Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2656

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (2):** 787-793 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Assessment of Genetic Diversity among the Accessions of Jeera (*Nigella Sativa* L.), from Afghanistan and India using Molecular Traits

Sayed Esmail Emran[1*], Rashad Ahmad Sherzad[2], Sayed Ali Yaqoobi[3], Sayed Ali Askar Musavi[4]

[1]Department of Plantation, Spices, Medicinal, and Aromatic Crops, College of Horticulture, UHS Campus, Bengaluru, Karnataka, India

[2]Department of postharvest technology, College of Horticulture, UHS Campus, Bengaluru, Karnataka, India

[3]Department of Horticulture, Faculty of Agriculture, Ferdowsi University, Mashhad, Iran

[4]Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, Japan

*Corresponding Author E-mail: emran50000@gmail.com

Received: 6.03.2017 | Revised: 18.03.2017 | Accepted: 19.03.2017

ABSTRACT

Estimation of genetic diversity is essential for breeding programs and for the conservation of genetic resources. Assessment of the genetic diversity in black cumin is therefore, of crucial importance for its genetic improvement. Molecular techniques have also had critical roles in studies of phylogeny and species evolution, and have been applied to increase our understanding of the distribution and extent of genetic variation within and between species. RAPD primers were used for molecular diversity analysis of 12 accessions representing diverse morphological clusters. The number of bands produced by each primer ranged from 1 to 11. The highest number of loci was amplified in I.15 and BH.2 primers and the lowest was in I.18, the gene diversity observed ranged from 0.00 in primer I.14 to 0.42 in primer I.1 with a mean gene diversity of 0.27, the Polymorphic information contents (PIC) of the 11 RAPD primers that were used varied between 0.00 and 0.35. Average PIC was 0.22. The genotypes from Afghanistan and India had similar similarity index values within each group (or country) than between them suggesting that short geographical isolation has low influence on the genetic diversity of the genotypes.

Key words: *Nigella sativa, Afghanistan, India, RAPD, Diversity, PIC*

INTRODUCTION

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of health care system. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe compared to modern allopathic medicines[7]. *N. sativa* is native to the geographic space associated with Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries of Middle Eastern Mediterranean region, South Europe, and in India, Pakistan, Syria, Turkey, Saudi Arabia[1]. Its black seeds and their oil have a long history and folklore of usage as food and medicine in Indian and Arabian civilization[3,4].

Cite this article: Emran, S.E., Sherzad, R.A., Yaqoobi, S.A. and Musavi, S.A.A., Assessment of Genetic Diversity among the Accessions of Jeera (*Nigella Sativa* L.), from Afghanistan and India using Molecular Traits, *Int. J. Pure App. Biosci.* **5(2):** 787-793 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2656

Estimation of genetic diversity is essential for breeding programs and for the conservation of genetic resources[12]. For any crop improvement program, a breeder depends on the variability present in the germplasm collections in order to advance productivity, bring about stability under different biotic and abiotic stresses[10]. Genetic resource conservation activities require characterization of the diversity present both in the gene pools and the gene banks[12]. Assessment of the genetic diversity in black cumin is therefore, of crucial importance for its genetic improvement. Molecular techniques have also had critical roles in studies of phylogeny and species evolution, and have been applied to increase our understanding of the distribution and extent of genetic variation within and between species.

MATERIALS AND METHODS

Thirty seven accessions collected from different places from Afghanistan (28 accessions) and India (Nine accessions) were included in this study. The experiment was taken up twice with first crop sown on November, 28, 2014 (Late *Rabi*) and the second crop sown on 5th October of 2015 (Early *Rabi* season). After germinating seeds and growing them for four weeks, young fresh leaves were collected separately from randomly selected individual plants from each accession and washed three times in sterile distilled water. Further, the leaf samples were frozen in liquid nitrogen and kept at -80 °C until further use. Based on data collected in two years clustering was done for all the 37 accessions. The 12 accessions were selected from those using the following objective criteria; (a) all clusters were represented by at least one genotype (as far as possible). (b) Number of genotypes from each cluster was also a function of the size of the cluster such that diversity is well represented. (c) There is consistence of genotypes in clustering with other genotype in both seasons and used for DNA analysis. The genomic DNA was extracted from leaf samples collected from 12 accessions of *Nigella Sativa.* The genomic DNA was prepared by method given by Krishna and Jawali[13] with minor modifications. The allelic data obtained from the RAPD markers were used to visualize the genetic relationships among twelve accessions of Jeera. The similarity was calculated using the simple matching (SM) coefficient[17].

