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Molecular Variability in 45 Indian Taro Cultivars

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ABSTRACT

Taro (*Colocasia esculenta* L. Schott), belonging to the family Araceae, is a popular tuber crop grown in India. Taro shows high variability in morphological characters with plants being mostly of green or purple type with a range of shades in between. To assess the genetic variability and diversity available in the germplasm available at CTCRI Trivandrum, 45 taro accessions which showed distinct morphology were evaluated for genetic diversity using 11 Random Amplified Polymorphic DNA (RAPD) primers. Only three primers showed bands and they were used to analyse 45 taro accessions. These three primers are considered highly informative because they amplified one or more polymorphic bands that distinguished between accessions. The accessions studied showed high variability with regard to number of DNA bands. The similarity between different accessions was quantified using the software package NTSYS-pc (Numerical Taxonomy Multivariate Analysis System). Similarity between accessions varied from 60 to 100%. A dendrogram grouped the 45 accessions into 4 clusters and one outlier. Accessions collected from same geographical areas tended to cluster together. The data on genetic distance between accessions is useful for planning a hybridization programme while DNA fingerprinting can be used as an additional character to indicate the distinctiveness of a variety.

Keywords: *Colocasia esculenta*, India, molecular variability, RAPD

INTRODUCTION

Taro (*Colocasia esculenta* L. Schott) is an important tuber crop grown in Asia, the Pacific and parts of Africa. As per an FAO estimate, taro is cultivated over an area of 1.80 million ha, producing 6.5 million tons of tubers (FAO 2006). In India taro is cultivated in almost all the states, right from the foot hills of the Himalayas to the southern plains of the peninsula, though official estimates regarding areas and production are not available. It is mostly cultivated as a popular crop in homestead gardens. Collection and conservation of germplasm and studies on genetic variability available in the germplasm are going on. Even though taro is a vegetatively propagated crop, it can also flower and set seed. Unlike other edible aroids, the extent of variability is higher in taro. The variability in Indian taro has been reported by Unnikrishnan *et al.* (1988), Thankamma Pillai and Unnikrishnan (1991), Pillai *et al.* (1999) and Lekhanpaul *et al*. (2003). Lebot and Aradhya (1991) studied the genetic variability in Asian and Pacific cultivars of taro using isozymes and found higher variation in Asian varieties. Irwin *et al.* (1998) analysed molecular variability in taro using RAPD markers and found that Asian cultivars were genetically distant from those from the Pacific. Kreike *et al.* (2004) studied the genetic diversity of taro from Asia and the Pacific using AFLP markers and found that the division of accessions into an Asian and a Pacific gene pool was obvious. The present study is an attempt to analyse the variability available in a subset of Indian taro using RAPD markers.

MATERIALS AND METHODS

Plant material

Forty five morphologically distinct taro accessions from the *ex vitro* collection of Indian germplasm available at Central Tuber Crops Research Institute, Trivandrum (India) were used in this study. Detailed morphological characters are given in **Table 1**. A photograph of three accessions showing variation in stem color is shown in **Fig. 1**. Young, unfurled leaves were used for extracting DNA.

Fig. 1 Taro (*Colocasia esculenta* L. Schott).

DNA extraction

DNA was extracted from 1.5-2 g of tissue using a modified CTAB (Banglore Genei) protocol (Murray and Thompson 1980; Bernatsky and Tanksley 1986). Extracted DNA was resuspended in TE (10 mM Tris, 1mM EDTA) buffer and the concentration estimated by electrophoresis on a 1% agarose gel with 1 Kb ladder (Bangalore Genei), and adjusted with TE buffer to 5 ng/ml before PCR amplification.

