

**PROTEOMICS VARIATION IN *NIGELLA SATIVA* L.  
(RANUNCULACEAE) GERMPLASM**

**MUHAMMAD SAJJAD IQBAL\* AND ABDUL GHAFOOR<sup>1</sup>**

*Department of Botany, University of Gujrat, Gujrat, Pakistan*

*Keywords:* Black seeds, Proteomics, Medicinal, Underutilized crops

**Abstract**

Study revealed a first report of proteomics variation in *Nigella sativa* L. based on analyzing 32 accessions through SDS-PAGE. Three prominent regions along eight subunits were identified. Intra specific variation was observed low whereas the sharpness of bands was high between first and second regions. It was noted that in second region there was no clear evidence of band formation in *N. sativa*. Prominent and sharp protein peptide bands were recorded in four accessions, namely PK-020561, PK-020609, PK-020620 and PK-020646. Further investigation of single seeds showed almost similar genetic pattern within the single accession. Five clusters were formed on the basis of Euclidean distance. Cluster-I & II contain 1, 1 accession each, likewise Cluster-III and C-IV contain 2, 2 accessions whereas Cluster-V was found diversified as consisted of 26 accessions. Two accessions PK-020878 and PK-020877 were recommended for polymorphism and crop improvement programs.

**Introduction**

The *Ranunculaceae* is a large family containing about 70 genera and at least 3000 species. About 20 annual species are known to be considered for genus *Nigella*, originated from Mediterranean region spread through West Asia to Northern India (Weiss 2002). In South-East Asia, seeds are used as flavoring dishes, salads, pickles and as medicinal (Benkaci-Ali *et al.* 2006, Iqbal *et al.* 2010). Biochemical constituent's diversity provides wealth of knowledge which would be used efficiently to manage genetic stocks and planning breeding experiments (Iqbal *et al.* 2017).

Plant varieties are usually characterized through agromorphological parameters but are controlled by multiple alleles/genes. To avoid such duplication, biochemical markers like proteins, enzymes, isozymes, and plant hormones, etc. received more attention in recent years for assessment of genetic variability because these are products of genes and their expression is more stable. The seed storage proteins are associated with traits of agricultural significance and are used in legal protection of cultivars (Knoblochova and Galova 2000). These proteins are divided into three groups: The 1<sup>st</sup> - storage proteins-gliadins and glutenins, the 2<sup>nd</sup> - proteins with a high metabolic activity - albumins and globulins, the 3<sup>rd</sup> - protein groups are structural proteins, very difficult to dissolve and fix in a cell structure (Dvoracek and Curn 2003).

Among biochemical markers, SDS-PAGE is widely used technique due to its validity and simplicity for describing genetic structure of various plants. Seed storage proteins have been used as genetic markers in four areas: (i) Intra and inter accession variability, (ii) plant adaptation, (iii) whole genome interaction and (iv) in crop improvement (Iqbal *et al.* 2009b). Considering its importance, 32 accessions were analyzed for banding pattern variation and phylogenetic relationship.

---

\*Author for correspondence: <drsajjad.iqbal@uog.edu.pk>. <sup>1</sup>Bioresource Conservation Institute, National Agricultural Research Centre, Islamabad, Pakistan.

DOI: <https://doi.org/10.3329/bjb.v50i2.54084>

### Materials and Methods

Fresh seeds of each of 32 accessions were separately bulked, ground and processed for SDS-PAGE seed storage protein analysis (Table 1). Accessions were collected throughout the country including two accessions, one from USA and another from Ukrain. In another experiment, single seed was grounded and protein analysis was performed under the same conditions of the gel electrophoresis. Protein extraction buffers were prepared according to the method provided in Iqbal (2009a).

