Indian J. Genet., 71(1): 87-90 (2011)

Short Communication

Genetic diversity in some south-Indian *Cinnamomum* Scha. species revealed by RAPD markers

A. M. Sandigawad and C. G. Patil*

Department of Botany (Genetics), Karnatak Science College, College Road, Dharwad 580 001

(Received: October 2010; Revised: December 2010; Accepted: January 2011)

The genus *Cinnamomum* Scha. consists of several species which have considerable economic importance. *Cinnamomum* fruit, bark and leaves are used as spice and also as a medicine. It is a valuable source of camphor, cinnamaldehyde and safrol oil. *Cinnamomum* is the largest genus in the family Lauraceae comprising 250 species, which are distributed in India, Sri-Lanka and Australia. In South-India, genus *Cinnamomum* is represented by 12 endemic species and an imported cultivar *C. verum.* Medicinally important *Cinnamon* oil of the world trade comes from three species *viz*., *C. zeylanicum, C.cassia* and *C. camphora.*

The major compounds present in both stem and root bark are Cinnamaldehyde (75%) and Camphor (56%) respectively [1]. Oil extracted from the stem bark possesses the aroma of the spice and a sweet pungent taste. It is employed mainly in food flavoring industry, meat and fast food seasonings, sauces, pickles, baked goods, confectionery, soft drinks, tobacco flavor and dental and pharmaceutical preparations.Since, the genus *Cinnamomum* includes large number of species and varieties, development of molecular markers have proved to be powerful tools in species identification. Molecular markers also proved to be the best in germplasm characterization, varietal identification, phylogenetic study and diversity analysis [2] and for quality assessment in the pharmaceutical industry [3]. Objective of the present investigation was to understand the genetic relationship between eight south-Indian species of the genus *Cinnamomum* using RAPD markers.

Eight *Cinnamomum* species were sampled from their natural distribution in Western Ghats of Karnataka, Tamil Nadu and Kerala. Genomic DNA was extracted from young leaves following the modified CTAB method [4].

A set of 40 random decamer primers (Operon Technologies, Almeda, California, USA) were used to amplify the genomic DNA [5]. PCR reactions were conducted in a 20µl reaction mixture consisting of 100ng geonomic DNA, 5pM/µl RAPD Primer, 10µM dNTPs, 0.5µl *Taq* DNA polymerase (3U/µl) (Bangalore Genei, Bangalore, India), 10 X PCR buffer and 1.5mM $MgCl_2$. DNA amplification was carried out (Corbett Research, Australia). Amplifications were carried out with initial denaturation for 4 minutes at 94^oC and each cycle with 15 seconds at 4^oC, 15 seconds primer annealing at 35^oC, 1.15 minutes for extension at 72^oC. The reaction was continued for 40 cycles followed by 7 minutes at 72^oC to ensure the completeness of the primer extension. The PCR products were analyzed on 1.5 % (w/v) agarose gels in 1X TBE buffer under constant voltage of 100 v for 3 to 4 hours. Experiments were repeated several times to ensure the reproducibility of results and the best gels of the replicates were used for band scoring. The gels were stained with ethidium bromide solution and documented in a gel documentation system.

*Corresponding author's e-mail: patil_cg@yahoo.com; Patil.cg1970@gmail.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

Polymorphic RAPD amplified product was considered to be a unit character and scored as binary data with presence of a band as "1" and absence as "0". Estimates of genetic similarity were calculated between all pairs of the species according to Jaccard's Similarity Index [6]. The matrix obtained was used to evaluate the genetic relationship among the species of *Cinnamomum* with cluster analysis using an Unweighed Pair Group Method with Arithmetic Averages (UPGMA) [7]. All the statistical analysis were performed with an aid of NTSYS-PC computer program version 2.0 [8].

In the present investigation, 111 reproducible and scorable DNA fragments were amplified, 98 % of which were polymorphic and consistently generated from 13 primers across *Cinnamomum* species. Primer OPB-17 yielded highest number of bands (16) while OPA-19 and OPB-16 amplified the lowest number of bands (3) with an average of 8.54 bands per primer (Table 1). The representative RAPD profiles of 8 species of *Cinnamomum* are shown in Fig. 1. The approximate size of the largest fragment was 4.2kb, where as the smallest recognizable fragment was 0.1kb in size.

