in fatty acids, particularly the unsaturated and essential fatty acids (linoleic and linolenic acid). Analysis of seeds protein hydrolysate showed the presence of 15 amino acids including all the 9 essential amino acids [16]. Essential amino acids cannot be synthesized within our body in sufficient quantities and are thus required from our diet. Black seed contains arginine which is essential for infant growth. Further, it contains carotene, which is converted by the liver into vitamin A, the vitamin known for its anti-cancer activity. The main function of these elements which are required in small quantity by body is to act as essential cofactors in various enzyme functions. These black seeds are also known to contain essential macro and micronutrients which play vital role as structural and functional components of metalloproteins and enzymes in the living cells [17]. Therefore, black seed is considered a valuable source of protein, carbohydrates, essential fatty acids, vitamins A, B1, B2, C and niacin as well as minerals such as calcium, potassium, iron, magnesium, selenium, magnesium and zinc.

So far, a large number of plants have been analyzed and some of these have been cultivated as new oil crops [18]. The objectives of this study were to quantify 13 mineral nutrients, to predict chemical constituent diversity and to select superior genotypes for low land farmers to increase their income which ultimately helps to overcome the poverty.

Results and Discussion

The contents of mineral nutrients *i.e.,* Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N in *Nigella sativa* L. are presented in the Table-1. The genotypes Pk-020877, Pk-020749, Pk-020876, Pk-020545, Pk-020561, Pk-020781 & Pk-020729, Pk-

Table-1: Quantification of 13 Mineral nutrient contents in 34 genotypes and 2 check genotypes of *Nigella sativa* L.

Genotypes	N	Fe	Ca	Cu	Mg	Pb	Zn	Co	Mn	Na	P	B	K
PK-020545	3.07	0.35	9.32	0.03	11.56	0.08	0.03	0.05	0.03	0.55	0.58	27.69	0.89
PK-020561	3.37	0.26	10.47	0.03	10.85	0.09	0.04	0.06	0.04	0.68	0.61	25.20	0.76
PK-020567	3.55	0.17	9.88	0.03	10.66	0.07	0.05	0.03	0.04	0.64	0.61	15.80	0.81
PK-020576	3.66	0.32	7.38	0.03	9.96	0.04	0.05	0.03	0.05	0.55	0.55	20.06	0.80
PK-020585	4.21	0.23	9.48	0.03	9.40	0.05	0.05	0.03	0.05	0.46	0.56	13.67	0.83
PK-020592	3.76	0.31	10.16	0.02	9.56	0.06	0.03	0.02	0.05	0.44	0.60	14.73	0.73
PK-020609	3.49	0.22	8.00	0.03	10.03	0.06	0.05	0.01	0.05	0.26	0.60	22.19	0.82
PK-020620	3.41	0.22	9.46	0.03	9.82	0.06	0.09	0.02	0.07	0.19	0.58	12.78	0.71
PK-020631	3.38	0.21	9.37	0.03	11.34	0.03	0.06	0.01	0.06	0.47	0.66	19.35	0.98
PK-020646	3.57	0.35	10.02	0.03	10.56	0.07	0.08	0.01	0.07	0.45	0.57	19.35	0.90
PK-020654	3.50	0.35	10.60	0.02	10.60	0.06	0.03	0.04	0.03	0.29	0.58	27.86	0.83
PK-020662	3.56	0.25	10.18	0.03	9.60	0.07	0.04	0.05	0.03	0.23	0.56	21.65	0.82
PK-020663	4.04	0.23	9.40	0.03	10.99	0.07	0.05	0.03	0.05	0.31	0.56	24.14	0.83
PK-020699	3.54	0.29	7.90	0.02	10.36	0.04	0.03	0.03	0.05	0.42	0.53	26.44	0.63
PK-020720	3.72	0.16	8.59	0.02	9.85	0.04	0.04	0.04	0.05	0.51	0.59	19.35	0.91
PK-020729	3.49	0.30	8.27	0.02	9.63	0.09	0.05	0.03	0.05	0.20	0.54	17.04	0.79
PK-020742	3.52	0.26	7.83	0.02	9.80	0.04	0.04	0.03	0.05	0.21	0.56	21.83	0.76
PK-020749	3.27	0.74	8.52	0.02	10.51	0.04	0.05	0.00	0.06	0.35	0.59	33.54	0.82
PK-020766	3.57	0.10	10.03	0.03	10.56	0.05	0.05	0.01	0.04	0.48	0.61	18.99	0.94
PK-020780	3.41	0.23	8.12	0.02	9.75	0.07	0.04	0.01	0.05	0.34	0.54	23.78	0.85
PK-020781	3.16	0.30	8.41	0.02	9.79	0.09	0.03	0.06	0.03	0.17	0.53	34.43	0.67
PK-020783	3.63	0.12	10.78	0.03	10.87	0.06	0.07	0.02	0.05	0.26	0.57	21.65	0.91
PK-020867	3.49	0.36	9.39	0.02	9.81	0.07	0.04	0.02	0.05	0.32	0.59	22.72	0.89
PK-020868	3.14	0.26	8.09	0.02	9.93	0.05	0.04	0.03	0.05	0.28	0.57	26.98	0.99
PK-020871	3.54	0.23	8.78	0.03	10.67	0.04	0.06	0.03	0.05	0.34	0.61	22.89	0.93
PK-020872	3.25	0.25	9.85	0.03	10.21	0.06	0.01	0.03	0.03	0.18	0.59	25.56	0.89
PK-020873	1.67	0.25	7.87	0.03	10.26	0.05	0.08	0.04	0.06	0.28	0.59	23.43	0.93
PK-020874	3.52	0.22	9.65	0.02	10.13	0.06	0.04	0.03	0.05	0.33	0.62	20.06	0.94
PK-020875	3.44	0.14	9.26	0.03	10.32	0.05	0.04	0.02	0.07	0.36	0.56	27.33	0.79
PK-020876	3.26	0.21	10.83	0.02	10.30	0.03	0.05	0.03	0.05	0.30	0.55	31.95	0.83
PK-020877	5.56	0.20	8.36	0.03	9.61	0.04	0.04	0.04	0.05	0.17	0.54	24.14	0.75
PK-020878	4.55	0.26	9.22	0.02	10.13	0.08	0.07	0.03	0.04	0.30	0.59	19.35	0.87
PK-020879	3.46	0.36	8.15	0.03	10.19	0.08	0.05	0.04	0.03	0.34	0.54	39.58	0.77
PK-020903	4.45	0.31	9.80	0.02	9.95	0.08	0.08	0.04	0.04	0.31	0.54	29.99	0.80
Check-1	3.94	0.27	8.11	0.02	9.46	0.08	0.04	0.04	0.06	0.32	0.51	26.09	0.77
Check-2	3.54	0.22	9.03	0.03	10.01	0.02	0.04	0.03	0.06	0.33	0.50	30.88	0.75