**Table 1. List of *Nigella sativa* L. accessions representing provinces, collection sites and altitudes.**

Sl. No.	Accessions*	Province	Collecting sites	Altitude	Sl. No.	Accessions*	Province	Collecting sites	Altitude*
1	Pk-020545	KPK	Haripur (Hattar)	580	17	Pk-020742	AJK	Mirpur	950
2	Pk-020561	Punjab	Lahore	290	18	Pk-020749	Punjab	Chakwal	525
3	Pk-020567	"	Faisalabad	230	19	Pk-020766	"	Multan	125
4	Pk-020576	"	"	230	20	Pk-020780	"	Lahore	290
5	Pk-020585	"	"	230	21	Pk-020781	"	Bahawalpur	190
6	Pk-020592	"	"	230	22	Pk-020783	"	Attock	430
7	Pk-020609	KPK	Peshawar	500	23	Pk-020867	"	Lahore	290
8	Pk-020620	"	"	500	24	Pk-020868	"	"	290
9	Pk-020631	Punjab	Narowal	290	25	Pk-020871	AJK	Muzaffarabad	810
10	Pk-020646	KPK	Kohat	510	26	Pk-020872	Punjab	Mianwali	250
11	Pk-020654	Punjab	Gujranwala	270	27	Pk-020873	"	Rawalpindi (MT)	530
12	Pk-020662		Ukrain		28	Pk-020874	"	Rawalpindi (Saddar)	521
13	Pk-020663		Pakistan		29	Pk-020875	Capital	Islamabad (Aabpara)	550
14	Pk-020699	KPK	D. I. Khan	230	30	Pk-020876	"	Islamabad (Karachi Co.)	550
15	Pk-020720	"	"	230	31	Pk-020877	Punjab	Rawalpindi (Kohuta)	600
16	Pk-020729	GB	Chilas	1450	32	Pk-020878	"	Lahore	290

Pk-020662 and Pk-020663 were received from United States of America; KPK, Khyber Pakhtun Khawa; D. I. Khan, Dera Ismail Khan; GB, Gilgit Baltistan; AJK, Azad Jammu & Kashmir; MT, Muslim Town. \*Accessions enlisted are preserved in National Gene bank of Plant Genetic Resources Program, National Agricultural Research Center, Islamabad, Pakistan (Iqbal 2009a).

For protein extraction, ten seeds were bulked and ground to fine powder in a mortar and pestle. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in eppendorf tube with a small glass rod. The extraction buffer contained the following final concentrations 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercapto-ethanol. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to watch the movement of proteins in the gel. To purify extraction, the homogenate samples were mixed thoroughly by vortexing and centrifuged at 15,000 rpm for 5 min at room temperature. The extracted crude proteins were recovered as clear supernatant, transferred into new 1.5 ml Eppendorf tubes and stored at -20°C until electrophoresis.

Total seed protein was analysed through slab type SDS-PAGE using 11.25% polyacrylamide gel following Laemmli (1970). The molecular weights of the dissociated polypeptides were determined by using molecular weight protein standards "MW-SDS-70 Kit" Sigma Chemical Company, USA following Iqbal *et al.* (2017). The electrophoresis box used was prepared by Auto Model AL 6500 (donated by JICA), Japan.

Depending upon the presence and absence of polypeptide bands, similarity index was calculated for all possible pairs of protein types as 1/0 and data were in a binary data matrix. Based on results of electrophoretic band spectra, Jaccard's similarity index (S) was calculated for all possible pairs of protein type electrophoregrams using the formula as of Sneath and Sokal (1973);  $S = W/A + B - W$ , where W is the number of band of common mobility, A the number of bands in protein type A and B is the number of bands in protein type B. The similarity matrix thus generated was converted into a dissimilarity matrix (Dissimilarity = 1-similarity) and used to construct cluster pattern by UPGMA through "Statistica" package.

### Results and Discussion

To get clear banding pattern and reliable results, different concentrations of gel were prepared and tested along with quantity of sample loading. SDS-PAGE of 11.25% polyacrylamide gel with 8  $\mu$ l loading sample protein yielded best results and gave quality products. Slab type gel containing seed protein banding pattern was divided into three regions where most of the banding patterns were observed between first and second regions. Protein peptide bands with sharpness were present in all regions while third region possessed low intensity bands as compared to first and second region. Fig. 1 represents proteomic profile in 10 accessions at right side. In total 8 subunits were observed and out of them a few were polymorphic.

