



## RESEARCH ARTICLE

### BIOCHEMICAL CHARACTERIZATION OF SELECTED ARECANUT (*A. CATECHU L.*) ACCESSIONS

<sup>1,\*</sup>Rajesh, B. and <sup>2</sup>Ananda, K. S.

<sup>1</sup>Postgraduate Department of Applied Botany, Alva's College, Moodbidri DK, Karnataka

<sup>2</sup>Central Plantation Crops Research Institute, Regional Station Vittal, DK, Karnataka

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#### ABSTRACT

The present effort has been made to characterize the arecanut accessions based on total phenolic content and native protein profiles. The study showed significant variations for total phenolic content in the mature fruits among the arecanut accessions. Accessions VTL-26 (Fiji), VTL-11 (Indonesia) and VTL-60 (West Bengal) recorded higher phenolic content while low content was noticed in VTL-56 (Hirehalli Dwarf). The study indicated that protein profiles could be used for identification of individual accession of arecanut and cluster analysis also did not show any correlation between geographic affinity and genetic affinity of arecanut accessions in the cluster diagram.

## INTRODUCTION

Arecanut is one of the most important cash crops of India having economic, religious and cultural importance. It is popularly known as 'supari'. Arecanut palm is cross pollinated crop. Evaluation of arecanut accessions contributed to identify the high yielding and location specific varieties like Mangala, Sumangala, Sreemangala, Mohithnagar and Suvarnamangala. The characterization of variability has relied mostly on phenotypic and yield parameters in arecanut (Ananda, 2004). Presently, biochemical (Phenol and isozymes) and molecular (DNA) markers play an important role in characterization of germplasm of any crop like arecanut also. Isozyme, the biochemical marker used to designate the multiple molecular forms of the given enzyme occurring members of same species and catalysing the same physiological reaction. The isozyme polymorphism study has been reported in various palms like coconut (Parthasarathy *et al.*, 2004, Geethalaxmi *et al.*, 2005). Similarly, phenols have been used as taxonomic markers mainly in Tea and Coffee and to limited extent in coconut (Niral, 1999). With this perspective the present effort has been made to characterize the arecanut accessions based on total phenolic content and protein profiles.

## MATERIALS AND METHODS

The study was carried out at the Central Plantation Crops Research Institute, Regional Station Vittal, DK, Karnataka. About 50 Arecanut accessions including 17 exotic and 33 indigenous accessions were used. Among these, one was a dwarf and rest of them were tall accessions. The palms were planted in RBD with three replications of eight palms.

**Estimation of Polyphenol in dry kernel:** About ten dried kernels from each palm (80 kernels from each accession) were collected randomly and ground for the estimation of total phenols. Exactly 0.05g of arecanut kernel powder was used and the phenol estimated based on Folin-Ciocalteu method (Bray and Thorpe, 1954).

**PAGE (Native protein profile):** Protein analysis was done using spindle leaf (1g leaf tissue in 5ml of extraction buffer) extracts of arecanut accessions. Extraction of protein was carried out with 0.1M Tris HCl (pH 7.8) containing Polyvinyl Pyrrolidone (PVPP), Calcium chloride, Triton X-100, beta Mercaptoenol and glycerol in cold condition. The extract was centrifuged at 10000rpm for 15 mins at 4°C. The supernatant (100µl) was then mixed with 3µl of 0.01 BPB tracking dye for loading. Clear extracts were used for electrophoresis (at constant power supply 25mA/gel) and processed at 4°C for 4 hrs or until the tracking dye had migrated to the bottom of the gel. The gel was carefully removed and stained for protein

**\*Corresponding author: Rajesh, B.**

Postgraduate Department of Applied Botany, Alva's College, Moodbidri DK, Karnataka.

activity. The gel was then incubated in 12.5%TCA solution at room temperature for one day and stained using 3% Commassive Brilliant Blue R solution for protein (PROT) bands. Then gel was destained using 7% acetic acid. The protein analysis was repeated for three times and the gels were stored in distilled water. Documentation was done by recording the number of samples in which a particular band was present and allelic frequency was calculated based on number of samples in which band was present/total number of samples analysed. Similarity index (Rohlf, 1993) was calculated and cluster analysis also worked out based on UPGMA using NTSYS software.