An interspecific genetic similarity index ranges from 0.17 between *C.zeylanicum* and C.*travancoricum* to 0.81 between *C.macrocarpum* and *C.travancoricum*. Cluster analysis using Jaccard's coefficient to generate a UPGMA dendrogram showing overall genetic relatedness among the *Cinnamonum* species is exhibited in Fig. 2. Two distinct clusters could be identified. First cluster (A) comprised of *C. malabatrum*, *C. sulphuratum, C. macrocarpum, C. travancoricum, C. wightii, C. nicolsonianum*, and *C. walaiwarense* and cultivated *C.zeylanicum* alone constituted the second cluster (B). Certain DNA fragments were found to be species specific with some primers and such bands could be used for species identification solely by developing a SCAR marker.

Table 1. Details of randomly selected decamer oligonucleotides

Primers	Nucleotide Sequence 5' → 3'	No. of polymorphic bands
OPA-4	AATCGGGCTG	10
OPA-9	GGGTAACGCC	11
OPA-17	GACCGCTTGT	9
OPA-19	CAAACGTCGG	3
OPB-07	GGTGACGCAG	11
OPB-08	GTCCACACGC	5
OPB-10	CTGCTGGGAC	12
OPB-11	GTAGACCCGT	10
OPB-13	TTCCCCCGCT	6
OPB-16	TTTGCCCGGA	3
OPB-17	AGGGAACGAG	16
OPB-19	ACCCCCGAAG	9
OPB-20	GGACCCTTAC	4
Total polymorphic bands :		109

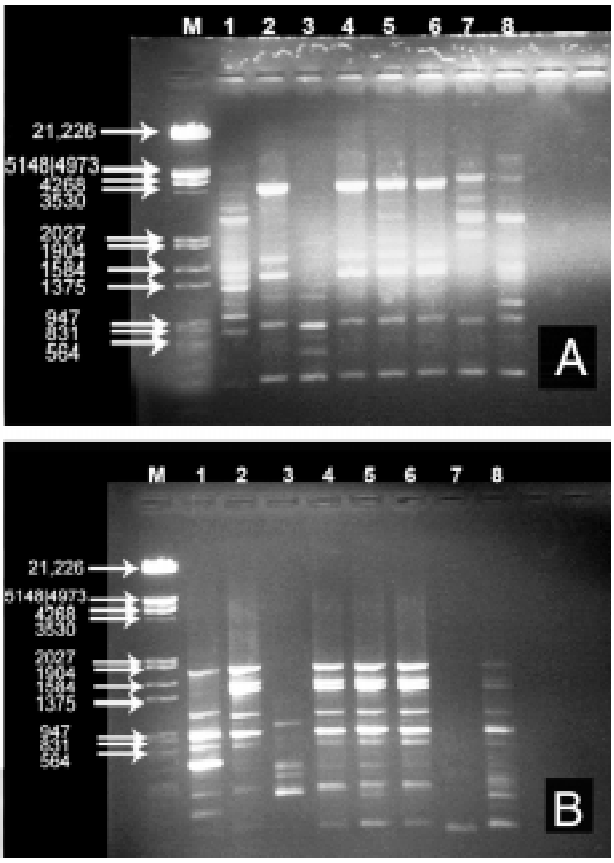

Fig. 1. RAPD profiles of 8 *Cinnamomum* species generated by primer (A) OPA-4 and (B) OPB-10. M-indicates the DNA molecular size marker. Lane numbers corresponds to the species serial number given in Table 1.

RAPD markers represent an efficient and inexpensive tool to generate molecular data and thus have been used successfully in various taxonomic and phylogenetic studies [9]. Genetic diversity analysis is traditionally done based on differences in morphological characteristics which are mostly influenced by environmental factors. Random amplified polymorphic DNA (RAPD) markers have been successfully used for establishing phylogenetic relationships among plant species [5] such as *Cicer* [9, 10], *Fagopyrum* [11], within *Digitalis* [12], *Hordeum* [13] etc.

