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CHARACTERIZATION OF *SOLANUM NIGRUM* L. VARIANTS BACK AND ORANGE FRUITS BY RAPD MARKER

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ABSTRACT

Randaom Amplified Polimorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationship among two variants of *Solanum nigrum* (black and orange fruited). Four primers were used in this study. A total of 48 bands were observed among the variants of *Solanum nigrum* L. In which 8% were polymorphic among the *S. nigrum* variants whilst, 92% of these bands were shared by the two variants. A total of 25 and 23 DNA products were scored for *Solanum nigrum* black and orange fruited respectively. Genetically distinct genotypes identified using RAPD markers could be potential sources of germplasm for *Solanum nigrum* improvement. Thus, RAPD markers combined with morphological analysis proved to be a quick, simple and significant testing methods to assess genetic diversity among *S. nigrum* variants (black and orange fruited).

KEYWORDS: *Solanum nigrum*, black nightshade, Solanaceae, Randaom Amplified Polimorphic DNA, Genetic diversity.

INDRODUCTION

Solanum nigrum L. the common name black nightshade or black berry. It is an annual herbaceous plant belonging to Solanaceae family. It grows up to 30-80 cm in height with an erect and divaricately branched stem, leaves are flat with white flowers appears in numbers from four to ten, the fruits of the plant are green in the first and then they become black or orange based on *variants* (Ogg et al., 1981). All parts of the plants are economically and medicinally important. It is used as an analgesic, antiulcer, antispasmodic, antiseptic, anti-cancerous (Saijo et al., 1998). They are the domestic remedy for liver pains, leukoderma, piles, diarrhoea, ulcer, vomiting, asthma and bronchitis (Krithiker and Basu1975). *Solanum nigrum* contain a number of alkaloids which are economically importance. The alkaloids α solasosonine and α solamargin are obtaine from fruits (Ridout *et al.*, 1989). Strack *et al.*, (2004) screed the genetic diversity of collection of *Solanum nigrum* in the Gaterleben gene bank. Operon primer sets OPD,OPE,OPF,OPAX AND OPAQ were used for the initial RAPD analysis to find out the best storable primers.

Earlier work (Dhasmana et al., 2007) revealed the presence of variation among populations of *Solanum nigrum.* They reported three variants viz. black, orange and yellow fruited. In Tamil Nadu and Pondicherry, we observed black and orange fruited variants. Both variants have been used as green and for treating diseases like ulcer (mouth and stomach) under a vernacular name Manathakkali or Milakuthakkali both as food and medicine one for the other without any prejudice.

The main goal of the present study is to detect RAPD variations in the species *nigrum* variants black and orange fruited in order to determine phylogenetic relationships at the variant level using RAPD analysis. Since Random Amplified Polymorphic DNA (RAPD) marker is described as fast, simple, easy and inexpensive for detecting polymorphisms, based on the amplification of random DNA segments with single primers at arbitrary nucleotide sequence (Williams et al., 1990).

MATERIALS AND METHODS

Plant material

Solanum nigrum L. is a medicinal plant belonging to the family Solanaceae has been selected for the present investigation.

Collection of plant sample

The whole plants of *Solanum nigrum* L. variants black and orange fruited were collected from Thirumalairayan Pattinam 609 606, Karaikal Region, Pondicherry, Union Territory of South India, located at latitude 10° 52′ 4″ N and longitude 79° 49′ 42″ E at 3m MSL during March – April 2018. Both plant samples were collected during same season and same time (evening) Plate-1&2.

Plant Identification

The identity of the plant specimens were confirmed by

using the Floras (Gamble, 1957; Matthew, 1983; Nair and Henry, 1983) and other treatises (Anonymous, 1992, Chatterjee, 1994; Kirtikar and Basu, 1935). Identity of the plant was confirmed with the help of type specimens available in the Herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamilnadu. The Herbarium specimens were prepared following the method of Jain and Rao (1976). The herbarium number in BSI is "BSI/SRC/5/23/2012-13/Tech.1480". The herbarium specimen was deposited at Tamil University Herbarium TUH-300(A) – *Solanum nigrum* L. (black fruited). TUH-300(B) – *Solanum nigrum* L. (orange fruited).