Beside inter-accession, single seeds were also used to extract protein from individual accession which showed extremely low differences (Fig. 1). The visible bands were compared with the standard marker which showed that most of the bands were of between 66.0 to 45.0 Kda and 18.4 to 14.3 Kda of molecular weight. Accessions PK020561 (Lahore, Punjab), PK-020585 (Faisalabad, Punjab), PK-020592 (Faisalabad, Punjab), PK-020609 (Peshawar, Khyber Pakhtun Khawa), PK-020620 (Peshawar, Khyber Pakhtun Khawa) and PK-020646 (Kohat, Khyber Pakhtun Khawa) showed high band intensity and sharpness while there were an evidence of first and second bands of light intensity. In accessions, PK-020609, PK-020620, PK-020631 (Narowal, Punjab) and PK-020646 (Kohat, Khyber Pakhtun Khawa) 1-2 bands were observed clearly as compared to other accessions. On the basis of single seed protein peptide banding pattern very clear bands were observed, in accession number PK-020592, the intensity and sharpness of the bands were very low as compared to other accessions.

Cluster pattern formed on the basis of proteomic profile divided into 5 clusters on 75% linkage distances (Fig. 2). Cluster I and Cluster-II consisted of one accession in each case, PK-020878 (Lahore, Punjab) and PK-020877 (Kohata, Rawalpindi, Punjab). Cluster-III consisted of two accessions PK-020871 (Muzaffarabad, Azad Jammu and Kashmir) and PK-020868 (Lahore, Punjab). Cluster IV consisted of two accessions PK-020783 (Attock, Punjab) and PK-020592 (Faisalabad, Punjab). Further, Cluster V consisted of 26 accessions of diverse origin, respectively.

In the present study, intraspecific variation was limited among *Nigella sativa* L. accessions. Due to low genetic diversity for proteomic profile, two-dimensional electrophoresis is suggested to separate various proteins observed on gel. SDS-PAGE may be used for interspecific diversity and phylogenetic relationships among various species rather than intraspecific variation. Low genetic diversity may be attributed to the narrow genetic base of the crop, or because one possibility is that *Nigella sativa* L. spreaded throughout the world from the same origin. Javaid *et*

*al.* (2004) also reported low level of diversity in groundnut. Hybridization between accessions from different groups, one with all bands and others with missing bands is suggested to investigate the inheritance and linkage of these bands. This may be linked with other agronomic traits that would help in planning of future experiments for marker-assisted breeding in *Nigella sativa* L. Variability intensity was observed in some bands that indicated the quantity of protein peptides cumulating at a particular molecular weight. Single seed protein banding pattern approach also illustrates the same banding pattern and sharpness of the bands, it helped to observe the facts more confidently at intraspecific level within one accession. Specific protein bands were observed for some accessions and hence these peptides may serve as markers for specific accessions as have been reported in legume crops (Iqbal *et al.* 2003, Ghafoor and Arshad 2008).

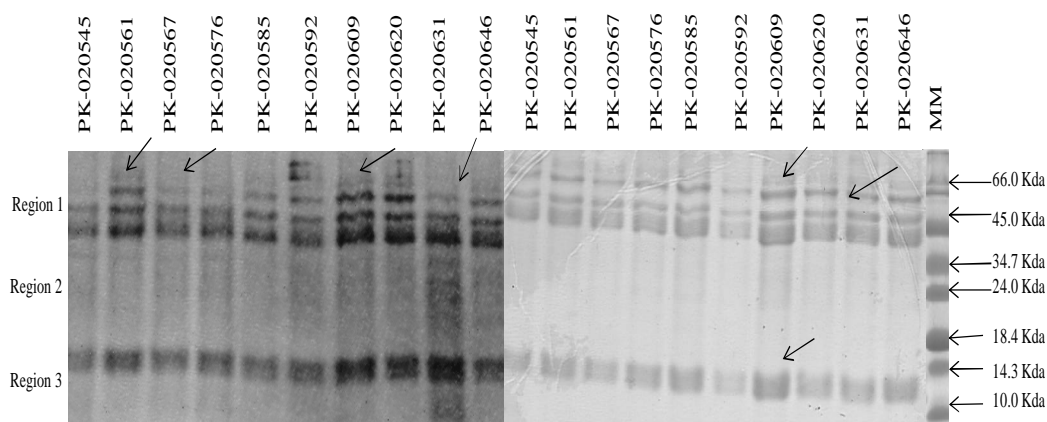


Fig. 1. Proteomics profile of *Nigella sativa* L. accessions; total seed storage protein banding pattern (left side) and single seed protein (right side). The molecular marker (MM) was SDS-70 Kit, Sigma.

