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Molecular characterization of traded black pepper (*Piper nigrum* L.) from India, Indonesia, Vietnam and Malaysia

Jaleel Kizhakkayil, Elizabeth Thomas and B Sasikumar*

Division of Crop Improvement and Biotechnology, Indian Institute of Spices Research, Marikunnu P O, Calicut 673 012, India

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Molecular profiling (RAPD) and clustering of traded black pepper samples from India, Indonesia, Vietnam and Malaysia revealed a comparatively high genetic similarity within the samples from a particular country than between any two countries. The UPGMA dendrogram constructed based on the similarity coefficient revealed a total of four groups in two different clusters. The two Indonesian samples form cluster I, while others form cluster II. The genuine Indian varieties and the traded pepper from India formed separate group in cluster II. Similarly, black pepper from Malaysia and Vietnam also formed distinct groups in cluster II. The aspect of genetic similarity was discussed in relation to the origin and spread of black pepper.

Keywords: black pepper, India, Indonesia, Malaysia, molecular technique, pepper, *Piper nigrum*, Vietnam

Introduction

Black pepper (*Piper nigrum* L), known as king of spices, is one of the most important agricultural commodities of commerce. Though, India is the centre of origin and diversity, the present major exporters of black pepper, besides India, are Vietnam, Brazil, Indonesia, Malaysia and Sri Lanka. In the past, India was the major producer and exporter of black pepper but, in recent years, the export from India declined and Vietnam tops in production and export of the commodity[1]. Dried berries of black pepper are traded globally on the basis of the geographical origin of the produce. Two of India's export grades are 'Malabar Garbled' (MG) and 'Tellicheri Garbled Extra Bold' (TGEB)[2]. Other major pepper grades entering into the international market are Lampung (Indonesia), Sarawak (Malaysia), Sri Lanka (Srilanka) and Vietnam (Vietnam). In the present scenario of globalization of trade, protection of reputed commodities, originating from a specific geographical region, not only sustain the trade but also restrict any unscrupulous trade practices. Geographical indication of goods envisaged in the WTO agreement is one of the instruments of protecting the globally traded commodities, such as spices. A reputed commodity eligible for geographical indication protection needs clear characterization based on physical/biochemical/molecular features, besides geographical specification, so as to define it accurately. The present work is an attempt to characterize the traded black pepper from India, Indonesia, Vietnam and Malaysia based on its DNA profiling (RAPD).

Materials and Methods

Plant Material

Export grade, dried berries of Indian (Malabar, TGEB and MG), Indonesian (two samples), Vietnamese (two samples) and Malaysian (two samples) black peppers used in the study were procured through Spices Board, Cochin, India or from other reliable sources. Genuine varieties were collected from the Gene Bank of Indian Institute of Spices Research Farm, Peruvannamuzhi, Calicut, Kerala.

DNA Extraction

DNA was isolated from dried powdered berries of black pepper by using a modified CTAB protocol developed in our laboratory[3]. Purity of the DNA was checked with Biophotometer and the quantity of the DNA was examined electrophoretically on a 1% agarose gel.

PCR Amplification and Electrophoresis

Twenty three random decamer primers (Operon Technologies, Almada, USA) were used for PCR

*Author for correspondence:
Tel & Fax: 91-495-2730294
E-mail: bhaskaransasikumar@yahoo.com

amplification following the procedure of Williams *et al*[4] with a few modifications. Amplifications were performed in 25 µL reaction volume with 35 ng genomic DNA, 0.2 m*M* dNTPs, 10 picomole primer, 2 m*M* $MgCl_2$ and 1U *Taq* DNA polymerase using a PTC-100 Programmable Thermal Controller (MJ Research, Inc, USA). After a pre denaturation step of 3 min at 94°C, amplification reactions were cycled 35 times at 94°C for 1 min, 37°C for 1 min, and 72°C for 1 min. A final amplification was allowed for 10 min at 72°C. The amplified products were loaded and visualized by running in 2% agarose gel containing 0.5 µg mL^{-1} of ethidium bromide and documented by a gel documentation system (Alpha Imager 2220, USA).