RAPD analysis of *Solanum nigrum* L. variants

Isolation of genomic DNA

The genomic DNA was isolated from plant samples by following the method of Moller *et al*., (1992). The plant powders were ground separately in pestle and mortar using liquid nitrogen. About 50 mg of the powdered plant material were transferred into a micro tube contained 500 µl of TES (100 mM Tris, pH 8.0, 10 mM EDTA, 2% SDS). To which, 50 µg Proteinase K was added and incubated for 1 hour at 60°C with occasional gentle mixing. To the above mixture, 140 µl of 5 M NaCl was added to adjust the salt concentration to 1.4 M. Then 65 µl of 10% CTAB (Cetyl Trimethyl Ammonium Bromide) was added and incubated for 10 min at 65°C. To the above mixture, 700 µl of chloroform and isoamyl alcohol (24:1) was added, mixed gently, incubated for 30 min at 0°C and centrifuged at 10000 rpm for 10 min at 4°C. The supernatant was transferred to a 1.5 ml tube; to which 225 µl of 5 M NH_4Ac was added, mixed gently, incubated on ice for 30 min and centrifuged at 10,000 rpm for 15 min at 4°C. Then the supernatant was transferred to a fresh tube, 510 µl of isopropanol was added to precipitate the DNA and centrifuged immediately for 10 min at 10,000 rpm. The supernatant was removed; the pellet was washed twice with cold 70% ethanol, air-dried and suspended in 50 µl TE buffer.

RAPD primers

In this study, the following RAPD primers (Operon Technologies, CA and USA) were used.

OP AP 07 5′-ACCACCCGCT-3′ OP AP 11 5′-CTGGCTTCTG-3′ OP X 05 5′-CCTTTCCCTC-3′ OP X 12 5′-TCGCCAGCCA-3′.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction was performed in a thermocycler to produce multi copies of a specified DNA using the following reaction mixture. Amplification was carried out with an initial denaturation at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 1.5 min, extension at 72°C for 1.5 min and final extension at 72°C for 5 min using a thermocycler (Eppendorf, Personal cycler, Germany). PCR products were analysed on 1.5% agarose gel for amplicons in 1X TAE buffer at 100 V and photographed using a UV transilluminator (Jayagen, Chennai, India). Amplified fragments of all the primers were scored by visual observations for their presence or absence.

RESULTS

The genomic DNA was successfully isolated from two variants of *Solanum nigrum* L. (Plate -3 -a).A total of 48 bands were observed among the variants of *Solanum nigrum* L. (Plate -3-b). Out of 48 bands, 8% were polymorphic among the *S. nigrum* variants whilst, 92% of these bands were shared by the two variants. A total of 25 and 23 DNA products were scored for *Solanum nigrum* black and orange fruited respectively. Hence based on the RAPD analysis the two variants of Solanum *nigrum* could be considered as one taxon since similarity observed at 92%.

Plate – 1 Habit of *Solanum nigrum* L. variant (Black fruited)

a) Habit.

b) Fruiting twig.

A

B

Plate – 2 Habit of *Solanum nigrum* L. variant (Orange fruited)

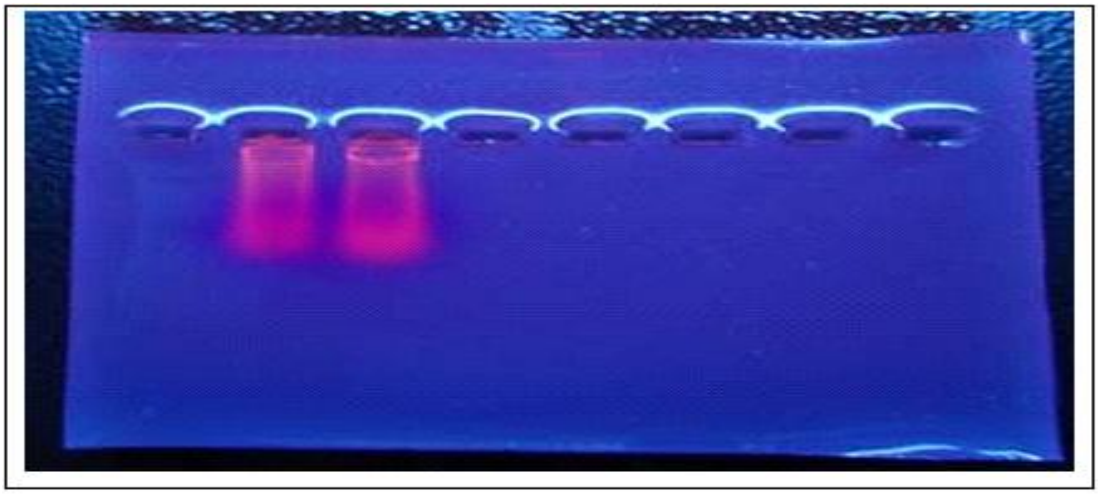
a) Habit.

b) Fruiting twig.