N (%), Fe (mg kg^{-1}), Ca (mg kg^{-1}), Cu (mg kg^{-1}), Mg (mg kg^{-1}), Pb (mg kg^{-1}), Zn (mg kg^{-1}), Co (mg kg^{-1}), Mn (mg kg^{-1}), Na (mg kg^{-1}), P(%), B (mg kg^{-1}) and K (%)

020620, Pk-020561, Pk-020631, Pk-020879, Pk-020868 produced the highest N (5.56), Fe (0.74), Ca (10.83), Mg (11.56), Pb (0.09), Zn (0.09), Na (0.68), P (0.66), B (39.58), and K (0.99), whereas genotypes Pk-020873 produced lowest N (1.67), Pk-020766 Fe (0.10), Pk-020576 Ca (7.38), Pk-020585 Mg (9.40), check-2 Pb (0.02), Pk-020872 Zn (0.01), Pk-020781 & Pk-020877 Na (0.17), check-2 P (0.50), Pk-020585 B (13.67) and Pk-020699 for K (0.63) mineral contents in the present material of *Nigella sativa* L.

There was a wide variation for mineral nutrient contents in the genotypic material evaluated in the present study. The B was recorded with highest mean ±SE (23.68±0.99) values with CV 25.21 % and ranged from 12.78 to 30.58 mg kg^{-1} (Table-2). Magnesium was found with mean±SE (10.20±0.09 mg kg^{-1}) followed by Ca (9.13±0.16) with CV 5.11 and 10.39 %, respectively. Percentage of N recorded was 3.57 ± 0.09 with range of 1.67 to 5.56 % and CV of 15.83 %. Mineral nutrients showed quantitative differences as well. Boron was found being in the highest amount followed by Mg and Ca as compared to other secondary and micro nutrients. Among other mineral nutrients, Fe, Na, P, N, and K were high in quantity, whereas micronutrients Cu, Pb, Zn, Co, and Mn were the lowest. Low level of SE (±) for the minerals (Mg, P, Ca, K) indicated that variation among the genotypes was low as were also indicated by lower range and CV values for these minerals (Table-2).

