Exploration of genotype specific fingerprinting of
Nigella sativa L. using RAPD markers

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Abstract: Nigella sativa L. has industrial, cosmetic, and pharmaceutical uses but has not been adequately characterized
in Pakistan. This investigation was carried out to explore genotype specific fingerprinting of 32 N. sativa L. genotypes
based on randomly amplified polymorphic DNA markers. From 58 random primers used, 15 primers generated 249
reproducible and scorable amplification products across all the genotypes, out of which 164 (66%) fragments were
polymorphic revealing a high level of polymorphism among these genotypes. The proportion of common bands was low
(34%). The size of the amplification products on agarose gels ranged between 0.5 and 10.0 kb. In 13 genotypes, 27 bands
of different masses (kilobases) were recorded and were considered specific to those genotypes. These specific/amplified
PCR products can be used as molecular markers for identification of germplasm and resource protection of
Nigella sativa L. genotypes. Specific bands were observed for individual primers that could resolve genetic diversity among
several genotypes (PK-020545, PK-020567, PK-020576, PK-020585, PK-020592, PK-020620, PK-020631, PK-020646,
PK-020663, PK-020729, PK-020742, PK-020749, and PK-020868). UPGMA cluster analysis indicated 7 distinct clusters,
1 (C-3) comprising 9 accessions of N. sativa L., while C-7, C-5, and C-6 included 7, 6, and 5 accessions, respectively. C-4
and C-2 included 2 accessions each. Cluster 1 remained distinct as it had only 1 accession (PK-020646), indicating its
higher genetic diversity from all other species. The overall grouping pattern of clusters corresponded well with principal
component analysis and confirmed overall patterns of genetic variability among the species. These genotypes could
be used as parents for random mutagenesis, or incorporated for gene recombination studies before marker assisted
selection/breeding can be used for crop improvement. Moreover, these DNA based markers can be suitable for genetic
distance estimation because they detected potentially large number of polymorphisms. Future research is needed to
identify molecular markers linked to important traits (yield and oil quality) and locating quantitative traits loci (QTL)
for improvement of N. sativa L. germplasm.

Key words: Biodiversity, genetic relatedness, genotype specific bands, PCR, polymorphism, RAPD, random priming,
UPGMA

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**Introduction**

The *Ranunculaceae* or buttercup of the order *Ranales* is a large family containing about 70 genera and at least 3000 species. The genus *Nigella* contains about 20 species of annual herbs indigenous to the Mediterranean region through West Asia to Northern India, and has long been domesticated (Weiss 2002). *Nigella sativa* L. (diploid, 2n = 12) is an erect annual herb considered important for both oil and bioactive compounds because its constituents have unique chemical properties and may augment the supply of edible oils or production of biodiesel (Ramadan and Morsel 2003; Iqbal et al. 2010).

Genetic variability is a prerequisite for selection program of germplasm; it is essential to detect and present the level of genetic variation present within and between plant populations. DNA marker-assisted fingerprinting can differentiate species rapidly employing a small quantity of DNA and therefore can help to derive reliable findings on their phylogenetic relativity. DNA markers are not influenced by environmental impacts and are useful in describing the levels of genetic variability among plant populations to discriminate the duplicated accessions within a specific collection of germplasms. Many researchers have used different techniques for DNA fingerprinting such as restriction of length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), simple sequence repeat (SSR) markers, and randomly amplified polymorphic DNA (RAPD) markers in combination with the analysis of morpho-physiological traits in various crops (Rafalski et al. 1991; Williams et al. 1993; Cheng et al. 2002). RAPD is a rapid and inexpensive method and non-dependent on plant genome information. It has been extensively applied to ascertain genetic polymorphism in several plants (Murai and Ohnishi 1996; Nevo et al. 1998). The variability of RAPD markers was measured by the proportion of polymorphic bands, in the sense that some natural populations produce a particular band, but others do not. This measurement of variability is highly dependent on the number of samples studied and on the ecological origins from where the samples were collected.