## RESULTS AND DISCUSSION

The total phenolic content of matured fruits as mg (catechol) per gram varied significantly among the accessions and it ranged from 120.55mg/g to 196.84mg.g with a mean of 151.89mg/g (Table 1). Among the tall accessions maximum polyphenolic content was recorded in VTL-26 (Fiji II) and minimum in VTL-89 (Hylakandi). Indigenous accessions VTL-60 (Mohithnagar), VTL-85 (Ratnagiri), VTL-82 (Mowlong), VTL-29II (Andaman), VTL-76 (Panicha), VTL-92 (Daukihills) and VTL-97 (Wyanad) recorded higher content of phenolics. Similarly, accessions VTI-11 (Indonesia), VTL-28III (Saigon), VTL-1 (Fiji), VTI-14 (Saigon) and VTL-9 (Indonesia) had higher total phenolic content among the exotic accessions. Similar results have been reported by Amudhan and Bhat (2002) in arecanut varieties. Accession VTI-56 (Hirehalli Dwarf- a natural mutant) had the lowest value (120.55mg.g) for this trait. Ananda (2005) also reported that the tall varieties had higher content of total phenolics in matured fruits than the Hirehalli Dwarf (VTL-56) and that the hybrids derived from the dwarf and tall varieties also had higher polyphenols. It was also reported in earlier studies that the IAA oxidase is inhibited by natural phenolics having two hydroxyl group in ortho position. As a result, with higher ODP, higher will be the IAA oxidase inhibition, higher the IAA concentration and taller the palms (CPCRI, 1992).

The content of total phenolics also varied with respect to maturity of fruit and it was reported that the immature fruit contain more polyphenols than the matured fruit (Mathew and Govindharjan, 1964). The leaf polyphenol content used as one of the trait for the descriptor data of coconut. The present study indicated the quantitative variation of total phenol in mature fruits of arecanut accessions studied which can be utilized for characterization of the accessions. And also it could be used for product diversification and development of value added products of arecanut. In some of the coconut cultivars, the higher phenol levels associated with relatively better tolerance to root disease (Chempakam and Ratnambal, 1993) and it can be incorporated in arecanut also for various diseases. The native protein profile study indicated the polymorphism and thirty two clear PROT bands were noticed among the arecanut accessions (Plate 1). Out of which band Nos. 4 (Rf value 0.09), 12 (Rf value 0.28), 16 (Rf value 0.44), 19 (Rf value 0.49), 28 (Rf value 0.72), 29 (Rf value 0.78) and 32 (Rf value 0.94) were monomorphic and rest of them polymorphic. Band (No. 32) with Rf value 0.85 was absent in accession VTL-56 (Hirehalli Dwarf) and present in all the tall accessions. Allelic frequency