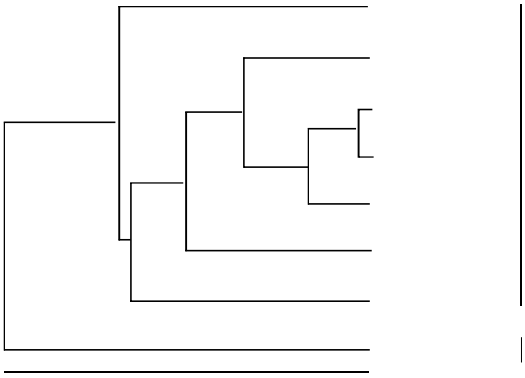

Fig. 2. Dendrogram showing the genetic relationship among 8 species of *Cinnamomum* generated by RAPD data using the UPGMA method

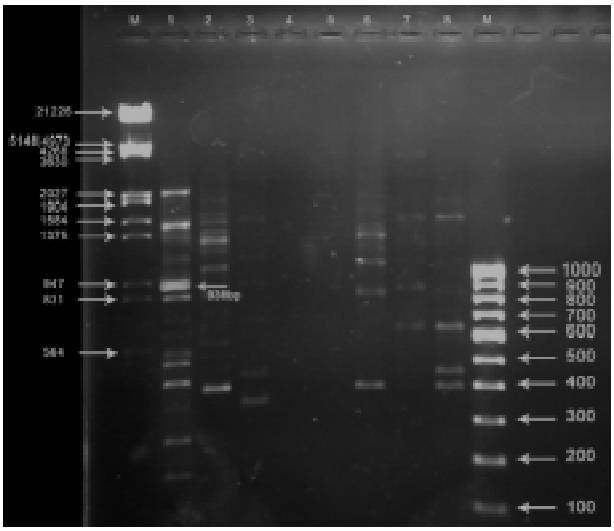

Fig. 3. RAPD Profiles of *Cinnamomum* species Amplified with primer OPB-17 on 1.5% Agarose Gel. Arrow indicates polymorphic band in C.*malabatrum.* M1: mole wt marker (Double digest), M2: mole wt marker (100bp), lane1:C.*malabatrum*, lane 2: C.*Sulphuratum*, lane 3: C.*zeylanicum*, lane 4: C.*macrocarpum,* lane 5: C.*wightii,* lane 6: C.*travancoricum* lane 7: C.*walaiwarense*, lane 8: C. *nicolsonianum*

Species placed within a cluster were more closely related to each other than to species present outside the cluster. UPGMA cluster analysis reveals that *C.macrocarpum* is genetically very closely related to *C.travancoricum* with 0.81 similarity index. Morphologically these two species are distinct and *C.macrocarpum* bears the largest fruits among all the Indian *Cinnamomum* species and the crushed leaves bears the smell of aniseed (safrol) and clove (eugenol). Our results of RAPD DNA finger printing could easily distinguish these two species at molecular level and suggest that both could be considered as distinct species. In flora of Karnataka [14], *C.malabatrum* is treated as a synonym of *C. macrocarpum*. These two species were treated as independent species [15] in Bulletin of the Botanical Society of India. However, the present molecular analysis supports the latter opinion. A unique DNA band of approximately 938bp length could be seen in *C.malabatrum* with OPB-17 primer. Development of a SCAR marker may provide a powerful molecular tool to identify the species, as the taxa has multiple medicinal uses and invariably used to adulterate stem bark and leaves of *C. zylanicum* which serve as major spices. *C. wightii* which forms the sub-cluster is genetically closer to *C.macrocarpum* and *C.travancoricum* with 0.76 and 0.69 similarity index respectively. *C. sulphuratum* and *C. nicolsonianum* forms separate sub-cluster and exhibit fair genetic relatedness with other species in cluster A. *C. sulphuratum* is closer to *C. travancoricum* with 0.65 similarity index and it is placed almost at the same distance from *C.macrocarpum* and *C. wightii* with 0.60 similarity index. *C. nicolsonianum* exhibits, similarity index ranging from 0.54 to 0.25 with *C. macrocarpum* and *C. zeylanicum* respectively. *C. malabatrum* and *C. walaiwarense* forms a sub-cluster in cluster A and are placed at 0.34 similarity index. *C. zeylanicum* which forms a separate cluster B (Fig. 2) and shows a poor genetic relationship with other *Cinnamomum* species in cluster A with maximum similarity of 0.29 with *C. sulphuratum* and minimum similarity of 0.17 with *C. travancoricum*.