RAPD involves the employment of short (10 bp) PCR primers of arbitrary sequence to amplify unknown genomic sequences (Khan et al. 2002). When the resulting products are separated in suitable electrophoretic gels, DNA-level polymorphisms (usually in primer recognition sites) can be uncovered (Williams et al. 1990; Welsh et al. 1991). RAPD techniques are still employed by many laboratories, as easy ways of screening potential molecular markers from many loci using small amounts of DNA (Ritland and Ritland 2000). Moreover, DNA fingerprinting is a powerful technique for assessing genetic diversity levels among germplasms (Lee 1995). Crops with improved output traits could have nutritional benefits for millions of people who suffer from malnutrition or other deficiency disorders. Identification has been carried out of all those genes that can modify and enhance the composition of carbohydrates (including starch), oils, and proteins in food/feed grains and root crops. The development of molecular markers based on polymorphism observable in protein or DNA has greatly facilitated research in phylogeny, ecology, plant breeding, and genetics.

Information regarding DNA fingerprinting and polymorphism in *Nigella sativa* L. germplasm is not widely available and demands its exploration. Due to applications of molecular markers, a number of genomes were sequenced and studied for different purposes. They were especially employed in the field of plant genetics and breeding, biotechnology, and bioinformatics. Study of DNA fingerprinting of *Nigella sativa* L. has been undertaken to explore the genetic relationship between its different species using RAPD markers in order to exploit the present germplasm efficiently.

**Materials and methods**

**Genomic DNA isolation**

For good quality and purity, genomic DNA was isolated/extracted following the method of Kang et al. (1998) using seeds of all the 32 accessions. Known weights of seeds (0.03 g each) were placed in 1.5 mL microcentrifuge tubes (Eppendorf tubes). Extraction buffer 400 μL (200 mM Tris-HCl, pH 8.0, 25 mM EDTA, 200 mM NaCl, 0.5% SDS) was added to all samples. Proteinase K (50 μg) was added to each tube to remove traces of proteins in
order to get pure genomic DNA. Then samples were incubated at 37 °C for 1 h. Then seed samples were ground with the addition of buffer with a glass rod. Four hundred microliters of 2% CTAB buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 2% CTAB (w/v), 1% PVP (polyvinyl pyrrolidone” Mr. 40,000) was added. Impurities usually create a problem during extraction of good quality DNA, especially in the case of medicinal plants, which have complex chemical compositions (Cheng et al. 2003). Then 600 μL of chloroform:isoamyl alcohol (24:1) with 5% phenol was added and mixed gently (manually) for about 2 min to get a homogenous mixture. It was then centrifuged at 12,000 rpm at 4 °C for 10 min. The aqueous layer was now recovered carefully and shifted into new 1.5 mL Eppendorf tubes. Seven hundred microliters of isopropanol, i.e. 2/3 (v), was added and incubated at room temperature for 10 min to precipitate DNA. Genomic DNA appeared in the form of threads. Then Eppendorf tubes were centrifuged at 12,000 rpm for 10 min and this time supernatant was removed. The DNA pellet was washed with 70% ethanol (500 μL) and again centrifuged at 12,000 rpm for 5 min at room temperature to remove ethanol. The DNA pellet was air dried for 5-10 min and resuspended in 100 μL of TE buffer for storage, quantification, and PCR application. RNase (10 mg mL⁻¹) 1 μL was added to remove RNA.

The purity and concentration of extracted genomic DNA were checked by spectrophotometer (Model Agilent 8453, USA). DNA concentrations were calculated using the formula \[ \text{DNA} = \text{OD}_{260} \times \text{dilution factor} \times \text{constant (50 μg mL}^{-1}) \]. DNA samples were diluted to a working concentration of 50-100 ng μL⁻¹ in sterile distilled water and stored at 4 °C. The integrity and concentration of the DNA was confirmed by running 1% (w/v) agarose gel electrophoresis for 45 min at 80 V and visualization under UV light after staining with ethidium bromide.