The significance of the particular banding pattern is evident for location identification of particular allele for a particular trait. The significance of these bands is that the cultivar identification becomes easy as one particular protein is present in one accession and this may be absent in the others. That particular protein may carry the information of the desirable trait. These specific marker peptides (proteins) could also be helpful in the genebank management of the germplasm. Five major bands were recorded, and most of the accessions were with common banding pattern and differences were observed between 45.0 and 60.0 Kda i.e., in region-I. In many accessions the single seed pattern was similar and low variability was observed which seems to be of the narrow genetic base. Therefore, diverse accessions based on SDS-PAGE are suggested to be acquired from various sources, preferably from center of diversity to build a broad based gene-pool with maximum variability. Since, the information obtained reflects the potential usefulness of the *Nigella sativa* L. germplasm collection, the database could be strengthened by characterizing the same accessions for other genetic/biochemical markers like morphological, isozymes, RFLP, AFLP, and SSR etc. The information generated from this database could be used by the geneticists and plant breeders for development of new cultivars and by the genebank managers to properly document and maintain the germplasm (Sultana *et al.* 2007). Because the analysis of proteins is the most direct approach to defining the function of their associated genes.

On the basis of proteomics profile, a low level of inter-collection diversity was observed and no clear differentiation on the basis of origin or source was observed. The accessions in one

cluster with similar agronomic traits were not necessarily belonging to the same source or origin. The indifferent clustering pattern of some accessions collected from Pakistan might be due to exchange of germplasm between the neighboring regions, and perhaps with same ancestors (Ghafoor and Ishtiaq 2001). The transfer of the germplasm across the boundaries is difficult to

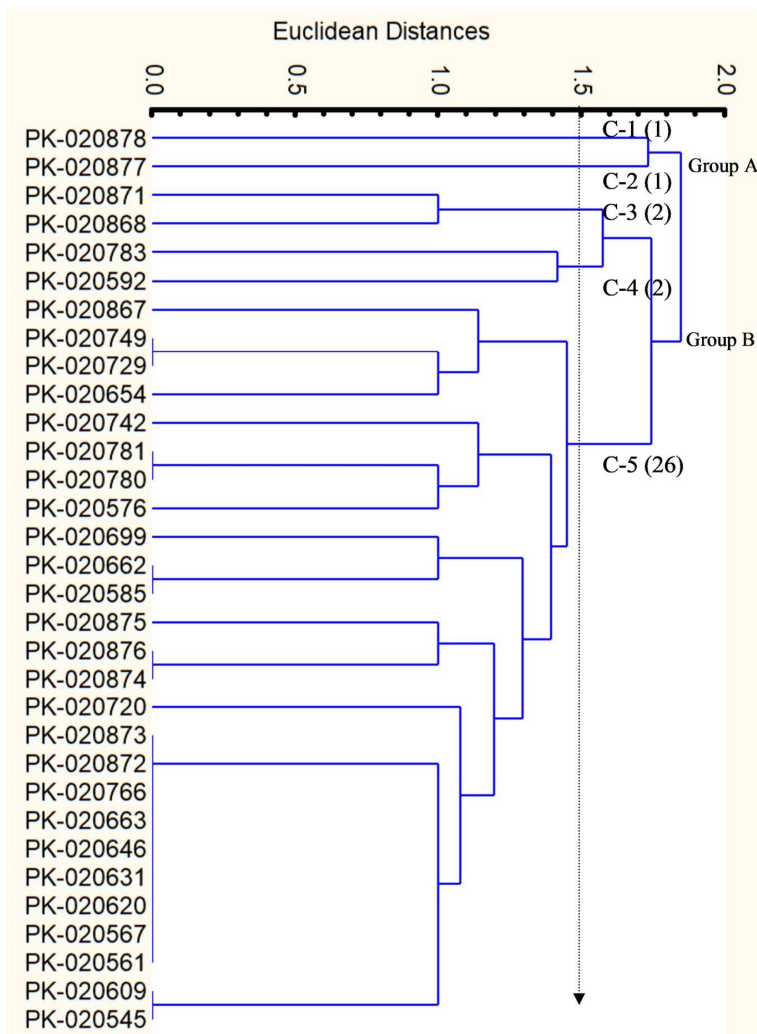


Fig. 2. Cluster diagram for proteomics profile constructed by UPGMA in *Nigella sativa* L. accessions.