RESULTS

In the present study RAPD primers were used to study diverse accessions, the RAPD primers produced totally bands. (Figure 1) genetic similarly measured through analysis of bands among 12 accessions. The similarity indices and consensus dendrogram were developed on the basis of score able banding pattern of twelve genotypes (0 for absence and 1 for presence). The similarity coefficients ranged from 0.43 to o.75 (Table 1). The genotypes AFB-DI and INB-M8 showed the lowest similarity index of 0.43, whereas AFG-QA and AFH-KO showed highest genetic similarity of 0.57. Most of the similarity values were between 0.51 and 0.70 (78.78 %; Figure 1). Indian lines showed higher similarity among themselves (0.50 to 0.73) while the Afghan lines showed a greater range of similarity values (0.43 to 0.75; Table 1).

The Polymorphic information content (PIC) values which denote allelic diversity and frequency among the genotypes had an average of 0.22 /marker and ranged from 0.0 to 0.35 with the highest PIC obtained by I.1 primer. The gene diversity was also calculated and ranged from 0.0 to 0.42 for the same primers used in the study and the highest gene diversity was with I.1 primer with an average of 0.27 per marker (Table 2). The percentage polymorphism ranged from 0 to 100 with polymorphic bands ranging from 3 to 10, Zero polymorphism was found with I.14 primer and 100 percent polymorphism was found with AB.10 and AB.13 primers (Table 3).

The dendrogram constructed using SHAN clustering separated the twelve varieties of *Nigella sativa* L. into two major clusters: the first major cluster (cluster-1) contained ten accessions (Viz., AFB-DI, AFF-PU, AFF-QA, AFG-QA, AFH-KO, AFH-KA, AFN-KA, INB-M4, INB-M6 and INB-M8)

and other cluster-2 contained two accessions (Viz., AFG-MU and AFG-NU) sharing a common node at a similarity coefficient of 0.55 (Figure 2). The first major cluster was again divided into two sub clusters viz., 1a and 1b at a similarity of 0.578; cluster 1a had seven while 1b had three accessions. Cluster 1a with seven clusters was further subdivided into two clusters, one with a solitary accession and other with the remaining six accessions.

Table 2: Polymorphic information content (PIC) among the 12 selected *Nigella sativa* L., accessions

Sl. No	Primer Name	Gene diversity	PIC
1	AB.4	0.12	0.10
2	AB.10	0.33	0.27
3	AB.13	0.33	0.27
4	I.14	0.00	0.00
5	AK.7	0.28	0.23
6	BH.2	0.22	0.19
7	BH.14	0.32	0.25
8	I.1	0.42	0.35
9	I.7	0.33	0.27
10	I.15	0.29	0.24
11	I.18	0.32	0.26
Mean		0.27	0.22

Table 1: Similarly coefficient matrix based on DNA analysis among the 12 selected *Nigella sativa* L., accessions

	AFB-DI	AFF-PU	AFF-QA	AFG-MU	AFG-NU	AFG-QA	AFH-KO	AFH-KA	AFN-KA	INB-M4	INB-M6	INB-M8
AFB-DI	1.00											
AFF-PU	0.60	1.00										
AFF-QA	0.66	0.71	1.00									
AFG-MU	0.56	0.56	0.59	1.00								
AFG-NU	0.50	0.44	0.53	0.58	1.00							
AFG-QA	0.47	0.63	0.64	0.60	0.55	1.00						
AFH-KO	0.44	0.65	0.59	0.56	0.52	0.75	1.00					
AFH-KA	0.46	0.67	0.63	0.58	0.50	0.64	0.71	1.00				
AFN-KA	0.58	0.69	0.65	0.60	0.56	0.61	0.60	0.67	1.00			
INB-M4	0.50	0.63	0.62	0.51	0.60	0.70	0.70	0.69	0.71	1.00		
INB-M6	0.52	0.54	0.55	0.52	0.52	0.64	0.61	0.66	0.70	0.73	1.00	
INB-M8	0.43	0.60	0.55	0.54	0.54	0.69	0.58	0.63	0.62	0.69	0.63	1.00

Table 3: Total bands, polymorphism and polymorphism percentage among the 12 selected *Nigella sativa* accessions