**PCR amplification kit and RAPD analysis**

For PCR-RAPD analysis, 10 to 12 base oligonucleotide primers were used, which were obtained from Operon Technologies Inc. USA (Table 1). PCR was performed by volume of 20 μL of 10× buffer, called master mix including 2 μL {Tris-HCl (100 mM), pH 8.8 with (NH₄)₂SO₄ (1 mL), 1.6 μL MgCl₂ (25 mM, 1 mL), 0.4 μL dNTPs [each of dATP, dCTP, dGTP, dTTP, (25 mM)], 0.8 μL of primer (15 ng μL⁻¹), 1 μL of genomic DNA (1 ng μL⁻¹), 0.15 unit of Taq polymerase and ddH₂O (13.85 μL)}. A total of 58 primers were used for screening of RAPD markers. Taq polymerase, together with 10× buffer, MgCl₂, and dNTPs, were obtained from Fermentas (Canada). PCR-based DNA amplification reactions were performed in Tpersonal Biometra DNA Thermocycler 480 (Germany).

**Optimization of RAPD Protocol**

The PCR-RAPD technology is sensitive to changes in experimental parameters. A total of 58 primers were initially screened against 10 plants selected from all populations (bulked seed DNA). The effects of magnesium, template DNA concentrations, pH values, and length of the denaturation stage of the amplification were all examined. When trying to optimize annealing temperatures, we ran the test reactions at 27 °C, 30 °C, 33 °C, 36 °C, and 37 °C. The decamer primers can be clearly amplified at 34 °C. A subset of 15 primers for further analysis was based on the following criteria: i) consistent, strong amplification products, and ii) production of uniform, reproducible fragments between replicate PCRs. PCR thermal cycler was programmed for first denaturation step at 94 °C for 1 min, followed by 40 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. The PCR tubes were kept at 72 °C for 7 min and then held at 4 °C until the tubes were removed.

**Gel Electrophoresis**

All visible and unambiguously scorable fragments were recorded. The fragments that were repeatedly present in one bulk and absent in the other were scored as polymorphic fragments. Polymorphic primers were used to verify the linkage of marker with the trait. RAPD fragments were separated electrophoretically on 0.9% agarose gels in 1× TBE buffer, stained with ethidium bromide, and photographed on a UV transilluminator using a digital camera. DNA from each plant was amplified with the same primer 3 times, and the banding patterns were compared.