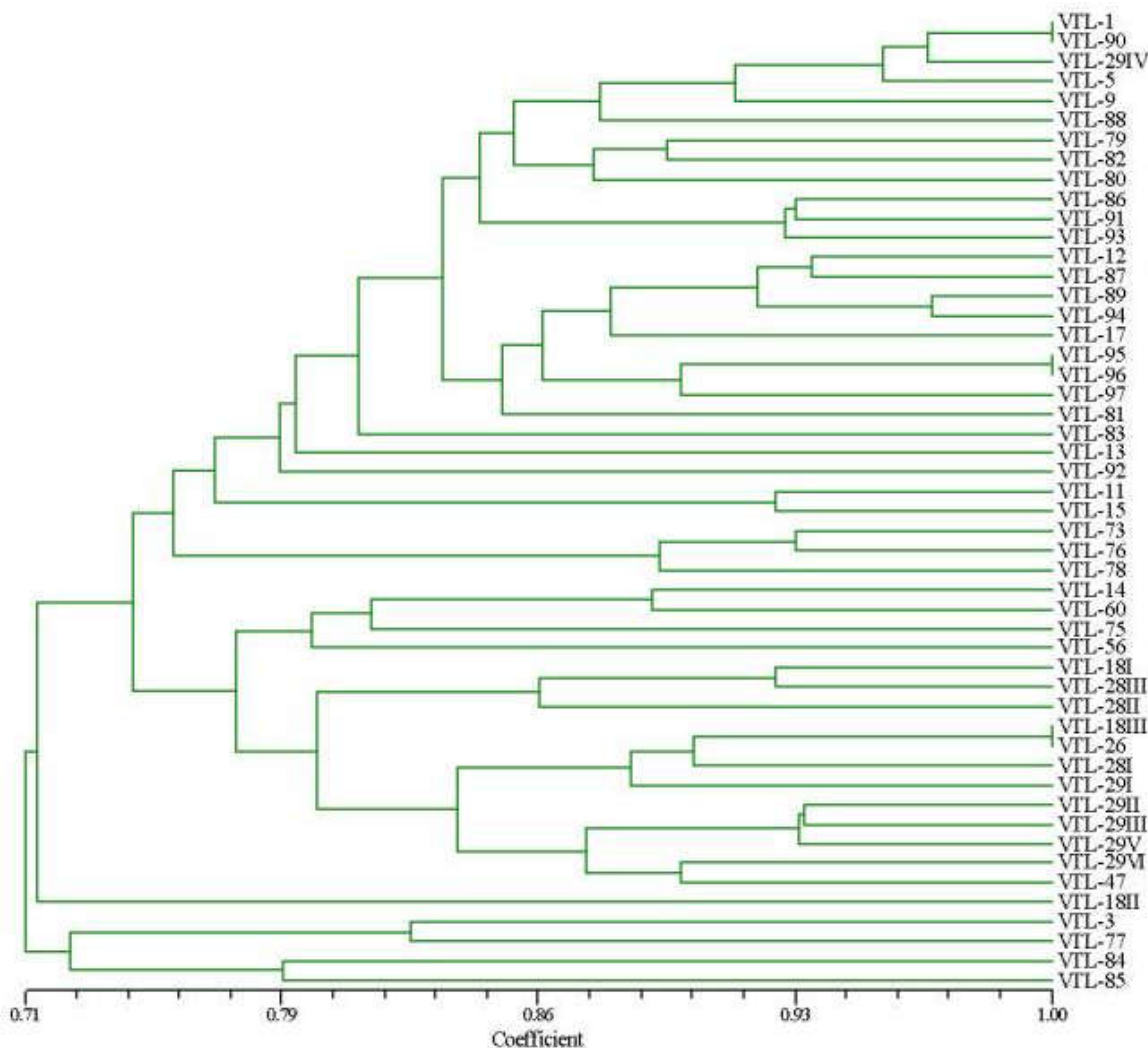
was highest in VTL-89 (0.94) and least (0.75) in VTL-18I, VTL-18II, VTI-77 and VTI-92 (Table 2). Similarly, allelic frequency for protein in VTL-56 was 0.81. Among the accessions, based on native protein data, the similarity index ranged between 53.10 per cent and 100 per cent. The similarity level between accessions VTL-1 (Fiji) and VTI-90 (Daukihills); VTL-95 (Jawai) and VTL-96 (Jawai) and also VTL-18III (BSI) and VTL-26 (Fiji) was 100 per cent. Among the accessions, VTL-15 (Sri Lanka) and VTL-18I (BSI) showed lowest similarity (53.10%). Among the tall accessions, VTL-29V (Anadaman) showed higher similarity (89.30%) with the dwarf type where as accession, VTL-18II (BSI) showed least similarity of 61.30 per cent with accession VTL-56 (Hirehalli Dwarf). The cluster analysis based on native protein profiles showed that the accessions grouped into two major groups (Fig. 1). Among the accessions four accessions (VTL-85, VTL-84, VTL-77 and VTL-3) grouped together while the remaining 46 accessions formed a separate group and most of the accessions sub-clustered separately in the group. Accession VTL-56 sub grouped separately from the other remaining tall accessions. However, accessions VTL-1 and VTL-90, VTL-95 and VTL-96 and VTL-18III and VTL-26 grouped together indicating the similarity among themselves. The cluster formation of arecanut accessions based on protein profiles also did not show geographic affinity. The study indicated that protein profiles could be used for identification of individual accessions of arecanut. Jayalekshmy (1999) also reported that proteins could be used as one of component for the characterization of coconut germplasm. The present cluster study indicated that the exotic arecanut accession have greater variability as these accessions were found scattered in many groups irrespective of their origin.