Data Analysis

The electrophoretic patterns were visually analysed and DNA bands were scored as '1' for presence and '0' for absence. Statistical analysis was performed using the NTSYS pc statistical package[5] and dendrograms were constructed[6].

Results and Discussion

Out of 60 random decamer primers screened, 23 primers which gave consistent amplification pattern were selected for RAPD analysis (Table 1). Mean number of amplified products ranged from 2.5-8.5 with a size range of 246-2076 bp (Fig. 1). Maximum number of markers were produced by the primer OPB10 (16) and minimum by primers OPA17 and OPB15 (4). Of the total 209 markers produced by the 23 primers, 162 were polymorphic. 100% of polymorphism was observed in case of the primers OPJ13 and OPJ18. Percentage similarity of the different black peppers is given in Table 2.

In the dendrogram constructed based on their similarity coefficient, a total of four groups spread over two clusters are obtained (Fig. 2). The two Indonesian samples with 81% similarity between them formed cluster I. In the cluster II, the genuine varieties like Karimunda, Panniyur-1 and Wynadan constitute the group I. Two Malaysian samples along with two Vietnam samples formed group II of this cluster. The last group in the cluster II is constituted by the three Indian samples namely MG, TGEB and Malabar (group III). Highest similarity (85%) was obtained between Malabar and TGEB, next in order being MG and TGEB (83%) followed by MG and Malabar (82%) in group III. The two Vietnam samples in group II (node 2) shared 82.7% similarity

Table 1—Sequence of RAPD markers, mean number, size of the product and percentage polymorphism in different black peppers

Primer	Sequence(5′-3′)	Mean no. of amplified product	Size range of amplified products (bp)	Total no. of markers	No. of polymorphic markers	% polymorphism
OPA-3	AGTCAGCCAC	6.4	263-714	7	5	71
OPA-17	GACCGCTTGT	3.0	250-700	4	3	75
OPA-18	AGGTGACCGT	8.5	267-1105	12	7	58
OPB-07	GGTGACGCAG	2.5	250-1100	5	4	80
OPB-10	CTGCTGGGAC	8.5	276-1518	16	12	75
OPB-11	GTAGACCCGT	6.5	246-1058	8	5	62
OPB-15	GGAGGGTGTT	2.75	250-800	4	2	50
OPB-17	AGGGAACGAG	4.3	300-1100	7	6	85
OPC-11	AAAGCTGCGG	7.4	281-1341	13	12	92
OPC-14	TGCGTGCTTG	4.5	370-1185	7	5	71
OPC-16	CACACTCCAG	4.75	491-1271	7	5	71
OPC-20	ACTTCGCCAC	4.5	317-1145	7	5	71
OPD-03	GTCGCCGTCA	6.1	292-1493	9	8	88
OPD-05	TGAGCGGACA	6.3	260-1468	11	8	72
OPD-07	TTGGCACGGG	7.75	276-1289	13	11	84
OPD-11	AGCGCCATTG	7.0	262-1588	12	11	91
OPD-20	ACCCGGTCAC	7.6	287-1493	11	8	72
OPE-04	GTGACATGCC	6.3	424-1086	8	4	50
OPE-14	TGCGGCTGAG	5.1	355-1518	9	6	66
OPE-15	ACGCACAACC	5.0	531-2076	9	8	88
OPJ-13	CCACACTACC	3.1	403-1730	9	9	100
OPJ-18	TGGTCGCAGA	7.25	258-1000	13	13	100
OPJ-19	GGACACCACT	5.5	301-869	8	5	62
Total				209	162	

