

Intra-Specific Genetic Relationship Analyses of *Cinnamomum trivancoricum* Based on GC-MS Volatile Oil Markers

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Abstract

Cinnamomum trivancoricum (Lauraceae) is an endemic species to India. The identification and genetic relationship of these species were studied based mainly on volatile oil constituents and their retention time. The color of essential oils was brown and the number of components of oils that were identified from Kodaiyar, Karaiyar, Thenmalai and Kodaikkanal samples were 22, 20, 19 and 18 which accounts 85.6%, 85.4%, 91.26% and 85% of their contents, respectively. Among them, terpinen-4-ol, *trans*- cinnamaldehyde, *a*-terpineol, and β -caryophyllene were the major compounds. A Dendrogram based on Jaccard's similarity coefficients indicated that the distribution pattern of the four accessions was coherent with their geographical origins. Most of the genetic variation (volatile oils Retention time data) occurred among clones within each region. However, the very close relationship between Kodaiyar and Thenmalai populations are statistically significant ($P < 0.001$). This is the first report regarding the inheritance of volatile oils elution time and their application on genetic diversity of wild populations, and it provides useful baseline data for optimizing sampling strategies in breeding. These results are important for future genetic improvement, identification, and conservation of *Cinnamomum trivancoricum* germplasm.

Key Words: *Cinnamomum trivancoricum*; Barks; Volatile oils; Chemical diversity; genetic diversity.

Introduction

Plant genetic resources for food and agriculture are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. The main aim of genetic resource conservation is to conserve as wide a representation as possible of the array of extent genetic variations of target taxa (Ferguson *et al.*, 1998). This is irrespective of the relative frequency of any gene or linked gene complex in germplasm. Satisfying this objective is dependent in part on the efficiency of selection of species and location for the sampling of the genetic diversity. Most species display a complex of genetic variations along their range of distribution (McCall *et al.*, 2004, Miller and Schaal, 2006). For landraces, this is a function of species characteristics, such as breeding system, migration and dispersal mechanisms, which determine

the movement of genes among populations (Erskine, 1997, Herlihy and Eckert, 2004) biotic pressure, for example, competition, predation and local anthropogenic influence and biotic selection intensities determined by location (Ferguson *et al.*, 1998). Genetic conservation strategies are initially concerned with understanding of the genetic variation within species and then by the geographical distribution of genetic variation (Frankel *et al.*, 1995; Ferguson *et al.*, 1998).

Cinnamomum trivancoricum Gamble (Family: Lauraceae) is an endemic plant, which is widely distributed in the higher elevation of Southern Western Ghats, South India and leaves and barks are used as additive in foods to offer aroma and flavor. Molecular markers have been applied to study of genetic diversity from natural populations and formulate efficient sampling strategies to capture maximum variation for genetic resources conservation. Among several characters such as phenolic compounds, flavonoids, glycosides and terpenoids have been used more commonly. Therefore, in the present study performed to GC-MS analysis of volatile oils constituents of four accessions of *C. trivancoricum* barks were collected in South India.

Materials and Methods

Plant material

The plant material (barks) of 4 accessions was collected during the flowering period (May-August, 2001) from the field collection of the Southern Western Ghats, Tamil Nadu, India. Voucher specimens of each field accession were deposited in the Animal Health Research Unit, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Essential oils analysis