**Data analysis for PCR-RAPD**

RAPDs behave as dominant markers (Hyman et al. 2003), thus they tend to be used with a bistate
Table 1. List of PCR-RAPD primers used for preliminary DNA screening and fingerprinting in 32 accessions of *Nigella sativa* L. germplasm.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>PCR response</th>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>PCR response</th>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>PCR response</th>
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<td>OPA-1</td>
<td>5’-CAGGCCCTTC-3’</td>
<td>+</td>
<td>OPC-27</td>
<td>5’-GAGGAGGTTAAA-3’</td>
<td>–</td>
<td>OPS-3</td>
<td>5’-CAGAGGTTCC-3’</td>
<td>+</td>
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<tr>
<td>OPA-2</td>
<td>5’-TGCCGAGCTG-3’</td>
<td>+</td>
<td>OPC-41</td>
<td>5’-CAGACAGGGTAT-3’</td>
<td>–</td>
<td>OPS-4</td>
<td>5’-CACCCCTTG-3’</td>
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<td>5’-AGTCAGCCAC-3’</td>
<td>+</td>
<td>OPC-44</td>
<td>5’-GGCAACATAGTA-3’</td>
<td>–</td>
<td>OPS-5</td>
<td>5’-TTTGGGGGCT-3’</td>
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<td>5’-AATCGGGCTG-3’</td>
<td>+</td>
<td>OPC-67</td>
<td>5’-CCAAGATCCATT-3’</td>
<td>–</td>
<td>OPS-6</td>
<td>5’-GATACCTCGG-3’</td>
<td>+/-</td>
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<td>5’-AGGGGTCTTG-3’</td>
<td>–</td>
<td>OPC-71</td>
<td>5’-TTCACATCGAC-3’</td>
<td>–</td>
<td>OPS-7</td>
<td>5’-TCCGATGCTG-3’</td>
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<td>5’-GTCCTCGAC-3’</td>
<td>+/-</td>
<td>OPC-75</td>
<td>5’-GATGGTGACGAA-3’</td>
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<td>OPS-8</td>
<td>5’-TCAGGGTGG-3’</td>
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<td>–</td>
<td>OPC-85</td>
<td>5’-ACTTTGAGACG-3’</td>
<td>–</td>
<td>OPS-9</td>
<td>5’-TCATGCTCC-3’</td>
<td>–</td>
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<td>5’-TGACGTAAG-3’</td>
<td>+</td>
<td>OPC-92</td>
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<td>5’-GGTACGACG-3’</td>
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<td>OPZ-1</td>
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<td>5’-GTGATCGAG-3’</td>
<td>+</td>
<td>OPC-97</td>
<td>5’-AAGACGTTGTA-3’</td>
<td>–</td>
<td>OPZ-2</td>
<td>5’-CCTACGAGGA-3’</td>
<td>+/-</td>
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<tr>
<td>OPA-11</td>
<td>5’-CAATCGGGCT-3’</td>
<td>+/-</td>
<td>OPC-98</td>
<td>5’-ACCAAGGTGAT-3’</td>
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<td>OPZ-3</td>
<td>5’-CAGACCGCA-3’</td>
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<td>+/-</td>
<td>OPF-22</td>
<td>5’-AAGATCAAGAC-3’</td>
<td>+</td>
<td>OPZ-4</td>
<td>5’-AGGCGTGCTG-3’</td>
<td>–</td>
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<tr>
<td>OPA-13</td>
<td>5’-CACACGAC-3’</td>
<td>+/-</td>
<td>OPN-1</td>
<td>5’-CTCGTGGCG-3’</td>
<td>+/-</td>
<td>OPZ-5</td>
<td>5’-TCCAGTGCT-3’</td>
<td>+/-</td>
</tr>
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<td>OPA-14</td>
<td>5’-TCTGTGCTG-3’</td>
<td>–</td>
<td>OPN-2</td>
<td>5’-ACCAGGGGCA-3’</td>
<td>+</td>
<td>OPZ-6</td>
<td>5’-GCAGTCTCA-3’</td>
<td>+/-</td>
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<td>OPA-15</td>
<td>5’-TCCGAAACCC-3’</td>
<td>–</td>
<td>OPN-3</td>
<td>5’-GCTACTTCCC-3’</td>
<td>–</td>
<td>OPZ-7</td>
<td>5’-CCAGAGGAG-3’</td>
<td>–</td>
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<tr>
<td>OPA-16</td>
<td>5’-AGCGAGCGAA-3’</td>
<td>–</td>
<td>OPN-4</td>
<td>5’-GACCGACCA-3’</td>
<td>–</td>
<td>OPZ-8</td>
<td>5’-GGCTGGGTAA-3’</td>
<td>+</td>
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<td>OPA-17</td>
<td>5’-GACGGCTGTG-3’</td>
<td>–</td>
<td>OPN-5</td>
<td>5’-ACTGAACGC-3’</td>
<td>–</td>
<td>OPZ-9</td>
<td>5’-CACCCAGTC-3’</td>
<td>+</td>
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<td>OPA-18</td>
<td>5’-AGGGTACCAG-3’</td>
<td>–</td>
<td>OPN-6</td>
<td>5’-GAGACGCCA-3’</td>
<td>–</td>
<td>OPZ-10</td>
<td>5’-CCGACAAACC-3’</td>
<td>–</td>
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<tr>
<td>OPA-19</td>
<td>5’-CAAGGTCGGG-3’</td>
<td>+/-</td>
<td>OPS-1</td>
<td>5’-CTACTCCTG-3’</td>
<td>–</td>
<td>OPZ-2</td>
<td>5’-CCTCTGACTG-3’</td>
<td>–</td>
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<td>OPA-20</td>
<td>5’-GTGGCGATCC-3’</td>
<td>–</td>
<td>OPS-2</td>
<td>5’-CCTCTGACTG-3’</td>
<td>–</td>
<td>OPZ-3</td>
<td>5’-CCTCTGACTG-3’</td>
<td>–</td>
</tr>
</tbody>
</table>