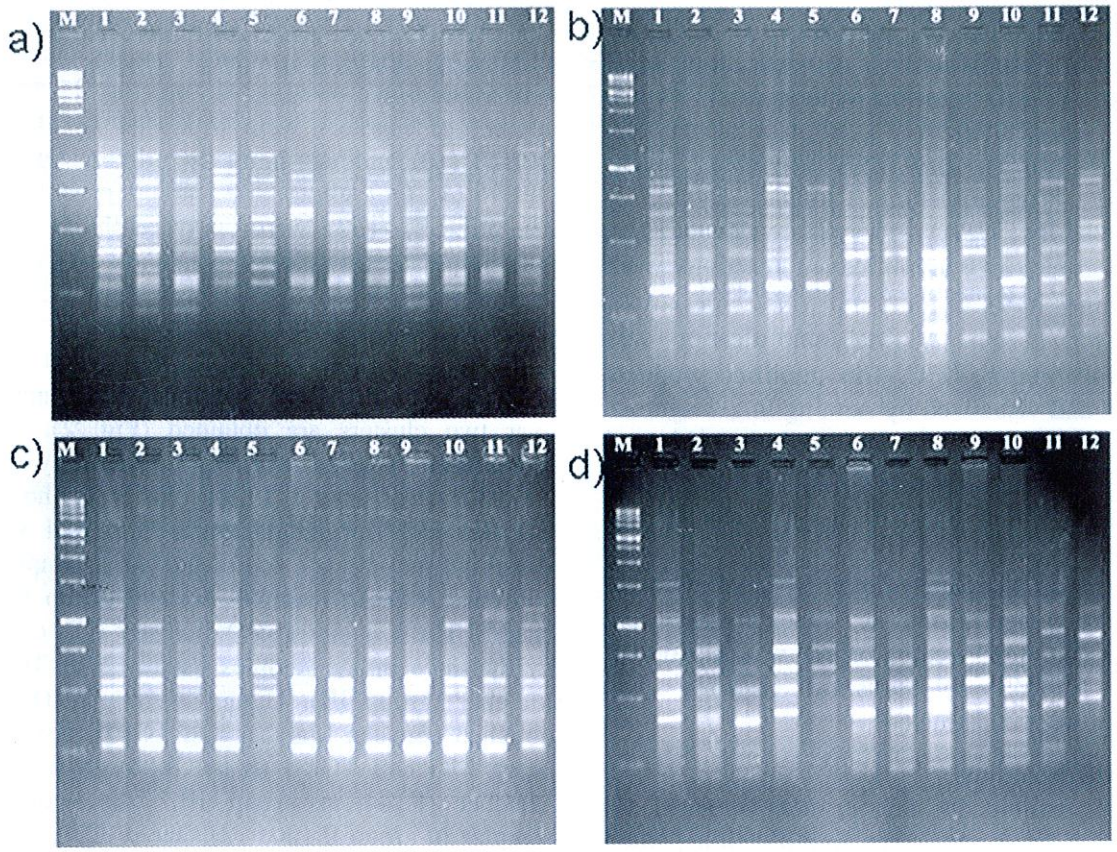

Fig. 1—RAPD profile of DNA isolated from dried berries of black pepper amplified with primers: a. OPA18, b. OPJ18, c. OPD07, and d. OPD11. M, Marker, and Lanes 1-12—Malabar, TGEB, MG, Indonesia-1, Indonesia-2, Vietnam-1, Vietnam-2, Malaysia-1, Malaysia-2, Panniyur-1, Karimunda, Wyanadan black peppers, respectively.

Table 2—Similarity coefficient of traded black pepper from different countries using RAPD markers

	Malabar	TGEB	MG	Indo-nesia-1	Indo-nesia-2	Viet-nam-1	Viet-nam-2	Mala-ysia-1	Mala-ysia-2	Panni-yur-1	Kari-munda	Wyn-adan
Malabar	1.000											
TGEB	0.855	1.000										
MG	0.817	0.832	1.000									
Indonesia-1	0.677	0.682	0.607	1.000								
Indonesia-2	0.630	0.692	0.636	0.813	1.000							
Vietnam-1	0.678	0.692	0.748	0.617	0.692	1.000						
Vietnam-2	0.654	0.696	0.762	0.603	0.650	0.827	1.000					
Malaysia-1	0.724	0.692	0.682	0.673	0.654	0.729	0.724	1.000				
Malaysia-2	0.687	0.673	0.691	0.607	0.598	0.757	0.752	0.757	1.000			
Panniyur-1	0.678	0.701	0.691	0.664	0.636	0.738	0.724	0.682	0.682	1.000		
Karimunda	0.696	0.664	0.692	0.533	0.542	0.738	0.678	0.710	0.692	0.748	1.000	
Wynadan	0.710	0.715	0.706	0.641	0.640	0.678	0.785	0.687	0.640	0.762	0.734	1.000