Table-2: Descriptive statistics for mineral nutrients in *Nigella sativa* L.

Traits	Units	Mean ± SE	Range	CV %
Nitrogen (N)	%	3.57 ± 0.09	1.67 –5.56	15.83
Iron (Fe)	mg kg^{-1}	0.26 ± 0.02	0.10 –0.74	39.51
Calcium (Ca)	mg kg^{-1}	9.13 ± 0.16	7.38 –10.83	10.39
Copper (Cu)	mg kg^{-1}	0.03 ± 0.00	0.02 –0.03	14.77
Magnesium (Mg)	mg kg^{-1}	10.20 ± 0.09	9.40 –11.56	5.11
Lead (Pb)	mg kg^{-1}	0.06 ± 0.00	0.02 –0.09	31.38
Zinc (Zn)	mg kg^{-1}	0.05 ± 0.00	0.01 –0.09	34.51
Cobalt (Co)	mg kg^{-1}	0.03 ± 0.00	0.00 –0.06	45.68
Manganese (Mn)	mg kg^{-1}	0.05 ± 0.00	0.03 –0.07	25.49
Sodium (Na)	mg kg^{-1}	0.35 ± 0.02	0.17 –0.68	36.64
Phosphorus (P)	%	0.57 ± 0.01	0.50 –0.66	5.83
Boron (B)	mg kg^{-1}	23.68 ± 0.99	12.78 –39.58	25.21
Potassium (K)	%	0.83 ± 0.01	0.63 –0.99	10.14

Mineral nutrients data were averages of triplicate determinations. SE- standard error, CV- coefficient of variability.

Coefficient of correlation studies revealed that Cu had significant positive correlation with Ca. Magnesium was significantly positively correlated with Ca and Cu while negatively correlated with N (Table-3). Association of Co was found significantly positive with Pb whereas it was negatively correlated with Zn. Phosphorus was found to be significantly positively associated with N, Ca, Cu, Mg, Co and Na where N and Co were negatively associated. Association of B was found significant and positive with Fe and Co while negatively associated with Zn, Mn, Na and P. Potassium was also found significantly positively associated with Cu, Mg and P whereas its correlation with N, Co and B was negative.

Four factors exhibited Eigen value > 1 that contributed 62 % of the variability amongst genotypes evaluated for 13 mineral nutrients (Table-4). Variations exhibited for component 1 were 23.57 %, for component 2, 17.28 % and 12.43 % of variations were deciphered for component 3, respectively. Characters that contributed more positively to PC_1 were Ca, Cu, Mg, Mn, Na, P and K, whereas Ca, Pb and Co were important for PC_2. The PC_3 was contributed through Fe and B, whereas Zn contributed more for PC_4.

Based on thirteen mineral nutrients, cluster diagram was constructed by UPGMA that revealed

Table: 3. Coefficient of correlation for 13 mineral nutrients in *Nigella sativa* L.

Minerals	N (%)	Fe	Ca	Cu	Mg	Pb	Zn	Cobalt	Mn	Na	P(%)	B ppm
Fe	-0.13											
Ca	0.09	-0.19										
Cu	-0.07	-0.12	0.25*									
Mg	-0.27*	0.02	0.34*	0.51**								
Pb	0.03	0.13	0.13	0.10	-0.04							
Zn	0.07	-0.09	0.10	0.17	0.02	0.02						
Co	0.05	-0.08	0.00	0.03	-0.09	0.43*	-0.25*					
Mn	-0.04	-0.06	-0.20*	0.01	-0.03	-0.48*	0.48*	-0.64**				
Na	-0.07	-0.02	0.18	0.12	0.43*	-0.01	-0.02	-0.02	-0.02			
P(%)	-0.22*	-0.07	0.34*	0.34*	0.48*	-0.09	0.11	-0.30*	0.01	0.36*		
B ppm	-0.16	0.40*	-0.16	-0.12	0.15	0.03	-0.29*	0.32*	-0.22*	-0.21*	-0.43*	
K (%)	-0.24*	-0.16	0.19	0.21*	0.37*	-0.15	0.18	-0.25*	0.11	0.13	0.59*	-0.20*

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

Table-4: Principal component analysis for 13 mineral nutrients in 36 genotypes of *Nigella sativa* L.