58 primers with 10 and 12 base pairs; primers, oligo-nucleotides; + represents amplification of primer; – no amplification and +/- represents low amplification response; only amplified primers were used in the present study.
(present-absent) type of scoring. Photographs from ethidium bromide stained polyacrylamide gels were used to score the data for RAPD analysis. Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored as present (1) or absent (0) for each of the primer-accession combinations. Since DNA samples consisted of a bulk sample of DNA extracted from individual plants, a low intensity for any particular fragment may be explained by the lesser representation of that specific sequence in the bulk sample of DNA. Therefore, the intensity of the bands was not considered and the fragments with identical mobility were scored as identical fragments. Only major bands were scored and faint bands were ignored. The molecular size of the amplification products was calculated from a standard curve based on the known size of DNA fragments of a marker \( X174/Hae \) digest. The presence and absence of the bands was scored in a binary data matrix. Pair-wise comparisons of the accessions based on the presence or absence of unique and shared amplification products were used to generate similarity coefficients. DNA bands shared by all the accessions were excluded from the data analysis since they are not informative (Hyman et al. 2003). The resulting similarity coefficients were used to evaluate the relationships among the accessions with cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA) and then plotted in the form of a dendrogram using STATISTICA version 6.0 for Windows. Genetic similarities were estimated using Nei’s standard (1973) genetic distances as Euclidean distances also using STATISTICA.

Results

Fifty-eight 10 to 12 mer primers were tested on 4 accessions (PK-020545, PK-020561, PK-020567, and PK-020576) for preliminary DNA amplification and screening. Out of 58 primers, 28 revealed amplification for present material (Figure 1 and 2). Out of amplified primers, 15 exhibited polymorphism and hence were used for fingerprinting of \( Nigella \) sativa L. germplasm for further studies. Some of the primers revealed characteristic fragments for some accessions that were not produced in others. Some of the primers generated several markers and were able to show high genetic diversity, while others produced few markers and suggested little variability. Fifteen primers generated 249 reproducible and scorable amplification products across all the accessions, out of which 164 (66%) fragments were polymorphic in one or other of the 32 accessions. Cluster pattern developed for PCR-RAPD markers divided into 8 clusters on 75% Euclidean distances (Figure 3). Accession (PK-020646) was present in cluster-1 and 2 accessions (PK-020631 and PK-020576) were present in cluster-2, while cluster-3 consisted of 9 accessions. Cluster-4 consisted of 2 accessions, and C-5 (6), C-6 (5), and C-7 consisted of 7 accessions of wide genetic base.

Figure 1. PCR-RAPD polymorphism generated by OPS-5, OPA-9, OPA-3, OPA-8, and OPS-4 in \( Nigella \) sativa L. germplasm. One kb DNA ladder visualized by ethidium bromide staining on a 0.9% TAE agarose gel. The digested DNA includes fragments ranging from 0.5 to 10.0 kilobases.
Exploration of genotype specific fingerprinting of *Nigella sativa* L. using RAPD markers

In the present study, the proportions of common bands were low (34%). Based on unweighted pair group method (UPGMA), 2 main groups and 7 sub-groups were distinguishable in the present material. Many primers generated accession specific amplification products and thus preliminary mapping (genetic maps) of *Nigella sativa* L. is suggested. The size of amplification products scored in 1.4% agarose gels ranged between 0.5 and 10.0 kb.
Twenty-seven bands were recorded in 13 genotypes, presented in Table 2. These bands were marker bands only located in these genotypes and there was no duplication of the genotype for a particular primer. In the Figure 4, the particular genotype specific bands are displayed and compared with molecular markers of 1 kb of DNA ladder. The bands were clear in visibility and sharpness. The highest genetic distances were present between accession PK-020576 (3.74) and accession PK-020875, followed by 3.66 between PK-020567 and PK-020877. The shortest distances were recorded between PK-020742 (1.00) and PK-

<table>
<thead>
<tr>
<th>No.</th>
<th>Primers</th>
<th>Sequences</th>
<th>Genotypes</th>
<th>Band size (kb)</th>
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<tr>
<td>1</td>
<td>OPS-3</td>
<td>5-CAGAGGTCCC-3</td>
<td>(PK-020620)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(PK-020631)</td>
<td>10.0, 4.5, 4.0</td>
</tr>
<tr>
<td>2</td>
<td>OPA-1</td>
<td>5-CAGGCCCTTC-3</td>
<td>(PK-020567)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(PK-020631)</td>
<td>2.0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>(PK-020868)</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>OPA-9</td>
<td>5-GGGTAACGCC-3</td>
<td>(PK-020592)</td>
<td>8.0, 5.0</td>
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<td></td>
<td></td>
<td></td>
<td>(PK-020631)</td>
<td>3.0</td>
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<td></td>
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020780, expressing linkage between them. High and short genetic distances recorded between different accessions might be helpful in the construction of genetic maps after further studies.