As most of the *Cinnamomum* species are used as a source of spice, the presence of a unique RAPD marker among the various *Cinnamomum* species indicate the utility of the approach for DNA fingerprinting purposes. RAPD fingerprinting has a number of potential applications and include the determination of cultivar purity, efficient use and management of genetic resources particularly the establishment of property rights (plant variety protection and patenting). This study represents only the first step in using RAPD markers as a tool to develop species specific DNA markers for molecular analysis of individual *Cinnamomum* species.

Acknowledgements

Authors are indebted to K.R. Prasad, RFO and Dr. C. Haleshi, for their help during the collection of germplasm in Westernghats of Karnataka and University Grants Commission, New Delhi for the financial aid under the Faculty Improvement Programme (FIP).

References

1. **Senanayake U. M., Lee T. H. and Wills R. B. H.** 1978. Volatile constituents of Cinnamon (*Cinnamomum zeylanicum*) oils. J. Agric. Food Chem., **26**: 822-824.
2. **Maughan P. J., Saghai Maroof M. A., Buss G. R. and Huestis G. M.** 1996. Amplified fragment length polymorphism (AFLP) in Soybean: species diversity, inheritance, and near –isogenic line analysis. Theor. Appl. Genet., **93**: 392-401.
3. **Sang-Bok Lee and Soren K. Rasmussen.** 2000. Molecular markers in some medicinal plants of the Apiaceae family. Euphytica, **114**: 87-91.
4. **Doyle J. J. and Doyle J. L.** 1990. Isolation of Plant DNA from fresh tissue. Focus, **12**: 13-15.
5. **Williams G. K., Kubelik A. R., Livak K. J., Rafalsky J. A. and Tingey S. V.** 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucl. Acids. Res., **18**: 6531-6535.
6. **Jaccard P.** 1908. Nouvelles researches sur la distribution florale. Bull. Vaudoise Sci. Nat., **44**: 223-270.
7. **Sneath P. H. A. and Sokal R. R.** 1973. Numerical Taxonomy. WH Freeman, San Francisco.
8. **Rohlf F. J.** 1998. NTSYS-PC, Numerical Taxonomy and Multivariate Analysis System, version 2.0, New York: Applied Biostatistics Inc.
9. **Iruela M., Rubio J., Cubero J. I., Gil J. and Milan T.** 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet., **104**: 643-651.
10. **Ahmad F.** 1999. Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theor. Appl. Genet., **98**: 657-663.
11. **Sharma T. R. and Jana S.** 2002. Species relationships in *Fagopyrum* revealed by PCR-based DNA fingerprinting. Theor. Appl. Genet., **105**: 306-312.
12. **Nebauer S. G., Del Castillo–Agudo and Segura J.** 2000. An assessment of genetic relationships within the genus *Digitalis* based on PCR generated RAPD markers. Theor. Appl. Genet., **100**: 1209-1216.
13. **Bustos A. D., Soler C. and Jouve N.** 1999. Analysis by PCR-based markers to study relationship between species of *Hordeum* (Poaceae). Genome, **42**: 129-138.
14. **Saldhana C. J.** 1984. Flora of Karnataka, Oxford IBH publishing company New Delhi, **1**: 61-63.
15. **Kostermans A. J. G. H.** 1983. The south Indian species of *Cinnamomum* Schaeffer (Lauraceae). Bull. Bot. Surv. India, **25**: 90-133.