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Exploration and collection of cassava (*Manihot esculenta* Crantz) in Western Ghats and characterisation for industrial use

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Abstract

In India cassava (Manihot esculenta Crantz.) is extensively cultivated as an annual tuberous root crop for both food and industrial uses. Study was aimed to explore and collect the adapted landraces of cassava available in Western Ghats of Tamil Nadu and to characterise the existing variability with particular reference to amylose for industrial applications. A total of 52 landraces were collected from various parts of Western Ghats and significant variations for morphological, root and root quality traits were observed. Analysis on quality parameters revealed that, amylose content ranged from 14.4% to 30.4% with an average of 22.0%. Landraces Adukku Muttan and Ullii chigappan showed low amylose content (14.4%) which is highly amenable for paper, textiles and plywood industries. Landraces with yellow pulp had high carotenoids can be used for culinary purpose by the local peoples. Cluster analysis grouped the landraces into five clusters and the number of accessions varied from 2 to 26. Knowledge on the variability in amylose content and phenotypic diversity for root traits increases the efficiency of the cassava breeding programmes by way of utilising them for industrial purpose and also helps in developing core collections which makes handling of germplasm easy for plant breeders. Diversity in amylose content may help in finding appropriate industrial utility and to formulate efficient quality improvement programme.

Keywords: Cassava, landraces, exploration, root morphology, diversity, amylose content.

Introduction

Cassava (*Manihot esculenta* Crantz.) is a perennial woody shrub of the Euphorbiaceae, native of South America that is extensively cultivated as an annual tuberous root crop in the tropical regions of Africa, Asia and Latin America as a subsistence crop on which more than 500 million people rely[1]. Globally cassava is grown in an area of 18.57 million ha producing 230.27 million tones with 12.4 t/ha of productivity[2]. Apart from its use as a high energy food, cassava starch has a wide range of applications in both food-related and nonfood-related industries. Cassava starch could be converted to maltotriose, maltose, glucose and other modified sugar and organic acid. Starch hydrolysate has been widely used as additive compound in food industries mainly in the preparation of candies, bread, canned food and frozen food. Outside of the food industry, cassava starch is used in the manufacture of paper, textiles and plywood, and more recently in the production of ethanol and biodegradable polymers[3]. Low amylose starch has high granule melting temperature and less retrogradation[4] and low amylase starch is highly amenable for non food industrial applications. India is one of the major Asian country growing cassava and it acquires significance in the global cassava scenario due to higher productivity in the world. Cassava cultivating area, production and productivity of India is 0.23 million ha, 8.06 million tones and 34.75 t/ha respectively[2]. Global efforts have been taken to improve the cassava based on its varied utility. Hence, diverse genotype of cassava is essential to start any crop improvement programme[5]. Existence of variability in quality parameters will aid in developing trait-specific cassava populations for use in food, feed and industrial purposes. India is the one of the richest biological diversity subcontinent in the world and Western Ghats regions retain its diversity due to its complex topography, high rainfall and relative inaccessibility. In the present study, we explored and collected the farmer-grown cassava landraces in Western Ghats of Tamil Nadu and assessed the extent of genetic diversity for root traits and amylose content to use them in cassava breeding programme for industrial applications.

Material and Methods

Exploration and maintenance of study material: An exploration was conducted during Sept-Oct. 2011 in the Western Ghats, covering the high altitude areas ranging from 250 to 2552 feet above MSL with the objective to collect and study the existing farmers-grown landrace diversity in cassava. The exploration was covered in 32 villages in the southern region of Western Ghats. The planning and logistics[6] and the sampling procedure[7] for the collection of germplasm was made as per the guidelines. A total of 51 landraces adapted to various rainfed situations with unique distinct morphological features were collected and planted with one improved commercial

hybrid, H226 at HRS, Pechiparai, Tamil Nadu as cassava clonal garden.