Variables	PC_1	PC_2	PC_3	PC_4
Eigen value	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

six clusters (Table-5 and Fig. 1). Cluster 1 consisted of two genotypes, cluster 2 five, cluster 3 seven, cluster 4 eleven and cluster 6 of 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 Ca, Cu, Mg, Pb, Co, Na and P, whereas cluster 3 consisting of seven genotypes (Pk-020567, Pk-020620, Pk-020631, Pk-020646, Pk-020663, Pk-020783 & Pk-020766) gave the highest performance for Cu, Zn and K. Similarly cluster 5 comprising only one genotype (Pk-020749) gave the best performance for Fe, Mn and B.

Scattered diagram (Fig. 2) obtained was divided into two factors, factor 1 constituted of (22.9 %) and factor 2 (17.5 %) of variability. Five genotypes were placed at the upper left side of the scattered diagram of which two of them were at extreme boundary which showed the extent of wide variation in these genotypes viz., Pk-020561 and Pk-020781, while other three (Pk-020663, Pk-020729 and Pk-020867) were near one another but away from center. On the right side of the scattered diagram three genotypes (Pk-020654, Pk-020875, and Pk-020877) were present near each other while genotype Pk-020592 was present at the extreme left corner showed higher level of N, Fe, Ca and Pb. Moreover, at the lower right side three genotypes Pk-020567, Pk-020868 and Pk-020873 were present away from the center due to low level of genetic variation present in these genotypes which distinct themselves from others.

The variation for nutritional characteristics and mineral nutrients reported in the germplasm provide baseline information for selection to develop better cultivars of *Nigella sativa* L. and increases its

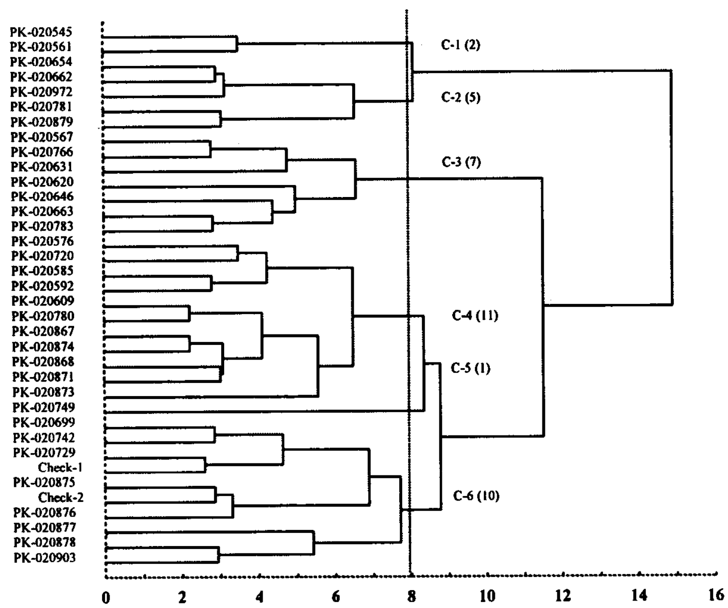

Fig. 1: Cluster diagram based on thirteen mineral nutrients in 36 genotypes of black cumin (*Nigella sativa* L).

Table-5: Average performance of 6 clusters obtained by UPGMA for mineral nutrients in *Nigella sativa* L. Clusters, frequency and Mean ± Standard deviation presented in 36 genotypes.