Arecanut accessions showed significant variation for total phenolic content in the mature fruits. Accessions VTL-26 (Fiji), VTL-11 (Indonesia) and VTL-60 (west Bengal) recorded higher phenolic content while low content was noticed in VTL-56 (Hirehalli Dwarf). The chali/dry kernel is the only economic product of arecanut crop, the phenolic bio-component could be used for product diversification and development of value added products of arecanut. The existing variability in total phenolics among the accessions studied could be used for developing varieties for industrial/pharmaceutical purposes. The phenolic contents in the different accessions could be correlated with tolerance to biotic/abiotic stresses of accessions, to determine the association between tolerance/resistance and phenolic contents, if any. Similarly, the polyphenol content could be used as one of the component trait for studying the genetic variability.

The cluster analysis based on protein profiles of arecanut accessions also did not show any correlation between geographic affinity and genetic affinity in the cluster diagram. The distant accessions with desirable traits identified in the present study will be of immense value in the arecanut improvement. The isozyme analysis of arecanut accessions also did not show any correlation between geographic affinity and genetic affinity in the cluster diagram. The distant accessions identified through cluster analysis based on enzyme systems could also be used for hybridization for obtaining highly heterotic progenies.

**Table 1. Polyphenol content in mature fruits of different areca nut accessions**

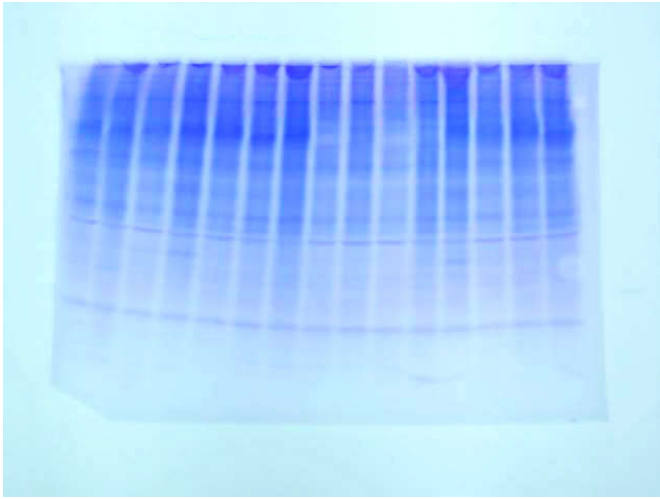
Accession	Country of collection	Total phenol content (mg/g)	Accessions	Country of collection	Total phenol content (mg/ g)
VTL-1	Fiji-I	160.41	VTL-75	Nalbari (Assam)	159.10
VTL-3	China	151.84	VTL-76	Panicha (Assam)	168.76
VTL-5	Sri Lanka-I	146.07	VTL-77	Saragoan-I (Assam)	145.14
VTL-9	Indonesia-IV	155.46	VTL-78	Saragoan-II (Assam)	144.65
VTL-11	Indonesia-VI	176.83	VTL-79	Shelleshella (Assam)	159.77
VTL-12	Saigon-I	145.40	VTL-80	Dangapara (West Bengal)	136.64
VTL-13	Saigon-II	139.76	VTL-81	Haldibari (West Bengal)	127.11
VTL-14	Saigon-III	165.43	VTL-82	Moralpara (West Bengal)	175.96
VTL-15	Sri Lanka-II	147.17	VTL-83	Thargira (West Bengal)	134.66
VTL-17	Singapore	130.47	VTL-84	Kashi and Jayanthi Hills (Meghalaya)	145.89
VTL-18I	British Solumn Islands-I	130.66	VTL-85	Ratnagiri (Maharastra)	182.54
VTL-18II	British Solumn Islands -II	139.66	VTL-86	Badarpur-I (Assam)	161.07
VTL-18III	British Solumn Islands-III	143.36	VTL-87	Badarpur-II (Assam)	133.59
VTL-26	Fiji-II	196.84	VTL-88	Badarpur-III (Assam)	143.80
VTL-28I	Saigon-V	136.79	VTL-89	Hylakandi (Assam)	124.42
VTL-28II	Saigon-VI	136.59	VTL-90	Cachhar (Assam)	139.81
VTL-28III	Saigon-VII	169.21	VTL-91	Dauki Hills-I (Meghalaya)	154.28
VTL-29I	Andaman-I	155.12	VTL-92	Dauki Hills-II (Meghalaya)	164.91
VTL-29II	Andaman-II	169.77	VTL-93	Mowlong-I (Meghalaya)	164.84
VTL-29III	Andaman-III	147.64	VTL-94	Mowlong-II (Meghalaya)	172.38
VTL-29IV	Andaman-IV	155.11	VTL-95	Jawai-I (Meghalaya)	139.95
VTL-29V	Andaman-V	161.29	VTL-96	Jawai-II (Meghalaya)	142.30
VTL-29VI	Andaman-VI	140.50	VTL-97	Wynad (Kerala)	162.70
VTL-56	Hirehalli Dwarf (Karmtaka)	120.55	VTL-47 (check)	S.K. Local (Karnataka)	165.67