PCR parameters and gel analysis

PCR amplification was performed according to Williams *et al.* (1990) using primers synthesized by Integrated DNA Technologies, Inc. (USA). The reaction mixture (25 µl) consisted of 10X buffer (Bangalore Genei), 100 mM each of dATP, dTTP, dCTP, and dGTP, 600 mM $MgCl_2$, 600 pM of each primer, 0.5 U *Taq* polymerase

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Original Research Paper

Table 1 List of 45 taro germplasm accessions used in the present study and their distinctive morphological characteristics.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Leaf margin color*	G	P	G	PG	G	G	P	G	G	P	P	P	G	P	G
Leaf color upper*	GG	G	G	G	G	G	GG	GG	GG	DG	GG	G	G	GG	G
Leaf color lower*	LG	LG	LG	LG	LG	G	LG	LG	LG	G	G	G	LG	LG	LG
Sinus color*	G	G	LG	G	G	G	P	G	G	P	P	G	G	G	G
Petiole color middle*	P	G	LG	G	G	G	G	G	G	PG	P	G	G	G	PG
Petiole color base*	G	G	G	G	G	G	G	G	G	PG	P	G	G	G	G
Corm shape**	Co	Cys	Co	Cys	Cys	Rs	Cs	Rs	Cs	Cs	Cs	Cys	Rs	Cs	Rs
Cormel shape**	Cs	Co	Cys	Cys Cs	Rs	Cs	Cys	Cys	Cys	Cs	Cs	Cys	Cys	Cys	Cs
Leaf length: breadth	1.3:1	1.2:1	1.3:1	1..3:1	1.9:1	1.3:1	1.3:1	1.3:1	1.4:1	1.3:1	1.4:1	1.4:1	1.4:1	1.3:1	1.3:1
Plant size***	T	T	M	M	M	M	D	M	D	M	M	M	D	M	M

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Leaf margin color*	P	PG	PG	G	PG	P	PG	P	G	G	P	P	G	P	G
Leaf color upper*	BG	LG	BG	GG	BG	G	G	BG	GG	G	G	G	DG	G	GG
Leaf color lower*	G	LG	BG	LG	LG	LG	LG	G	LG	LG	LG	LG	LG	LG	LG
Sinus color*	G	G	G	G	P	G	P	G	G	G	G	G	P	G	G
Petiole color middle*	P	G	P	G	P	PG	G	G	G	G	G	G	G	PG	G
Petiole color base*	P	G	P	G	P	PG	G	G	G	G	G	G	G	PG	G
Corm shape**	Cys	Cys	Cs	Cs	Rs	Cs	Cs	Cys	Rs	Rs	Cys	Cys	Rs	Rs	Cs
Cormel shape**	Cs	Cys	Cys	Cys	Cys	Cs	Cs	Cs	Cys	Cs	Rs	Cys	Cys	Cs	Cs
Leaf length: breadth	1.2:1	1.2:1	1.3:1	1.4:1	1.2:1	1.4:1	1.1:1	1.4:1	1.4:1	1.4:1	1.4:1	1.2:1	1.4:1	1.1:1	1.4:1
Plant size***	D	M	M	M	M	D	T	M	T	M	M	M	D	T	M

	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Leaf margin color*	P	G	G	G	P	P	G	P	G	P	G	P	P	G	P
Leaf color upper*	G	G	GG	GG	GG	G	G	G	GG	G	G	LG	GG	G	GG
Leaf color lower*	G	LG	LG	LG	G	LG	LG	G	LG	LG	LG	LG	LG	LG	LG
Sinus color*	G	G	G	G	P	G	G	G	P	G	G	P	P	G	G
Petiole color middle*	LG	G	G	P	G	G	G	PG	G	G	P	P	G	P	P
Petiole color base*	LG	G	G	P	G	G	G	G	G	G	G	P	P	G	P
Corm shape**	Rs	Cys	Rs	Cs	Cs	Rs	Rs	Cys	Cs	Cys	Cys	Rs	Cs	Cs	Cs
Cormel shape**	Cs	Cs	Cys	Cs	Cys	Cys	CS	Cys	Cs	Cys	Cys	Cys	Cs	Cys	Cs
Leaf length: breadth	1.4:1	1.4:1	1.3:1	1.6:1	1.6:1	1.5:1	1.3:1	1.4:1	1.4:1	1.4:1	1.5:1	1.5:1	1.5:1	1.6:1	1.4:1
Plant size***	T	M	T	M	M	M	T	M	D	D	D	D	D	M	M