Traits	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6
			Frequency			
	2	5	7	11	1	10
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

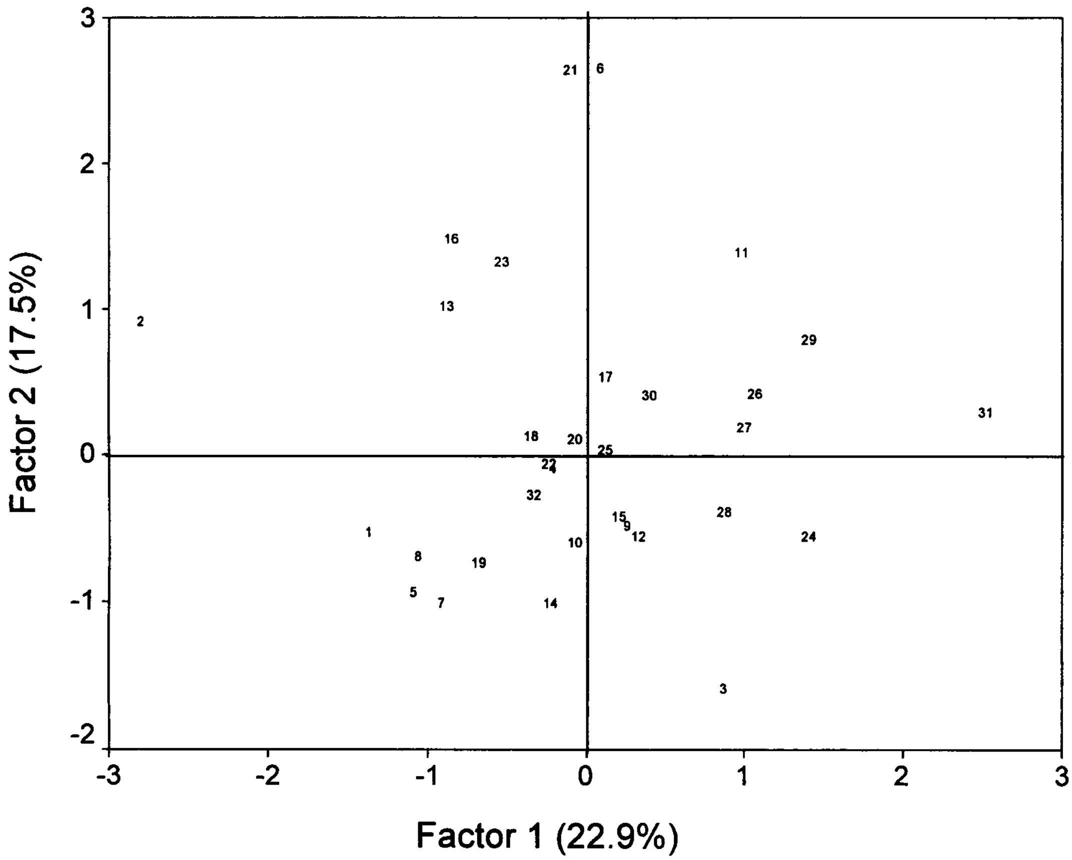

Fig. 2: Scattered diagram obtained for *Nigella sativa* L. genotypes based on 13 mineral nutrients. The legends 1,2,3,4,5... represents genotypes as, PK-020545, PK-020561, PK-020567, PK-020576, PK-020585, PK-020592, PK-020609, PK-020620, PK-020631, PK-020646, PK-020654, PK-020662, PK-020663, PK-020699, PK-020720, PK-020729, PK-020742, PK-020749, PK-020766, PK-020780, PK-020781, PK-020783, PK-020867, PK-020868, PK-020871, PK-020872, PK-020873, PK-020874, PK-020875, PK-020876, PK-020877, and PK-020878.

value to use as supplementary food/feed for essential nutrients [19]. Most of the mineral nutrients except Pb in *Nigella sativa* L. have been reported [20, 21]. Lead (Pb) in traces is found to be helpful for enzymatic functions. In general the order of concentration of different nutrients found in the present investigation (B >Mg >Ca >N >K >P >Na >Fe >Pb >Zn >Mn >Cu >Co) is quite in agreement with previous literature [17]. Determination of higher level of NPK in *Nigella sativa* L. is expected to save abundant use of fertilizers for successful crop production. In another study, we observed that use of NPK as fertilizer did not produce more yield as compared to control [22].

Depending on performance, specific genotypes could be selected for exploitation under various agro-ecological zones to identify the best ones. The Pk-020663 genotype was found promising one and was identified as elite source to be used as a cultivar or in other breeding experiments to exploit the best combinations.