Morphological characterisation using descriptors: Tuber samples from all the accessions were collected and it can be used for morphological characterization of roots as per the cassava descriptors[8]. Seven morphological characters *viz.,* root constriction, root shape, external colour of root, colour of the root pulp, colour of root cortex, cortex: ease of peeling and texture of root epidermis were recorded and converted into binary data[9].

Estimation of Amylose Content: Amylose, the basic unit of the starch was determined using method[10] for all the 52 genotypes and expressed as per cent on dry weight basis. Two determinations were made for each sample and mean values were used for grouping based on the amylose content as low (10-20%), medium (20-25%) and high amylose (25-30%) genotypes.

Genetic diversity and cluster analysis: The binary data obtained for 52 cassava genotypes were used to generate similarity matrix using the SIMQUAL programme of NTSYS-pc software, version 2.02i[11]. The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair-Group method (UPGMA)[12].

Results and Discussion

Variability at morphological level: In the present study, an attempt has been made to determine the extent of diversity using the seven evaluated traits and to characterise the amylose content among 52 cassava accessions studied (table 1). The easily observable root traits are useful for preliminary evaluation as they offer a fast and useful approach for assessing the extent of diversity in cassava. Analysis of variance revealed the presence of significant variability in the experimental material. Descriptors based root constriction study revealed that, 26 accessions were found to possess few to no constriction, 13 were found to possess some and 13 were found to possess many constrictions in roots. Tuber shape: 21 genotypes exhibited cylindrical, 14 conical-cylindrical, 21 irregular and 5 conical. For external skin colour of the storage root, 34 accessions were found to be of brown in colour, 15 were cream and 3 were of yellow in colour.

Table-1
Details of cassava lines explored in Western Ghats of Tamil Nadu

Sl. No.	Cassava Landrace	Ac. No.	Collection site	Observations							
				Root Constriction	Root Shape	Root Skin colour	Pulp colour	Root cortex colour	Cortex peeling	Root texture	Amylose content (%)
1	*Ullii chigappan*	CL1	*Pechiparai*	Many	Conical-cylindrical	Light brown	Yellow	Pink	Easy	Smooth	25.4
2	*Laxhmi vellai*	CL2	*Tiruvarambu*	Some	Cylindrical	Cream	Yellow	Cream	Difficult	Rough	20
3	*Karialai porian*	CL3	*Pechiparai*	Many	Cylindrical	Light brown	Yellow	Pink	Easy	Rough	24.4
4	*Ottai moodu*	CL4	*Koruvakkuzhi*	Few to none	Conical	Light brown	Yellow	Pink	Difficult	Intermediate	23.4
5	*Karialai porian*	CL5	*Thanikunndu*	Some	Conical-cylindrical	Light brown	Yellow	Pink	Easy	Intermediate	21.2
6	*Karialai porian*	CL6	*Mothiramalai*	Few to none	Irregular	Light brown	Yellow	Pink	Easy	Intermediate	30.4
7	*Karialai porian*	CL7	*Vazhi battu kadavu*	Some	Conical	Cream	White	Cream	Easy	Intermediate	25.4
8	*Karialai porian*	CL8	*EB.Pachiparai*	Some	Irregular	Dark brown	Cream	Pink	Easy	Intermediate	22.4
9	*Dwarf vellai*	CL9	*Maramalai*	Some	Cylindrical	Cream	White	Cream	Easy	Intermediate	22.4
10	*Dwarf Ullii chigappan*	CL10	*Naval kadu*	Some	Irregular	Cream	White	Cream	Easy	Intermediate	25.4
11	*Kattu (NT)*	CL11	*Koruvakkuzhi*	Some	Irregular	Light brown	Yellow	Pink	Difficult	Intermediate	17.2
12	*Ullii chigappan*	CL12	*Koruvakkuzhi*	Some	Irregular	Light brown	White	Pink	Easy	Intermediate	14.4
13	*Kerala thadi muttan*	CL13	*Pathanamthittai*	Many	Conical-cylindrical	Cream	Cream	Pink	Easy	Smooth	18.4
14	*Ullii chigappan*	CL14	*Thanikunndu*	Many	Conical-cylindrical	Cream	Cream	Pink	Easy	Intermediate	15.2
15	*Adukku muttan*	CL15	*Thanikunndu*	Many	Conical-cylindrical	Cream	Yellow	Cream	Easy	Rough	14.4
16	*Karialai porian*	CL16	*Mookirakall*	Many	Conical	Cream	Yellow	Pink	Difficult	Rough	15.3
17	*Laxhmi vellai*	CL17	*Mallamuthan karai*	Many	Conical	Cream	Cream	Cream	Easy	Intermediate	17.2
18	*Tall-kattu*	CL18	*Mallamuthan*	Many	Cylindrical	Dark	White	Yellow	Difficult	Smooth	16.1