One hundred gm of fresh barks were subjected to hydrodistillation for 3hr at 100°C in a Clevenger apparatus for extraction of the essential oil. The essential oil was subsequently cooled, separated from the condensation water and analyzed by the GC-MS. Sample (0.1µl) of oil diluted with pentane (1:10,000,v:v) were analysed on an HP-GCD apparatus equipped with an HP5 (30m x 0.25mm) fused-silica capillary column using helium(1ml/min) as a carrier gas. The injector and detector temperatures were 250 and 280 °C, respectively, and the oven conditions were 70 °C for 2 min, then rising from 70 to 200 °C at a rate of 4 °C/min and subsequently held at 200 °C for 10 min. The mass range was recorded from 45 to 450 m/z, with ionization energy of 70 eV. Major components were identified by co-injection with authentic standards and by with recorded from computerized libraries. Quantification of compounds was based on a comparison of the total ion chromatographic peaks size with those obtained with internal standard. The constituents of the oil were identified by the combination of mass spectral and retention indexes and

they were compared with both those of reference authentic compounds and from library spectra data and literature (Adams, 1995; Jennings and Shibamoto, 1980).

Data analysis

The chromatogram peaks were converted into a “1” and “0” matrix, to indicate the presence or absence of a peak, respectively. Genetic similarities (GS) were estimated for all comparisons of each accessions samples according to Nei (1972) as $GS=2nxy/(nx+ny)$ in which nx and ny are the total numbers of peaks in the chromatograms of the samples x and y , respectively, and nxy is the number of peaks shared by the two samples. To examine the genetic relationships between populations, a dendrogram was constructed by an unweighted paired group method of cluster analysis using arithmetic averages (UPGMA) option of the SPSS (version 11.0) software. After this process, GC-MS chromatograms of all *C. trivancoricum* barks were made and then data with different peak retention times from each accession were compared.

Results and Discussion

A comparison is shown in Table -1 of volatile oil of *C. trivancoricum* bark distilled from the different accessions (Kodaiyar, Karaiyar, Thenmalai and Kodaikkanal). The hydrodistillation of the barks yielded (%) brown oil with an aromatic odour (Table-1). The essential oils from five natural populations of *C. trivancoricum* in South India were analyzed for quality and quantity using GC-MS (Table- 2). Twenty-seven essential oil compounds were obtained, in which twenty five were identified. The identified compounds represented in Table-2. Among them, terpinen-4-ol, *trans*- cinnamaldehyde, *α*-terpineol, and β -caryophyllene were the major compounds. Among the four accessions, the maximum percentages of terpinen-4-ol over the total compounds 25.6%, 23.5%, 24.9%, and 26.1%, respectively (Table-1).

GC/MS analysis resulted in detection and identification of volatile constituents of four accessions of different locality (Table-2). Volatile constituents showed high variability among four accessions of *C. trivancoricum* (Table-3). On the based on retention time of 27 constituents were cluster analysis of four accessions represented in (Fig.1), thus indicating close resemblance in the chemical content of *C. trivancoricum* around the Kodaiyar and Thenmalai. The genetic distances, Jaccard's coefficient of similarity, among *C. trivancoricum* accessions were based on retention time of elution of volatile compounds (Tab-2). Jaccard's similarity coefficients ranged from 0.83 (between an accession from Thenmalai and accessions from the Kodaiyar, and Karaiyar) to 0.76 (between the accession from Karaiyar and accessions from the Kodaikkanal), with an average of 0.65%. The dendrogram produced using distance matrix on average linkage (Fig. 4) shows three groups of germplasm; one group consisted of very closely related germplasm from Kodaiyar and Thenmalai region, except the accession from Karaiyar and Kodaikkanal. However,

the degree of this variability is different with the first time using tools of volatile oils (Retention time). The Mantel test, used to compare essential oil content to the genetic matrices, indicated a low, did not significant relation between the matrices, suggesting that there was no chemotypic differentiation. The genetic differentiation of accessions of *C. trivancoricum* could be broadly explained as a result of abiotic (geographical, e.g., hydrographic connections, or climatic differentiation. e.g., annual rainfall differences) and biotic (pollination between populations and seed dispersal) factors. The percentage of polymorphism i.e., 49.61 was higher in comparison to other endangered plants, e.g. *Lactoris fernandeziana* (Lactoridaceae) (24.5%) (Brauner et al., 1992), *Paeonia suffruticosa* (22.5%) and *Paeonia rockii* (27.6%) (Pei et al., 1995), *Cathaya argyrophylla* (32%) (Wang et al., 1996), and *Dacydium pierrei* (33.3%) (Su et al., 1999). This shows that the species genetic diversity by itself is low, but relatively higher when compared to other endangered species as stated above and it should be able to adapt to the environmental variation. The present results represented the volatile oils content variability of different accessions of different altitude populations of *C. trivancoricum*, which exposed a high variation in the chemical composition and quantity of essential oil. These results concluded that differences in ecological factors (growth season and other environmental variables) and genetic differences among chemotypes most likely affected the essential oil constituents measured in this study.