* G = green; P = purple; PG = green with purple streaks; GG = glaucous green; LG = light green; BG = bluish green
** Co = conical; Cys = cylindrical; Rs = round shape; Cs = club shape
*** D = dwarf, M = medium, T = tall

(Finnzymes, Finland) and 25 ng DNA. PCR was carried out in a thermal cycler (MJ Research PTC-100 (USA), under the following condition: an initial denaturation at 94°C for 4 min followed by 40 cycles of 94°C for 1 min each, 35°C for 1 min and 72°C for 2 min and a final extension at 72°C for 5 min. Approximately 10 µl of the amplified RAPD products were loaded onto a 2% agarose gel and separated by electrophoresis in TBE buffer at 80 V. Gels were stained with ethidium bromide (10 mg/ml) and products were visualized by UV light (Syngene). A 1 Kb ladder was included in all gels as a molecular weight standard.

Scoring gels and data analyses

Each band was scored as present (1) or absent (0) and cluster analysis of the RAPD data was performed with the assistance of the SIMQUAL programme of NTSYS software, version 2.10 (Applied Biostatistics Inc., Setauket, NY, USA). Similarity matrices were generated using DICE and simple matching coefficients. An Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster analysis was produced from similarity matrices constructed for RAPD data and resulting dendrogram was compared.

RESULTS AND DISCUSSION

Eleven RAPD primers were used in DNA amplification. Only three primers gave scorable bands. The number of scorable bands per primer ranged from 1 (**Fig. 2A**, Lane 4) to 15 (**Fig. 2B**, Lane 9). The band size ranged from 1000 to 6000 bp. A total of 472 strongly staining DNA bands were scored, of which 42 were polymorphic (**Table 2**). The largest number of polymorphic bands (12) was obtained from primer B11, and the lowest number of bands (9) was obtained from primer AN2. A dendrogram was generated from the UPGMA cluster analysis of the RAPD data (**Fig. 3**). In the dendrogram 45 accessions formed 4 clusters and one outlier (**Table 3**). Cluster I consist of 13 accessions. These accessions are collected from different parts of India. It consists of accessions with different type of corm shape as well as cormel shape. Cluster II consists of 24 accessions. They are collected from Northern part of India comprising the states of Orissa, Bihar and Madhyapradesh. Cluster III consists of 3 accessions. They are collected from the state of Kerala. Cormel shape is cylindrical in all the 3 accessions. Cluster IV also consists of 3 accessions. They are also collected from Kerala. However, their cormels are club shaped. The length versus breadth ratio is 1.4:1 in all the three accessions. The accession C2, which doesnot come under any cluster is characterized by cormels of conical shape. It is a high yielding accession with good cooking quality. It is also a triploid accession. However, other triploid accessions like

Table 2 Nucleotide sequences of 3 selected primers and the number of polymorphic bands detected by RAPD analysis.

Primer	Sequence (5′ to 3′)	№ of bands polymorphic
B11	GTAGACCCGT	12
AN2	CACCGCAGTT	9
AM3	CTTCCCTGTG	21

Table 3 List of varieties into different clusters.

Cluster №	№ of varieties
1	13 (C- 1,15,3,4,7,8,9,10,12,14,11,13,6)
2	24 (C-16,33,37,34,43,44,45,35,42,40,17,29, 18,22,30,21,23,24,19,20,25,26,28,27)
3	4 (C-5,41,36,38)
4	3 (C-31,32,39)
Outlier	1 (C-2)