			karai			brown					
19	*Tall-Chilly kallan*	CL19	*Mallamuthan karai*	Many	Irregular	Cream	White	Cream	Easy	Intermediate	16
20	*Tall- Karialai porian*	CL20	*Mallamuthan karai*	Many	Irregular	Cream	Yellow	Cream	Easy	Rough	20
21	*Adukku muttan*	CL21	*Mallamuthan karai*	Some	Conical-cylindrical	Cream	White	Cream	Easy	Intermediate	26.4
22	*Yeathan chivalai*	CL22	*Vaiyana challai*	Many	Conical-cylindrical	Cream	Cream	Pink	Easy	Intermediate	15.2
23	*Pachai konntai*	CL23	*Koruvakkuzhi*	Many	Cylindrical	Dark brown	White	Cream	Easy	Smooth	16
24	*Nadan karialai*	CL24	*Chittar*	Few to none	Cylindrical	Light brown	Cream	Cream	Easy	Intermediate	20.4
25	-	CL25	*Allan chollai*	Few to none	Conical-cylindrical	Dark brown	Cream	Pink	Easy	Intermediate	16
26	*Laxhmi vellai*	CL26	*Chittar*	Some	Conical-cylindrical	Dark brown	Yellow	Yellow	Easy	Intermediate	25.4
27	*Kaichi kuttai*	CL27	*Chittar dam*	Some	Cylindrical	Dark brown	Yellow	Pink	Easy	Intermediate	30.4
28	*Nooru muttan*	CL28	*Allan chollai*	Few to none	Conical-cylindrical	Dark brown	Yellow	Pink	Easy	Intermediate	20.4
29	*Aana karialai*	CL29	*Neduman kaadu*	Few to none	Cylindrical	Dark brown	Cream	Cream	Difficult	Intermediate	22.4
30	*Kailady*	CL30	*Valia yela*	Few to none	Cylindrical	Light brown	Cream	Pink	Easy	Intermediate	19.4
31	*Black karialai*	CL31	*Valia yela*	Some	Irregular	Light brown	Yellow	Pink	Easy	Intermediate	26.4
32	-	CL32	*Mantharam puthur*	Few to none	Irregular	Dark brown	Yellow	Yellow	Easy	Intermediate	27.4
33	-	CL33	*Kottaram*	Few to none	Irregular	Dark brown	Cream	Pink	Easy	Intermediate	22.4
34	-	CL34	*Kottaram*	Few to none	Cylindrical	Dark brown	Yellow	Pink	Easy	Intermediate	14.9
35	-	CL35	*Achan kulam*	Few to none	Conical-cylindrical	Dark brown	Cream	Pink	Easy	Intermediate	24.4
36	*Arriam vellai*	CL36	*Kaata vilai*	Many	Irregular	Light brown	Cream	Pink	Easy	Intermediate	25.4
37	*Tall-muttan*	CL37	*Kaata vilai*	Few to none	Irregular	Dark brown	Yellow	Pink	Easy	Intermediate	20.4
38	*Karun karialai*	CL38	*Mudavan pottai (Hill)*	Few to none	Cylindrical	Light brown	Cream	Pink	Easy	Intermediate	20.4
39	*Chengambai*	CL39	*Valayamthukki*	Few to none	Conical-cylindrical	Dark brown	Cream	Cream	Easy	Rough	22.4
40	*Pachai konda*	CL40	*Valayamthukki*	Few to none	Cylindrical	Yellow	Cream	Cream	Easy	Smooth	24.4
41	*Vellai porian*	CL41	*Valayamthukki*	Few to none	Conical-cylindrical	Yellow	Yellow	Pink	Easy	Intermediate	23.4
42	*Karialai porian*	CL42	*Kayarkarai*	Few to none	Cylindrical	Dark brown	Yellow	Pink	Easy	Intermediate	26.4
43	*Adukku muttan*	CL43	*Kkayarkarai*	Few to none	Conical-cylindrical	Dark brown	Cream	Cream	Easy	Intermediate	24.4
44	*Chengambai*	CL44	*Kayarkarai*	Few to none	Cylindrical	Dark brown	Yellow	Cream	Easy	Intermediate	26.4
45	*Pachai konda*	CL45	*Kkayarkarai*	Few to none	Cylindrical	Cream	Yellow	Pink	Easy	Intermediate	24.4
46	*Karialai porian*	CL46	*Kayarkarai*	Few to none	Cylindrical	Dark brown	Yellow	Pink	Easy	Rough	28.4
47	*Karialai porian*	CL47	*Kayarkarai*	Some	Cylindrical	Yellow	Cream	Pink	Easy	Intermediate	24.4
48	*Karialai porian*	CL48	*Verkilambi*	Few to none	Cylindrical	Dark brown	Yellow	Pink	Easy	Intermediate	28.4
49	*Adukku muttan*	CL49	*Arasan seri*	Few to none	Cylindrical	Dark brown	Yellow	Yellow	Easy	Intermediate	24.4
50	-	CL50	*Mathur*	Few to none	Conical	Light brown	Yellow	Pink	Easy	Intermediate	28.4
51	*Ullii chigappan*	CL51	*Mathur*	Few to none	Cylindrical	Dark brown	Cream	Pink	Easy	Intermediate	28.4
52	*White rose*	CL52	*Salem*	Few to none	Cylindrical	Cream	Cream	Cream	Easy	Intermediate	28.5