Conclusion

As a result of this comparative investigation of different accessions of essential oils of *C. trivancoricum* in Southern India, it was found that the composition of these oils differed significantly only in quantity and quality. For the first time, my preliminary genetic chemical diversity study of volatile oils constituents of *Cinnamomum trivancoricum* using GC-MS analysis volatile markers. Further study should be going on primary metabolites using genetic diversity of *Cinnamomum* species of India.

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Fig. a and b. *Cinnamomum trivancoricum* barks and fruits.

Table 1: Comparison of oil yields (oven dry weight basis) for different accessions of *C. trivancoricum*

Accessions No.	Locality	Yield (%)
1	Kodaiyar	0.85
2	Karaiyar	0.81
3	Thenmalai	0.76
4	Kodaikkanal	0.82

Table-2: Volatile oils composition (%) of different accessions of *C. trivancoricum* barks

Peak No.	Active constituents	Composition (%)			
		Kodaiyar	Inzhikuzhi	Kodaikkanal	Thenmalai
1	<i>a</i> -thujene	0	2.6	0	2.5
2	<i>a</i> -pinene	0	1.2	0	0.3
3	linalool	2.5	1.4	3.5	3.5
4	camphene	1.9	1.8	2.9	1.8
5	<i>trans</i> -cinnamyl acetate	1.1	0	0	0
6	<i>a</i> - humulene	1.7	3.5	2.5	0
7	germacrene-D	0	0.5	0	0
8	<i>a</i> -muurolene	1.2	0	3.1	1.4
9	<i>trans</i> - cinnamaldehyde	12.5	13.5	14.36	12.5
10	myrcene	0.5	0.3	3.1	1.2
11	unknown	1.6	0	0	0
12	isomenthone	3.5	3.4	3.5	5.4
13	<i>a</i> -copaene	0	0	0.9	0.7
14	camphor	2.4	2.5	2.3	0
15	<i>g</i> -elemene	1.5	0	0	0
16	bornyl acetate	4.6	1.5	3.8	2.8
17	terpinen-4-ol	25.6	23.5	24.9	26.1
18	β -elemene	0.5	0.9	3.4	3.1
19	dihydrocarvone	0.7	0	0	0.5
20	β -caryophyllene	6.9	6.5	5.6	7.6
21	germacrene B	0.8	0.5	2.5	0
22	unknown	1.6	0	1.3	0
23	β -terpineol	6.9	8.5	6.5	7.5
24	safrole	2.5	2.8	1.5	3.7
25	eugenyl acetate	3.2	5.4	4.1	4.3
26	β -eudesmol	1.9	2.8	0	0
27	myristicine	0	2.3	1.5	0.3
Total		85.6	85.4	91.26	85.2

Table-3: Converted data on elution of active constituent's retention time of essential oils peaks in different accessions of *C. trivancoricum* barks

Peak No	Rt Time	Constituents	Accessions ID			
			Kodaiyar	Inzhikuzhi	Kodaikkanal	Thenmalai
1	0.45	<i>a</i> -thujene	0	1	0	1
2	1.3	<i>a</i> -pinene	0	1	0	1
3	2.3	linalool	1	1	1	1
4	3.5	camphene	1	1	1	1
5	4.1	<i>trans</i> -cinnamyl	1	0	0	0
6	4.9	acetate	1	1	1	0
7	5.2	<i>a</i> - humulene	0	1	0	0