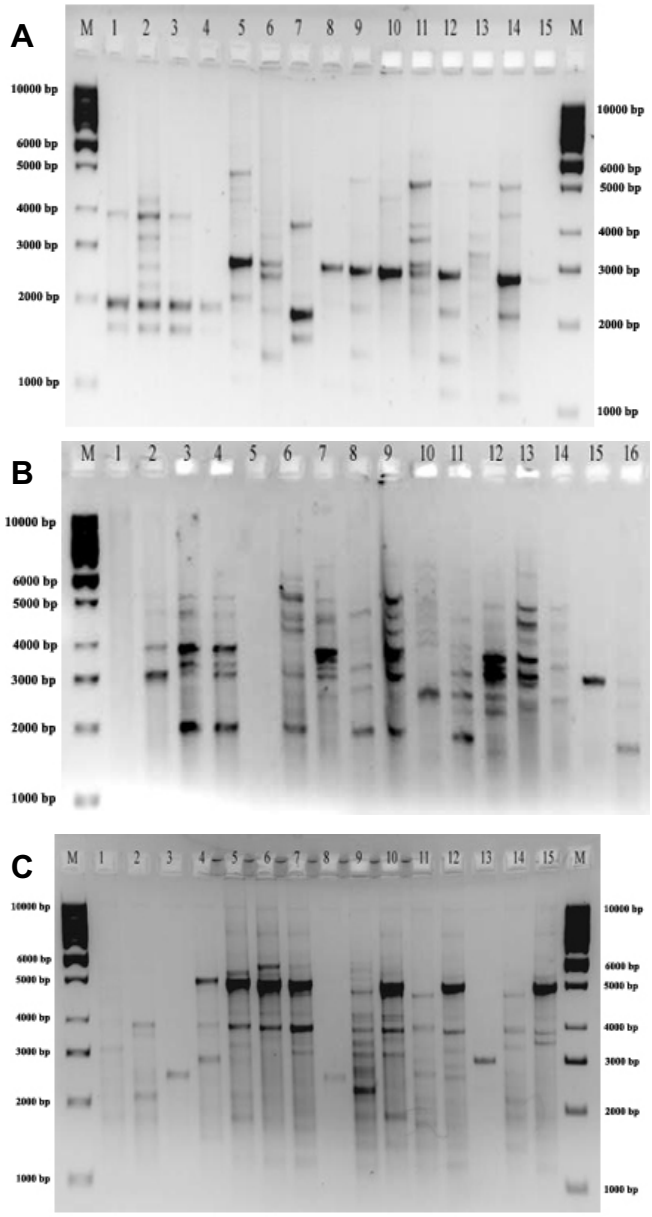

Fig. 2 (**A**) Representative gel showing RAPD pattern produced from DNA amplification of 15 accessions using the primer B11; Lane M showing 1Kb molecular weight marker; Lane 1-15 showing RAPD pattern of *Colocasia* accessions. (**B**) Representative gel showing RAPD pattern produced from DNA amplification of 16 accessions using the primer AM3; Lane M showing 1 Kb molecular weight marker; Lane 1-16 showing RAPD pattern of *Colocasia* accessions. (**C**) Representative gel showing RAPD pattern produced from DNA amplification of 16 accessions using the primer AN2; Lane M showing 1Kb molecular weight marker; Lane 1-16 showing RAPD pattern of *Colocasia* accessions.