Regarding the pulp colour, 18 accessions were found to possess pulp cream and 25 were possessing yellow pulp. In the case of root cortex colour, 32 accessions were having the pink colour, 17 were of cream colour and 3 accessions were to be of yellow colour. With respect to peeling of cortex, 46 accessions were peeled easily and 6 accessions were found difficult to peel. Regarding the tuber surface texture, 4 accessions were of smooth, 41 accessions were of intermediate and 7 accessions found to possess rough surface (table 2. and figure-1). Descriptor based root diversity study revealed the presence of enormous diversity for tuberous root colour, cortex colour, pulp colour as well as tuberous root shape and texture. The reason may be, a farmer very rarely discards even a low-yielding variety, but maintains it at a low frequency for its other quality or adaptability traits. This corresponds to a strategy of risk management in uncertain farming conditions, but the practice is also motivated by social or cultural reasons because diversity is prized for its own sake. This much variability would have been created through volunteer cassava or through exchange of clones within the community, or with other villages. The present study revealed the existence of high level of morphological diversity among the cassava genotypes as reported earlier in cassava[13], providing scope for improvement through hybridization and selection.

Table-2
Cassava variability for root morphological traits and amylose content

Sl.No.	Characters	No. of Genotypes
1	Root constrictions	
	Few to none	26
	Some	13
	Many	13
2	Root shape	
	Conical	5
	Conical-cylindrical	14
	Cylindrical	21
	Irregular	12
3	External skin colour of root	
	White or cream	15
	Yellow	3
	Light brown	13
	Dark brown	21
4	Colour of root pulp	
	White	9
	Cream	18
	Yellow	25
5	Colour of root cortex	
	White or cream	17
	Yellow	3
	Pink	32
6	Cortex: ease of peeling	
	Easy	46
	Difficult	6
7	Texture of root epidermis	
	Smooth	4
	Intermediate	41
	Rough	7