**Discussion**

As a complement to morphological, biochemical, ecological, and genetic information, molecular markers can contribute greatly to the use of genetic diversity through the descriptive information they provide on the structure of gene pools and accessions. The use of RAPD techniques for germplasm characterization facilitates the conservation and utilization of plant genetic resources, permitting the identification of unique accessions or sources of genetically diverse germplasm (Brown-Guedira et al. 2001; Li et al. 2001; Vieira et al. 2001). The ability of this method to distinguish between taxa also has useful implications in botanical quality analysis (Kapteyn and Simon 2002).

The technique was found useful for characterization of present germplasm, and its ability to sample any portion of the genome of *Nigella sativa* L. could help to study markers on all the linkage groups, genotypes distinctness, and classification of accessions into specific groups. The RAPD meets all these requirements, although it suffers from other serious drawbacks such as the dominant nature of markers, non-reproducibility of patterns, and difficulty in establishing homology of amplification products with similar molecular weights, which reduce the quality of information obtained (Korzun et al. 2001).

In the present study, RAPD markers were used to determine genetic diversity in *Nigella sativa* L. germplasm and it was concluded that the technique was an effective tool in identifying *Nigella sativa* L. germplasm and determining their genetic relationships. The present study revealed that working germplasm is not sufficient to build a comprehensive database for *Nigella sativa* L. The information generated, however, will be helpful to expand the scope of further research by adding new accessions not only from local growing conditions but also from international markets and gene banks. As some primers revealed characteristic fragments for some accessions that were not produced in others, these accessions could be utilized as new breeding material for local needs. Some of the primers generated several markers and were able to show high genetic diversity, while others produced few markers and detected little variability. Fifteen primers generated 249 reproducible and scorable amplification products across all the accessions, out of which 164 (66%) fragments were polymorphic.
in one or other accessions under study. The level of polymorphism varied with different primers among various accessions. The RAPDs have also been used to estimate genetic diversity among crop germplasm (Kresovich et al. 1992; Farnham 1996), for plant breeding and seed testing programs (Jianhua et al. 1996) and for tagging agronomical traits (Joshi and Nguyen 1993). Halward et al. (1991) used molecular markers in peanut but did not observe significant level of polymorphism. This could have been due to the material and primers used in these experiments, as selection of primer in such studies is very important.

In 13 genotypes, 27 bands of different mass (kilobases) were recorded that were considered specific to that genotype. These specific/cloned PCR-RAPD products can be used as molecular markers for germplasm identification and resource protection of Nigella sativa L. genotypes. Moreover, these products could be used for sequencing that might be successfully converted into SCAR (sequence characterized amplification region) markers. The developed RAPD-DNA fingerprinting and specific genetic markers could provide useful ways for the identification, classification, and resource protection of the Nigella sativa L. genotypes, being one of the important medicinal herbs listed among underutilized species of enormous economic importance. The information could be used to start research for the establishment of medicinal plant genomic databases.

Diverse germplasm is vital for any crop improvement program and we have preserved the evaluated accessions under the same accession numbers in the gene bank at 15 °C with <40% relative humidity for coming years. These are available for research and development work to researchers. The identified accessions of Nigella sativa L. could be used as raw material for herbal, pharmaceutical, nutraceutical, and cosmetic industry including edible oils to enhance farmers’ productivity and income. Collection missions are proposed to be arranged within the areas where genetic diversity has not been yet gathered. Probably there are some variety-specific RAPD/RFLPs in Nigella sativa L. useful for fingerprinting purposes.

References


Exploration of genotype specific fingerprinting of *Nigella sativa* L. using RAPD markers