A

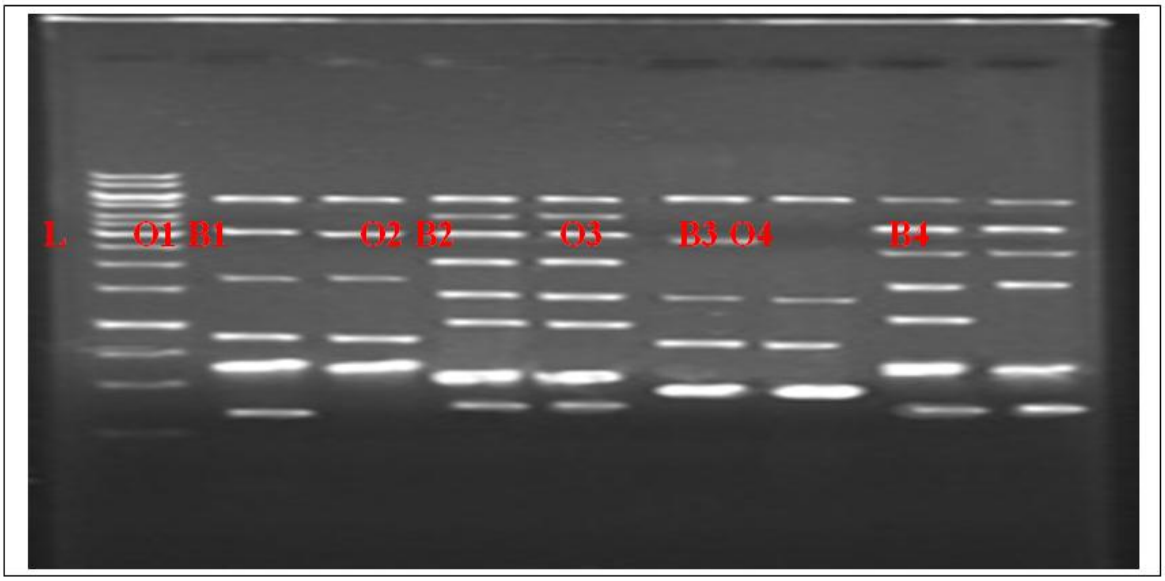
B

Plate-3



a: Genomic DNA of *Solanum nigrum* L. variants

Lane 1. Variant orange fruited
Lane 2. Variant black fruited



A: PCR amplification of *Solanum nigrum* L. variants using RAPD primers

Lane L: 100 bp DNA Ladder; O: orange fruit; B: black fruit; 1: OPAP 07 primer; 2: OPAP 11 primer; 3: OPX 05 primer; 4: OPX 12 primer

DISCUSSION

The present study confirms the existence of genetic diversity within the collection of *Solanum nigrum* variants (black and orange fruited). Assessment of genetic variability within a germplasm is of interest in modern plant research. The use of the Random Amplified Polymorphic DNA (RAPD) technique (Welsh and Mccleand 1990, Williams *et al*. 1990) to investigate genetic diversity between different plant groups has been reported in several studies (Cardeiro *et al*, 2000, Ray Choudhury *et al.*, 2008, Refoufi and Esnult 2008, Yang *et al*,. 2008). The traditional method of identifying species by morphological character is now been accompanied by DNA profiling for the better reliability towords identification and authentication of plant species.

The genus *Solanum* shows diversity in habitat, morphology and ethno medicinal use. The knowledge on phylogenetic relationship and genetic diversity among the species of the genus *Solanum* is rather scanty. In the present study the genetic similarity among genotypes was calculated as being 92% (Plate -3 a&b) in addition emphasizing the effectiveness of RAPD markers in detecting polymorphisms. However the polymorphism obtained by the RAPD markers used to produce the greatest number of bands and showed great potential to discriminate polymorphic DNA segments. The identification of more Polymorphic RAPD markers may

provide a better characterization for genotypes. This correlates with the observations found in other plants like rice, bean (Muthusamy *et al.,* 2008) and Barley (Fernandez *et al.,* 2002, Tikunov *et al.,* 2000. Likewise Kumar *et al.* (2005) reported morphological, cytological and molecular characters of Indian variant tetraploid, diploid and hexaploid. Randomly Amplified Polymorphic DNA (RAPD) marker was identified for the origin of Indian variant hexaploid *S. nigrum* and interrelationship with diploid and tetraploid of the complex. Out of 60 random primers 45 were used to examine 362 bands for all 3 variants. Fifteen did not amplify or showed unclear amplification across all variants. It has been concluded on the basis of RAPD data that Indian variant of tetraploid and diploid were involved in origin of hexaploid Indian variant *S. nigrum*. However tetraploid forms of *S. nigrum* play an important role in hexaploid origin. The present information on genetic diversity in medicinal plant species is important for efficient utilization of plant genetic resources.

CONCLUSION

The results of the present study suggest that the application of molecular fingerprinting (RAPD) provides a rapid and sensitive tool for detecting genetic variations among the different variants of the species *nigrum* variants (black and orange fruited) as well as other genera of the family Solanaceae. Results confirm that DNA analysis by RAPD is an efficient method for the exploration of genetic diversity in *Solanum nigrum.* The study effectively revealed the use of RAPD and morphological markers in analyzing genetic diversity in *S. nigrum*, which could be the step toward efficient germplasm studies among medicinal plants for further exploration of traditional medicinal knowledge and towards diversity conservation. RAPD analysis of two variants of *Solanum nigrum* showed 92 % similarity with 48 bands studied proving the similarity among the two variants at molecular level.

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