8	Amylose content (%)	
	Low (10-20)	18
	Medium (20-25)	18
	High (>25)	16

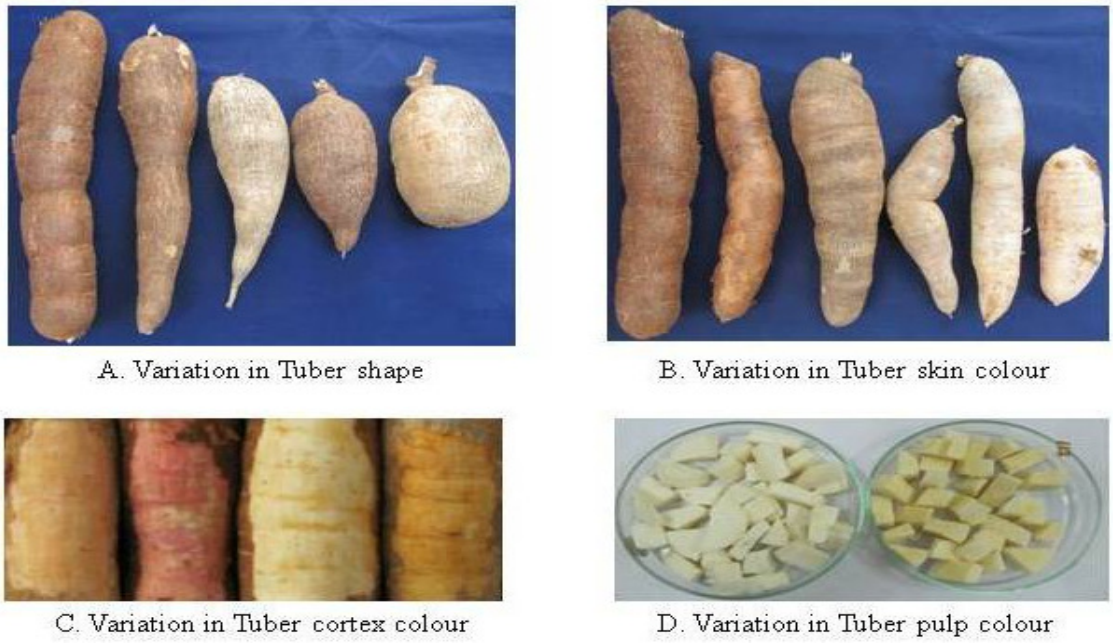

Figure-1
Variation in root morphological characters in cassava

Diversity in amylose content: Estimation of amylose content in the 52 genotypes revealed that the amylose content ranged from 14.4% (*Ullii chigappan* from *Koruvakkuzhi*) to 30.4% (*Karialai porian* from *Mothiramalai*) with an average of 22.0% (table 1). Amylose content in cassava genotypes were grouped as high (25.4%–30.4%), intermediate (20.4%–24.4%) and low (14.4%–20.0%). Among the 52 accessions, 18 cassava accessions had low amylose content, 18 were medium and 16 genotypes possessed high amylose content (table 2). Estimation of amylose content indicates the existence of variation among the landraces studied (figure-2). The extreme amylose lines can be used in cassava breeding programme to develop high or low amylose genotypes that has wider applications in food processing industry and paper and textile industries respectively.

Principal component analysis: The Principal Component Analysis was performed using the observations recorded for seven root morphological traits and amylose content. The values of the Eigen vectors and their contribution to total variation are presented in table 3. The first three principal components accounted for 61.8% of the total variance. The first principal component (PC1) accounted for 30.23% of total variance and had high contributing factor loadings from root skin colour, pulp colour and root cortex colour. The second principal component (PC2) accounted for 17.95% variation and had high contributing factor loadings from traits namely pulp colour, cortex peeling and root texture. The third principal component (PC3) accounted to 13.6% of the total variation, with high factor loadings for root cortex colour. The contribution of maximum variability to total variation by root skin colour, pulp colour, cortex peeling and root cortex colour were reported earlier[13,14].