VTL-60	Mohitnagar (West Bengal)	186.89	Grand mean		151.89
VTL-73	Kahikuchi-II (Assam)	135.65	CD (+/- 0.05)		17.62



**Fig. 1. Dendrogram based on Jaccard's similarity level (protein) showing the genetic relationship between the arecanut accessions**

Table 2. Allelic frequency for Proteins (PROT) in arcanut accessions

Accession/ Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Mean		
Rfvalue	0.02	0.04	0.05	0.09	0.11	0.13	0.15	0.16	0.21	0.23	0.24	0.28	0.34	0.35	0.36	0.44	0.45	0.47	0.49	0.52	0.55	0.59	0.61	0.66	0.67	0.68	0.70	0.72	0.78	0.81	0.85	0.94			
VTL-1	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	
VTL-3	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-5	1.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-9	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	
VTL-11	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.78	
VTL-12	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	
VTL-13	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	
VTL-14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.81	
VTL-15	0.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.78	
VTL-17	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88
VTL-18I	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.75	
VTL-18II	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.75		
VTL-18III	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	
VTL-26	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	
VTL-28I	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	
VTL-28II	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-28III	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.81	
VTL-29I	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.78	
VTL-29II	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	
VTL-29III	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-29IV	1.00	1.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	
VTL-29V	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-29VI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88
VTL-47	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL-56	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.81	
VTL 60	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.78	
VTL 73	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88
VTL 75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	
VTL 76	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.81	
VTL 77	0.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.75	
VTL 78	1.00	1.00	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.78	
VTL 79	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	
VTL 80	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00																							



**Plate 1. Profiles of proteins (lanes left to right): VTL-1, VTL-3, VTL-5, VTL-9, VTL-11, VTL-12, VTL-13, VTL-14, VTL-15, VTL-17, VTL-18I, VTL-18II, VTL-18III, VTL-26, VTL-56**

Similarly, variations were observed for polyphenol content in mature fruits among the accessions and could be used as one of the bio-component for germplasm characterization and exploited to diversify the value added products. Further, the study also confirmed the usefulness of isozymes for studying the variations among the arecanut accessions.

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