S/N	Primer name	Total bands	Polymorphism	(%) polymorphism
1	AB.4	7	3	42.8
2	AB.10	10	10	100
3	AB.13	9	9	100
4	I.14	7	0	0
5	AK.7	9	8	88.8
6	BH.2	11	9	81.8
7	BH.14	10	9	90
8	I.1	8	7	87.5
9	I.7	8	6	75
10	I.15	11	10	90.9
11	I.18	6	5	83.3

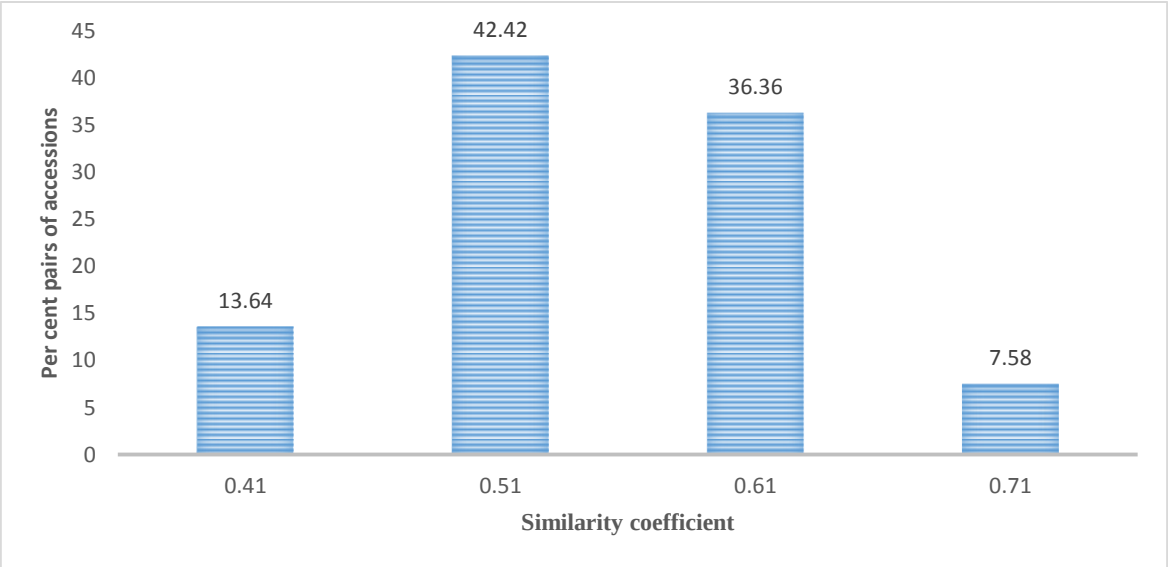

Fig. 1: Percent pairs of accessions with varying similarity coefficient values based on DNA analyses