C1, C2, C4, C8, C17, C18, C22, C23, C26, C27, C29, C32, C33, C34, C42 and C43 fell into different clusters. As such these primers could not distinguish between diploid and triploid accessions. The similarity index between pairs of accessions varied from 60 to 100% (**Table 4A, 4B**). Accessions numbering 26 and 28 showed 100% similarity. But morphologically they are not very similar. Using more number of RAPD primers or more powerful markers like SSR or AFLP, it may be possible to distinguish the morphological variants. However, Lakhanpaul *et al*. (2003), after analyzing a set of Indian taro using 13 RAPD primers, reported that morphological similarity among the morphotypes was not reflected in terms of similarity of RAPD patterns, although some accessions belonging to the same phenotype grouped together. Garcia (2000) also reported variation between morphological data and DNA banding pattern after analyzing 40 accessions from Vanuatu (Pacific Island) for AFLP markers. Our study could quantify the genetic similarity between 45 taro accessions collected from within India. This study could also distinguish a variety C2, which is significantly superior to others. Crosses between genetically distant parents are expected to yield wide variability in the hybrid progeny. As such, this information can be utilized for recombination breeding. In general, high variability was noticed in the accessions studied. The North Eastern region of India is believed to be one of taro's centers of origin. Some of the accessions in the study are collected from this region (Pillai *et al.* 2000). Kuruvilla and Singh (1984) studied protein variability in local varieties from the North Eastern Hill Region and confirmed the possibility of this region being the centre of origin of taro. Lebot and Arodhya (1991) used isozymes to differentiate a collection from Asia and Oceanic and found greater variation in Asia than Oceania. Pillai *et al.* (1995) conducted ANOVA for 10 traits and found significant variation in 11 yield contributing characters among a set of 22 genotypes. Irwin *et al.* (1998) used RAPD analysis in taro and found high genetic diversity in an Indonesian collection. They could distinguish diploid and triploid varieties based on RAPD markers and also a Hawaii collection from others. However, Kreike *et al*. (2004) could not discriminate between diploid and triploid accessions with AFLP markers. Ochiai *et al.* (2001) reported the use of isozyme and RAPD analysis in Japanese collections and found that RAPD analysis was highly capable of evaluating genetic variability in Asian taros. Noyer *et al.* (2002) identified SSR markers for analysis of molecular variability in taro collected from different countries. Wide diversity was noticed in the 105 accessions of taro analyzed. Kreike *et al.* (2003) used AFLP markers to study the diversity of a core sample of taro germplasm collected from seven countries like Vietnam, Thailand, Indonesia, Malaysia, The Philippines, Papua New Guinea and Vanuatu. Most accessions could be clearly differentiated by using three primer pairs and a few duplicates were found. Shen *et al*. (2003) analysed 28 accessions of taro collected from China using isozyme, RAPD and AFLP markers and found significant genetic diversity among the group. The high variability of taro found in this Indian collection supports the hypothesis that India may be one of the centers of origin of taro.

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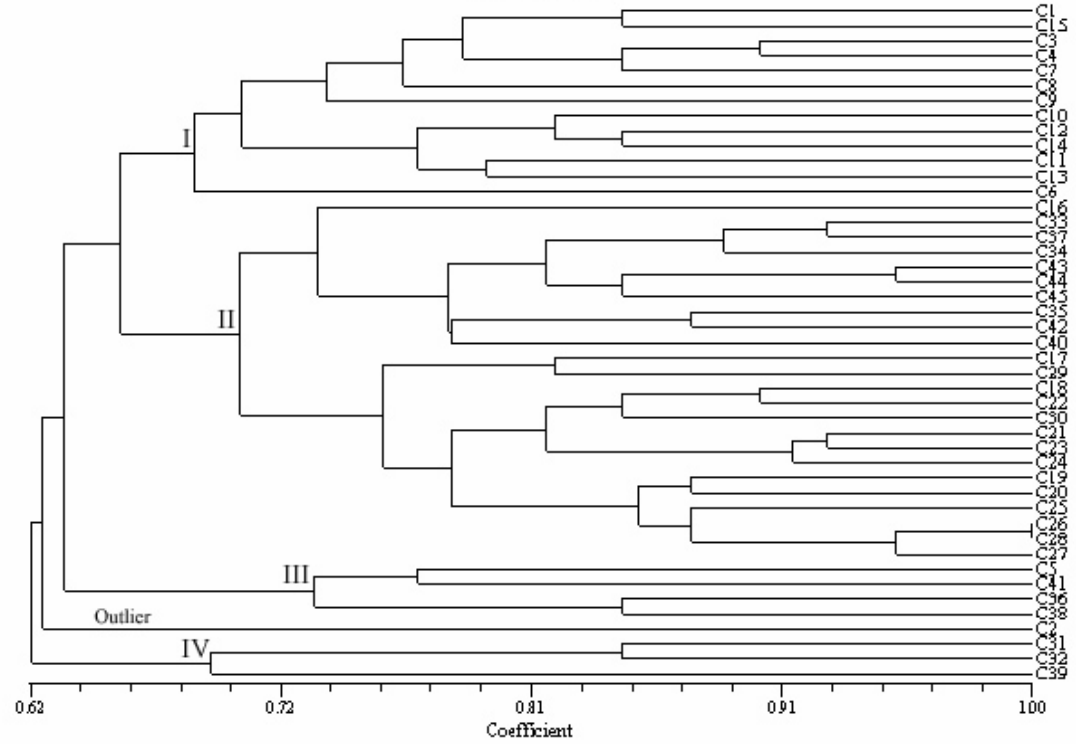