between them, whereas the two Malaysian samples which formed node 3 of this group had 75.7% similarity between them. Among the three genuine varieties, the highest similarity obtained between 'Wyanadan'-'Panniyur-1' (76.2%) followed by 'Karimunda'-'Panniyur-1' (74.8%) and 'Wyanadan'-'Karimunda' (73.4%).

It was expected that the genuine Indian varieties such as Panniyur-1, Karimunda and Wyanadan have more percentage of similarity with the traded black pepper from India, while the pattern obtained did not exactly support this concept. Although the percentage of similarity between Panniyur-1 with the three Indian grades (Malabar, MG and TGEB) was higher

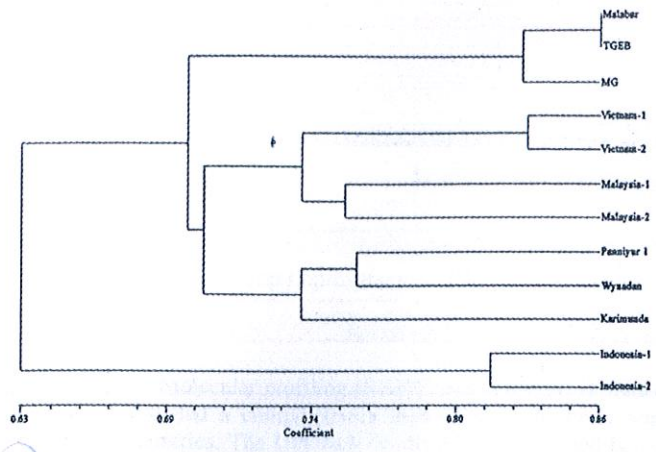

Fig. 2—Dendrogram of the traded samples of black pepper obtained by UPGMA cluster analysis.

compared to the Indonesian and Malaysian produces, Panniyur-1 had maximum percentage of similarity with the Vietnam samples. When the black pepper of genuine Karimunda variety was compared with the produce from other countries, one sample from Vietnam registered a very high percentage of similarity (73.8%) with Karimunda, followed by Malaysian produce. In case of Wyanadan variety also, one sample from Vietnam showed maximum similarity (78.5%) over other produces. However, the similarity between the Indian produces and Wyanadan was more than that of Indonesia and Malayasia.

A comparison of the traded black pepper from different countries indicates that the smilarity between any two samples from two countries was less as compared to samples within the country. The MG from India had least similarity with the Indonesian produces (60-63%). However, MG and Vietnam-2 sample shared a similarity as high as 76.2%. A mixed trend was observed in the similarity of the Indian produces with that of the other countries.

P. nigrum is considered to be originated in India from where it has spread to different countries[7]. Colonists from India are believed to have introduced pepper cultivation to Indonesia about 100 B.C[8]. Black pepper was introduced into Malaysia probably by the European settlers during the early 17th century[9]. Over the years these materials would have evolved into genetically distinct entities. Thus, the present day black pepper cultivated in other countries, such as Vietnam, Malaysia, Indonesia, etc, may be a mixture of the locally adapted Indian genotypes and some improved varieties carrying the original genes from the Indian diversity in their pedigree, and of course the traded produce from these countries must be also a mixture of the berries harvested and marketed from these varieties. This may probably be the reason for the comparative high percentage of similarity observed between the genuine Indian varieties, such as Panniyur, Karimunda and Wyanadan, and traded produces from countries like Vietnam, Malaysia, Indonesia.

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