8	7.9	germacrene-D	1	0	1	1
9	8.1	α -muurolene	1	1	1	1
10	9.1	caryophyllene oxide	1	1	1	1
11	9.3	unknown	1	0	0	0
12	11.12	myrcene	1	1	1	1
13	16.4	isomenthone	0	0	1	1
14	19.7	α -copaene	1	1	1	0
15	22.16	γ -elemene	1	0	0	0
16	25.3	camphor	1	1	1	1
17	27.35	bornyl acetate	1	1	1	1
18	28.31	terpinen-4-ol	1	1	1	1
19	31.87	β -elemene	1	0	0	1
20	40.9	dihydrocarvone	1	1	1	1
21	43.1	caryophyllene	1	1	1	0
22	44.5	germacrene B	1	0	1	0
23	44.7	unknown	1	1	1	1
24	45.9	α -terpineol	1	1	1	1
25	47.0	safrole	1	1	1	1
26	51.4	eugenyl acetate	1	1	0	0
27	52.1	β -eudesmol	0	1	1	1
		myristicine				
Total peaks			22	20	19	18

Table- 4: Percentage of similarities between four accessions of *C. travancoricum* basing on volatile oils retention time

Accessions	Kodaiyar	Karaiyar	Thenmalai	Kodaikkanal
Kodaiyar	1.0000			
Karaiyar	0.76	1.0000		
Thenmalai	0.83	0.82	1.0000	
Kodaikkanal	0.65	0.79	0.81	1.0000

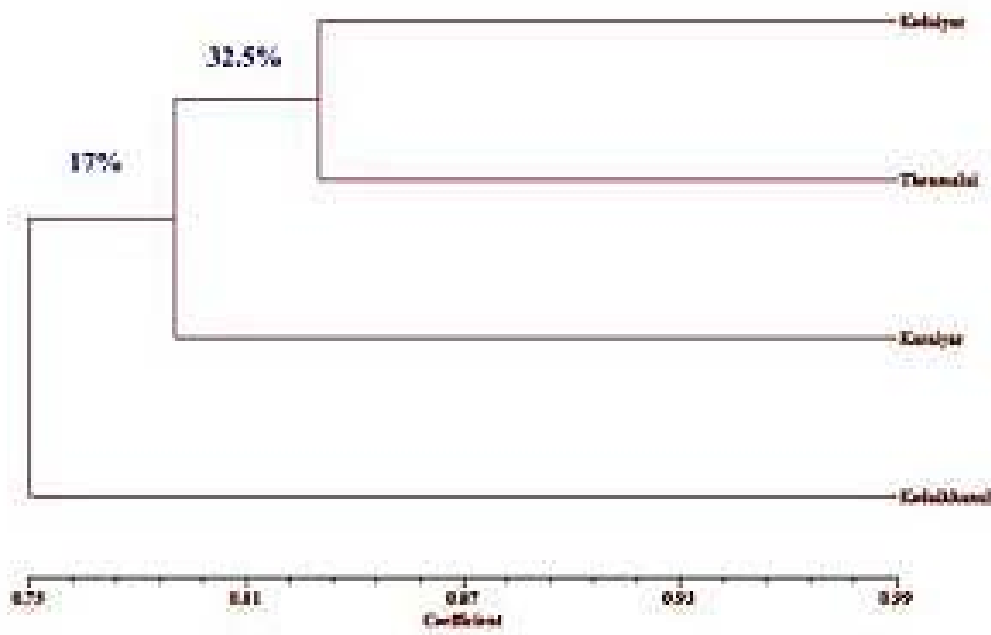


Fig.1: Dendrogram showing intra genetic relationships of *C. travancoricum* based on retention time of volatile oils constituents