Fig. 3 Unweighed Pair Group Method with Arithmatic average (UPGMA) dendrogram of 45 accessions of *Colocasia* based on the RAPD data. The dendrogram was constructed from the matrix of Dices similarity coefficients.

Table 4a Genetic similarity matrix of 45 taro genotypes based on RAPD markers.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22
C1	1.0																					
C2	0.90	1.0																				
C3	0.87	0.90	1.0																			
C4	0.79	0.82	0.85	1.0																		
C5	0.82	0.79	0.82	0.84	1.0																	
C6	0.87	0.87	0.84	0.82	0.82	1.0																
C7	0.76	0.69	0.73	0.71	0.77	0.79	1.0															
C8	0.74	0.74	0.71	0.79	0.76	0.80	0.73	1.0														
C9	0.79	0.82	0.79	0.84	0.84	0.85	0.74	0.76	1.0													
C10	0.88	0.79	0.76	0.80	0.80	0.85	0.80	0.76	0.84	1.0												
C11	0.82	0.79	0.73	0.74	0.77	0.79	0.74	0.69	0.80	0.90	1.0											
C12	0.77	0.77	0.68	0.63	0.66	0.71	0.73	0.61	0.66	0.79	0.85	1.0										
C13	0.61	0.71	0.68	0.63	0.63	0.65	0.69	0.61	0.60	0.60	0.63	0.71	1.0									
C14	0.60	0.66	0.66	0.65	0.71	0.69	0.74	0.69	0.71	0.61	0.61	0.66	0.76	1.0								
C15	0.65	0.65	0.65	0.63	0.69	0.71	0.76	0.68	0.69	0.69	0.63	0.65	0.74	0.76	1.0							
C16	0.68	0.61	0.61	0.66	0.69	0.68	0.79	0.65	0.66	0.76	0.76	0.74	0.71	0.63	0.71	1.0						
C17	0..76	0.79	0.79	0.74	0.71	0.73	0.68	0.69	0.74	0.74	0.74	0.73	0.79	0.71	0.73	0.73	1.0					
C18	0.85	0.82	0.85	0.74	0.74	0.79	0.74	0.63	0.74	0.80	0.80	0.76	0.66	0.58	0.63	0.73	0.77	1.0				
C19	0.82	0.82	0.85	0.77	0.74	0.82	0.74	0.66	0.77	0.84	0.80	0.73	0.66	0.65	0.69	0.73	0.77	0.90	1.0			
C20	0.84	0.80	0.84	0.73	0.76	0.80	0.69	0.65	0.76	0.79	0.76	0.68	0.65	0.69	0.71	0.65	0.79	0.82	0.92	1.0		
C21	0.74	0.77	0.77	0.76	0.76	0.77	0.69	0.71	0.82	0.79	0.76	0.68	0.68	0.73	0.68	0.68	0.79	0.73	0.82	0.80	1.0	
C22	0.82	0.79	0.76	0.77	0.80	0.79	0.74	0.73	0.80	0.87	0.84	0.76	0.66	0.71	0.63	0.73	0.84	0.77	0.84	0.85	0.88	1.0

Table 4b Genetic similarity matrix of 45 taro genotypes based on RAPD markers.