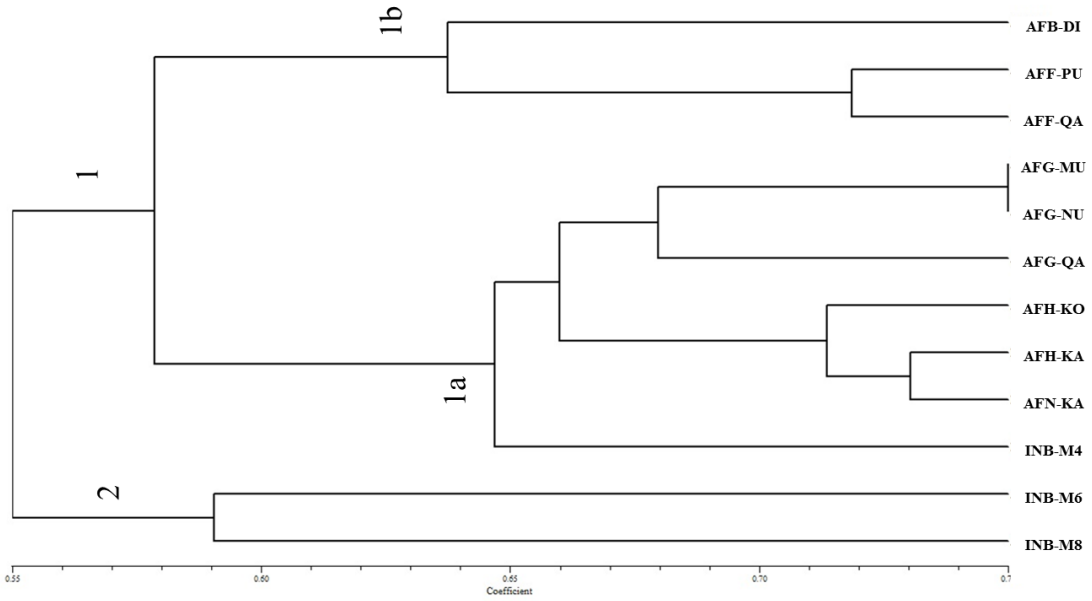

Fig. 2: UPGMA Dendrogram representing relationship among 12 selected accessions of *Nigella sativa* L.

DISCUSSIONS

Since past few decades black cumin has not been subjected to systematic genetic improvement program for the following three reasons. Firstly, this crop has still not been brought under systematic and intensive cultivation in parts of Asia such as Afghanistan. In fact it is still being harvested from the wild and marketed in Afghanistan, Pakistan etc[2]. Secondly, the complete genetic diversity available among the global population, of *Nigella Sativa* is yet to be studied; this is because the accessions are not yet collected from areas of it's occurrence in Afghanistan, Pakistan etc., owing to poor information and other political problems. Consequently, the full genetic potential of the crop is not yet understood. Thirdly, owing to lack of attention by the breeders and other concerned scientists in the areas of its abundant occurrence, especially in North West part of Asia, no systematic genetic improvement has been attempted in this crop. RAPD is widely used in many plant species

owing to its low cost, multi loci analysis and rapid detection of polymorphisms[5,6]. It has been suggested that the use of the RAPD seem more appropriate when the objective was to cluster genotypes in many other plant species; it showed similarities between the individuals[16]. RAPD marker have been extensively used for DNA fingerprinting[8,14], genetic diversity studies[9]. Population genetic studies[18,19].

In the present study, RAPD primers were used for molecular diversity analysis of 12 accessions representing diverse morphological clusters. The number of bands produced by each primer ranged from 1 to 11. The highest number of loci was amplified in I.15 and BH.2 primers and the lowest was in I.18 (Table 2). Such a variation in the number of fragments produced by these arbitrary primers may be attributed to the differences in the binding sites throughout genome of the genotypes included. The gene diversity observed ranged from 0.00 in primer I.14 to 0.42 in primer I.1 with a mean gene diversity of 0.27. The mean gene diversity was moderate with a limited number of primers used in this study suggesting that there is a lot of variation in the genotypes studied. It is expected as the genotypes were collected from geographically different locations of India and Afghanistan. The Polymorphic information contents (PIC) of the 11 RAPD primers that were used varied between 0.00 and 0.35. Average PIC was 0.22. The selected primers displayed moderate frequencies of polymorphic bands. When an overall evaluation is made, it was observed that PIC values of the primers used in the study were quite low. Normally if the diverse genotypes are used the PIC values are expected to be more. The low PIC could be due to the ploidy level of the species. It has also been reported in other crops with higher ploidy levels that the SSR and cytochrome P450 gene based markers would be a better tool than RAPD markers for phylogenetic studies[15]. The present study based on the RAPD technique varied from those obtained in previous studies on different plant species, like barley (77.06%), rice bean (70.30%), Vigna species (48%) and maize (76.14%) where the PIC values were more.

Clustering of genotypes tended to follow pedigree relatedness, geographical isolation, and origin of genotypes in many plant species. Ability of these markers to discriminate closely related lines is evident from the dendrogram. The similarity coefficient matrix based on DNA markers suggested that the genotype AF-GOA and AF-HKO are very similar with a similarity coefficient value of 0.75, while the genotype AF-BDI and IN-BM8 are highly diverse with a similarity value of 0.43. Thus the similarity values seem to represent the geographical origin of the genotypes. The genotypes from Afghanistan and India had similar similarity index values within each group (or country) than between them suggesting that short geographical isolation has low influence on the genetic diversity of the genotypes. However, the Indian and Afghan lines tended to separate in the dendrogram suggesting that long geographic distances have influenced the genetic structure of black cumin. The tendency for genetic diversity to increase with geographic distance has been reported in many earlier studies in different crop plants[11] (Inoue and Kawahara, 1990). The UPGMA phenograms suggested that the genotypes from different geographical regions, especially from India and Afghanistan showed tendency to cluster separately suggesting that there is restricted movement of genes across countries (between India and Afghanistan). However, the dendrograms showed that samples collected from Afghanistan did not separate based on their origin with in the country. This may be because we have sampled only one or two population(s) from each cluster and also we have used the population pools for marker analysis, we might have underestimated the genetic distance between two populations within the country.