provide uniform and pure seed supply. Seed quarantine measures are needed to be implemented properly for the control of mixing germplasm. Lack of proper checking systems at airports or at the time of shipment is major hindrance for their smooth and pure seed supply and production. The need is to exchange the material legally across boundaries with the official permission and seal of the quarantine department and the user should have relevant qualification to ensure seed purity and quality. New seeds of medicinal herbs should be trial tested before its use and multiplication to grow in field conditions.

### Acknowledgements

Authors acknowledge the funding by HEC, Pakistan project titled “A proteomic evaluation of *Nigella sativa* L. for seed protein profile and multivariate analysis” and PGRP, NARC, Islamabad, Pakistan for provision of germplasm.

### References

- Benkaci-Ali F, Baaliouamer A and Meklati BY 2006. Kinetic study of microwave extraction of essential oil of *Nigella sativa* L. seeds. *Chromatograp.* **64**: 227-231.
- Ghafoor A and Arshad M 2008. Seed protein profiling of *Pisum sativum* L. germplasm using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. *Pak. J. Bot.* **40**: 2315-2321.
- Ghafoor A and Ishtiaq M 2001. Use of biochemical markers for genetic diversity and determining QTLs in *Vigna mungo* germplasm. International symposium on managing biodiversity in agricultural ecosystems, 8-10 November 2001, held at Montreal, Canada.
- Iqbal MS 2009a. Biodiversity in Black cumin (*Nigella sativa* L.) for morpho-physiological traits and biochemical markers. Ph. D. Dissertation. Quaid-i-Azam University, Islamabad, Pakistan. pp. 1-268.
- Iqbal MS, Ghafoor A, Asif J, Shahzad A, Khushi M and Fatima S 2017. Finger printing analysis of industrially important medicinal plants through protein and DNA markers. Workshop Manual. HEC-Department of Botany, University of Gujrat, Gujrat, Pakistan. 45pp.
- Iqbal MS, Ghafoor A and Qureshi AS 2010. Evaluation of *Nigella sativa* L. for genetic variation and *ex situ* conservation. *Pak. J. Bot.* **42**: 2489-2495.
- Iqbal MS, Ghafoor A, Qureshi AS and Ahmed Z 2003. Genetic dissimilarities in cowpea (*Vigna unguiculata* L. Walp.) for protein peptides and their significance for quantitative traits loci. *Pak. J. Bot.* **35**: 377-386.
- Iqbal MS, Ghafoor A, Qureshi AS, Khan MR and Chaudhary MI 2009b. Quantification and diversity in the black seeds (*Nigella sativa* L.) gene stock of Pakistan for their composition of mineral nutrients. *J. Chem. Soc. Pak.* **31**: 793-800.
- Javaid A, Ghafoor A and Anwar R 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak. J. Bot.* **36**: 25-29.
- Knoblochova H and Galova Z 2000. High molecular weight glutenin subunits variation and relative quantitation in winter spelt wheat cultivars. *Rostl. Vým.* **46**: 255-260.
- Dvoracek V and Curn V 2003. Evaluation of protein fractions as biochemical markers for identification of spelt wheat cultivars (*Triticum spelta* L.). *Plant Soil Environ.* **49**: 99-105.
- Laemmli UK 1970. Cleavage of structural protein during the assembly of the head of bacteria phage T<sub>4</sub>. *Nature* **227**: 680-685.
- Sneath PHA and Sokal RR 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. Freeman WF & Co, San Francisco. 573 pp.
- Sultana T, Ghafoor A and Ashraf M 2007. Genetic variability in bread wheat (*Triticum aestivum* L.) of Pakistan based on polymorphism for high molecular weight glutenin subunits. *Genet. Resour. Crop. Evol.* **54**: 1159-1165.
- Weiss EA 2002. Spice Crops. CABI Publishing. CABI International, Wallingford, Oxon, UK.

(Manuscript received on 8 June, 2019; revised on 6 May, 2020)