	C45	C44	C43	C42	C41	C40	C39	C38	C37	C36	C35	C34	C33	C32	C31	C30	C29	C28	C27	C26	C25	C24	C23
C1	0.84	0.79	0.82	0.77	0.80	0.80	0.79	0.79	0.76	0.79	0.76	0.77	0.80	0.79	0.84	0.85	0.79	0.76	0.77	0.84	0.84	0.79	0.77
C2	0.87	0.82	0.79	0.80	0.77	0.80	0.79	0.73	0.76	0.79	0.82	0.77	0.80	0.85	0.80	0.85	0.79	0.76	0.74	0.87	0.87	0.73	0.84
C3	0.80	0.79	0.79	0.84	0.77	0.77	0.76	0.69	0.73	0.82	0.76	0.77	0.77	0.79	0.77	0.82	0.76	0.79	0.74	0.90	0.90	0.69	0.77
C4	0.76	0.77	0.77	0.76	0.76	0.73	0.74	0.74	0.71	0.68	0.74	0.79	0.76	0.80	0.79	0.80	0.74	0.77	0.79	0.79	0.79	0.68	0.73
C5	0.76	0.77	0.80	0.79	0.76	0.79	0.84	0.80	0.74	0.68	0.71	0.76	0.82	0.80	0.85	0.87	0.80	0.74	0.74	0.79	0.79	0.74	0.76
C6	0.80	0.79	0.79	0.80	0.77	0.80	0.79	0.79	0.79	0.76	0.79	0.77	0.74	0.79	0.77	0.92	0.85	0.73	0.69	0.84	0.84	0.82	0.87
C7	0.66	0.74	0.80	0.76	0.76	0.72	0.71	0.72	0.68	0.68	0.68	0.73	0.73	0.68	0.73	0.74	0.74	0.71	0.65	0.76	0.76	0.71	0.73
C8	0.68	0.76	0.73	0.77	0.71	0.71	0.79	0.73	0.69	0.63	0.66	0.74	0.68	0.76	0.74	0.79	0.76	0.73	0.82	0.74	0.74	0.69	0.77
C9	0.79	0.84	0.84	0.88	0.79	0.76	0.77	0.74	0.77	0.74	0.80	0.82	0.82	0.87	0.85	0.87	0.80	0.74	0.76	0.85	0.85	0.74	0.76
C10	0.76	0.80	0.84	0.76	0.79	0.76	0.77	0.74	0.80	0.77	0.71	0.79	0.79	0.77	0.82	0.80	0.77	0.74	0.79	0.79	0.77	0.80	0.76
C11	0.79	0.80	0.84	0.79	0.82	0.76	0.74	0.84	0.80	0.80	0.74	0.79	0.82	0.77	0.82	0.77	0.76	0.73	0.68	0.79	0.77	0.77	0.76
C12	0.77	0.80	0.76	0.71	0.77	0.74	0.66	0.80	0.79	0.76	0.76	0.71	0.77	0.73	0.74	0.66	0.69	0.69	0.58	0.71	0.66	0.66	0.74
C13	0.68	0.76	0.66	0.68	0.65	0.77	0.73	0.73	0.73	0.73	0.66	0.65	0.71	0.69	0.65	0.66	0.66	0.68	0.69	0.71	0.66	0.63	0.71
C14	0.63	0.69	0.68	0.76	0.69	0.66	0.68	0.69	0.65	0.61	0.68	0.66	0.69	0.71	0.69	0.71	0.68	0.66	0.58	0.73	0.71	0.58	0.69
C15	0.61	0.74	0.60	0.71	0.58	0.68	0.73	0.61	0.63	0.60	0.63	0.58	0.65	0.66	0.68	0.73	0.60	0.73	0.65	0.68	0.69	0.63	0.71
C16	0.68	0.63	0.76	0.65	0.71	0.77	0.73	0.66	0.79	0.73	0.63	0.71	0.77	0.69	0.75	0.69	0.69	0.84	0.69	0.65	0.66	0.76	0.68
C17	0.76	0.73	0.77	0.76	0.72	0.82	0.80	0.82	0.80	0.80	0.71	0.76	0.79	0.80	0.79	0.74	0.71	0.80	0.73	0.82	0.77	0.71	0.68
C18	0.79	0.77	0.80	0.79	0.79	0.79	0.77	0.77	0.77	0.87	0.71	0.76	0.79	0.74	0.76	0.74	0.71	0.77	0.73	0.85	0.80	0.74	0.76