REFERENCES

1. Abdel-Fattah, A.M., Matsumoto, K. And Watanabe, H., Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone in mice. *Eur. J. Pharmacol*., **400:** 89–97 (2000).
2. Ahmad, Z., Ghafoor, A. and Aslam, M., *Nigella sativa*–A potential commodity in crop diversification traditionally used in health care. Project on Introduction of Medicinal herb and species as crop. Ministry of food, agriculture and livestock, *Pakistan*, 6-10 (2004).
3. l-Awadi, F., Fatania, H. and Shamte, U., The effect of a plant's mixture extract on liver gluconeogenesis in streptozotocin-induced diabetic rats. *Diabetes Res.,* **18:** 163–168 (1991).
4. Badary, O.A., Nagi, M.N., Al-Shabanah, O.A., Al-Sawaf, H.A., Al-Sohaibani, M.O. and Al-Bekairi, A.M., Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. *Can. J. Physiol. Pharmacol.,* **75:** 1356–61 (1997).
5. Bruel, D.C., Carpentieri-Pipolo, V., De Fátima Ruas, C., Gerage, A.C. And De Souza, S.G.H., Assessment of genetic diversity in maize inbred lines using RAPD markers. *Crop Breed. Appl. Technol.*, **7(2):** 173 (2007).
6. Carvalho, S.M.P., Heuvelink, E., Cascais, R. and Van KooteN, O., Effect of day and night temperature on internode and stem length in chrysanthemum., *Ann. Bot*., **90:** 111–118 (2002).
7. Debas, H.T., Laxminarayan, R. and Straus, S.E., Complementary and alternative medicine. *Disease control priorities in developing countries*, 2 (2006).
8. Gilbert, D.E. and Feigon, J., Multistranded DNA structures. *Current Opinion in Structural Biology*, **9(3):** 305-314 (1999).
9. Hoz, M.S.D.L., Davila, J.A., Loarce, Y. and Ferrer, E., Simple sequence repeat primers used in polymerase chain reaction amplifications to study genetic diversity in barley. *Genome.*, **39(1):** 112-117 (1996)
10. Ibrahim, A.A., Mohammad A.B, Hasseb, A.K., Ahmad, H.A., Ali, A.A., Ali, H.B., Mohammad, A. and Mohammad, S., A brief review of molecular techniques to assess plant diversity. *Int. J. Mol. Sci*., **11:** 2079-2096 (2010).
11. Inoue, K. and Kawahara, T., Allozyme differentiation and genetic structure in island and mainland Japanese populations of *Campanula punctate* (*Campanulaceae*). *American J. Bot.*, pp.1440-1448 (1990).
12. Karp, A., Molecular tools in plant genetic resources conservation: a guide to the technologies. *Bioversity International*, **2:** pp 381-390 (1997).
13. Krishna, T.G. and Jawali, N., DNA isolation from single or half seeds suitable for random amplified polymorphic DNA analyses. *Analytical Biochem.*, **250(1):** 125-127 (1997).
14. Moreno, S., Martín, J.P. and Ortiz, J.M., Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica.*, **101(1):** 117-125 (1998).
15. Panwar, P., Nath, M., Yadav, V.K. and Kumar, A., Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn.). *J. Genet.*, **89(2):** 21-133 (2010).
16. Ristic, M.I.O.D.R.A.G., Anaplasmosis. *Infectious blood diseases of man and animals*, **11**, pp.473-542 (2013).
17. Sneath, P.H. and Sokal, R.R., *Numerical taxonomy. The principles and practice of numerical classification*., 322-331 (1973).

18. Wolf, H.T., Zundorf, I., Winckler, T., Bauer, R. and Dingermann, T., Characterization of *Echinacea* species and detection of possible adulterations by RAPD analysis. *Planta Med*., **65:** 773-774 (1999).

19. Wu, S., Jia, M., Ruan, Y., Liu, J., Guo, Y., Shuang, M., Gong, X., Zhang, Y., Yang, X. and Zhang, D., Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biological Psychiatry.*, **58(1):** 74-77 (2005).