Diversity of crop species is useful for analyzing and monitoring germplasm during the maintenance phase and predicting potential genetic gain in breeding programmes [23]. Although previous studies indicated the presence of most of the minerals but little information is available at intra specific level in *Nigella sativa* L. on extent of diversity for these important traits [21, 24-26]. In the present study, we have reported the variation in mineral nutrients at intra-specific level that is a unique systematic report on *Nigella sativa* L. The differences reported in this study are useful to identify promising genotypes for yield potential and quality and to start research on genetic improvement that has not yet initiated in this crop. In general agro-botanical traits, nutritional characteristics and mineral nutrients are additive in nature with continuous variation that is suggested to exploit through successive selection.

Although *Nigella sativa* L. is a new crop in Pakistan but on account of reasonable acceptance from the farmers and market enterprisers due to high economic return, hence there is a need to develop suitable genotypes with optimum levels of nutritional and mineral contents. Scattered diagram revealed that both Pk-020561 and Pk-020781 were rich in Ca, Co, Na, P, and B contents which differentiate them from other genotypes. Genotypes present on the right side of the scattered diagram (Pk-020654, Pk-020875, and Pk-020877) grouped closely which might be due to some similarities in their nutrients composition while genotype Pk-020592 present at the extreme of left corner contain higher level of N, Fe, Ca and Pb. The genotypes present at the lower right side (Pk-020567, Pk-020868 and Pk-020873) were due to low level of nutrients that differentiates them from other ones. The genotypes present in diverse groups could be used as source material for planning breeding strategy for *Nigella sativa* L. to develop high yielding and enriched mineral nutrient contents cultivars for future cultivation. These genotypes could also be used as source material for minerals as well as food supplements in number of bakery & industrial products. *Nigella sativa* L. has contained both important utilities as medicinal herb and underutilized crop for low land farmers and marginal lands to get more cash as compared to other crops in short time. The exploitation of unique genotypes with enriched mineral nutrients could be a new source of medicinal wealth.

Experimental

Thirty four test genotypes of *Nigella sativa* L. along with 2 check genotypes were grown under field conditions 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 study lasted for consecutive three years, *viz.* from 2002-03 through 2004-5. Crop was sown during the last week of November and harvested at the end of April each year. Seeds of these genotypes were obtained and utilized for the determination of 13 mineral nutrients. The test genotypes were PK-020545, PK-020561, PK-020567, PK-020576, PK-020585, PK-020592, PK-020609, PK-020620, PK-020631, PK-020646, PK-020654, PK-020662, PK-020663, PK-020699, PK-020720, PK-020729, PK-020742, PK-020749, PK-020766, PK-020780, PK-020781, PK-020783, PK-020867, PK-020868, PK-020871, PK-020872, PK-020873, PK-020874, PK-020875, PK-020876, PK-020877, PK-020878, PK-020903, PK-020879 and checks were check-1 and check-2.

For the 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 [27]. Seed sample of 0.50 g of each genotype was crushed, grinded and powdered with pestle and mortar and added into 50 mL conical flask. Digestion was performed in the presence of 70 % perchloric acid and 70 % nitric acid mixture (HNO_3-$HClO_4$ in 2:1 ratio). Digest was stored in plastic bottles washed with deionized water. Flame atomic absorption measurements were made for required elements except N and B with Varian AA240 FS Fast sequential atomic absorption spectrometer following specific instrumental conditions [28]. Calibration of the instrument was repeated periodically during operation to minimize error. Wavelength and particular flame type were as followed, 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).

Phosphorus was determined by developing vanadomolybdo-phosphoric acid yellow color on spectrophotometer. Potassium and sodium was determined by flame photometer [29]. All the analyses were performed in triplicate samples to minimize error. The reagents and solvents used in the study were of the highest purity and analytical grade (Sigma Chemical Company, St. Louis, MO, USA).

Seeds were burned to ash for boron (B) determination using the method described by [30]. The ash was dissolved in hot water. The B concentration was determined colorimetrically on spectrophotometer using azomethine-H method of [31].

Statistical Analyses

The data were analysed for mean, coefficient of variability (CV) and correlation using computer software MS Excel and numerical taxonomic techniques following the methods of cluster and principal component analyses [32] with the help of computer software 'Statistica' version 6.0 and 'SPSS' version 10.01 for windows XP Professional. For cluster analysis, means of each character were standardized prior to analysis to avoid the effect due to difference in scale.

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