C19	0.79	0.77	0.77	0.79	0.79	0.79	0.77	0.77	0.77	0.84	0.71	0.76	0.76	0.74	0.76	0.77	0.74	0.76	0.77	0.82	0.84	0.74	0.73
C20	0.77	0.77	0.76	0.80	0.77	0.77	0.76	0.77	0.73	0.79	0.73	0.74	0.74	0.73	0.77	0.82	0.73	0..76	0.84	0.80	0.85	0.73	0.79
C21	0.74	0.76	0.82	0.80	0.80	0.77	0.79	0.76	0.79	0.79	0.69	0.87	0.80	0.82	0.77	0.76	0.82	0.77	0.82	0.84	0.83	0.79	0.77
C22	0.79	0.82	0.82	0.76	0.85	0.82	0.80	0.76	0.84	0.80	0.71	0.82	0.82	0.80	0.85	0.77	0.80	0.69	0.71	0.79	0.80	0.80	0.80
C23	0.80	0.84	0.79	0.74	0.74	0.84	0.82	0.84	0.82	0.79	0.79	0.74	0.71	0.79	0.77	0.82	0.82	0.68	0.79	0.77	0.76	0.79	1.0
C24	0.73	0.79	0.77	0.69	0.76	0.82	0.77	0.79	0.77	0.77	0.65	0.79	0.73	0.71	0.76	0.80	0.84	0.77	0.82	0.73	0.77	1.0	
C25	0.76	0.74	0.80	0.88	0.79	0.79	0.84	0.84	0.74	0.84	0.74	0.79	0.79	0.74	0.79	0.80	0.74	0.82	0.80	0.88	1.0		
C26	0.80	0.84	0.85	0.93	0.84	0.77	0.82	0.74	0.76	0.85	0.79	0.84	0.84	0.85	0.77	0.82	0.76	0.76	0.80	1.0			
C27	0.80	0.85	0.85	0.84	0.84	0.71	0.69	0.73	0.76	0.79	0.76	0.87	0.84	0.76	0.84	0.79	0.82	0.76	1.0				
C28	0.82	0.82	0.87	0.82	0.85	0.79	0.74	0.73	0.77	0.77	0.74	0.88	0.88	0.80	0.85	0.77	0.77	1.0					
C29	0.82	0.87	0.84	0.76	0.79	0.85	0.77	0.80	0.80	0.74	0.74	0.88	0.79	0.77	0.82	0.84	1.0						
C30	0.82	0.84	0.80	0.85	0.79	0.82	0.80	0.80	0.77	0.74	0.80	0.79	0.79	0.80	0.85	1.0							
C31	0.84	0.80	0.88	0.80	0.84	0.80	0.79	0.84	0.83	0.76	0.79	0.84	0.90	0.82	1.0								
C32	0.85	0.85	0.84	0.82	0.79	0.79	0.80	0.82	0.77	0.74	0.77	0.82	0.85	1.0									
C33	0.84	0.87	0.92	0.84	0.87	0.80	0.79	0.77	0.82	0.79	0.76	0.87	1.0										
C34	0.84	0.88	0.92	0.84	0.90	0.80	0.76	0.82	0.79	0.79	0.76	1.0											
C35	0.85	0.92	0.80	0.85	0.79	0.76	0.82	0.82	0.80	0.77	1.0												
C36	0.82	0.80	0.84	0.82	0.82	0.82	0.74	0.68	0.90	1.0													
C37	0.74	0.80	0.80	0.84	0.76	0.80	0.85	0.80	1.0														
C38	0.80	0.82	0.80	0.84	0.73	0.84	0.88	1.0															
C39	0.71	0.79	0.80	0.77	0.76	0.84	1.0																
C40	0.76	0.76	0.82	0.82	0.77	1.0																	
C41	0.80	0.84	0.87	0.87	1.0																		
C42	0.82	0.85	0.88	1.0																			
C43	0.79	0.80	1.0																				
C44	0.80	1.0																					
C45	1.0																						