Table-3
Principal components analysis showing the contribution of root morphological traits in cassava

Variables	PC1	PC2	PC3
Root constrictions	-0.794	0.115	0.336
Root shape	0.131	-0.400	0.277
External skin colour of root	0.783	-0.214	0.190
Colour of root pulp	0.511	0.685	0.172
Colour of root cortex	0.489	0.197	0.713
Cortex: ease of peeling	-0.164	0.466	0.161
Texture of root epidermis	0.037	0.694	-0.378
Amylose content	-0.794	0.106	0.395
EIGEN VALUE	2.419	1.436	1.088
% VARIANCE	30.2	18.0	13.6
CUMULATIVE % VARIANCE	30.2	48.2	61.8

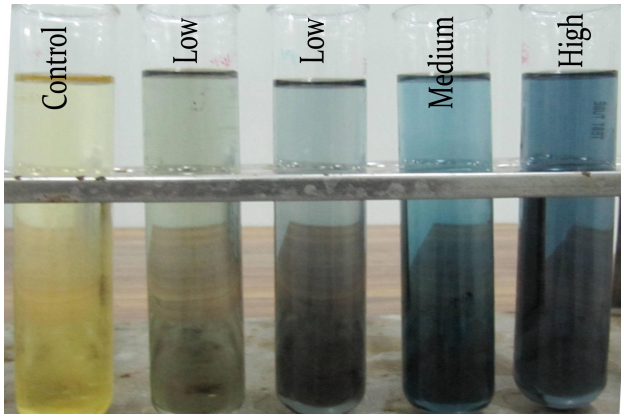

Figure-2
Variation in amylose content among the cassava genotypes

Cluster analysis: The similarity coefficients generated in the SIMQUAL programme of NTSYS-pc 2.02i were used to construct the hierarchical clusters and resulting dendrogram is presented in figure 3. The 52 cassava accessions formed 5 clusters at similarity index of 0.71. The details of five clusters along with the accessions and average amylose content were presented in table 4. Among the different clusters, the cluster size varied from 2 to 26. The cluster II possessed a maximum of 26 genotypes with intermediate amylose and less root constrictions. A minimum of two genotypes with low amylose represented in cluster V. Cluster I contains 10 genotypes and the tubers possessed light brown colour outer skin. Four accessions with characters like yellow pulp and rough texture were grouped in Cluster III. Ten accessions having unique characters like cylindrical tuber shape, white pulp and smooth texture were grouped in Cluster IV. Similar studies have been carried out previously[15-18] for the estimation of genetic diversity in cassava based on morphological traits and reported high degree of genetic variability among 94 cassava accessions of Brazilian origin[14] and African and Latin American accessions[19]. This trait based clustering will be helpful in handling large populations and to develop core collections and trait based collections for the breeders to develop a working germplasm. Also, cassava hybrid breeding programme involving parents from diverse amylose content clusters will be useful in exploiting and fixing heterosis.

Sequencing of candidate gene: Molecular breeding program has been initiated by retrieving the Expressed Sequence Tag (EST`s) sequences for cassava (*Manihot esculenta* Crantz.) granule bound starch synthase gene 1 *(GBSS 1)* (X74160.1) along with the amino acid sequences from the gene bank database of NCBI and primers were designed. Using the primers the candidate gene was sequenced through primer walking strategy and gene model was developed. Gene model for *GBSS 1* gene revealed it has 13 exons and 12 introns (figure- 4). It was in accordance with the *GBSS 1* gene model of potato[20] and sweet potato[21]. Comparing the sequence difference in two extreme genotypes is underway. After sequencing, it can be compared to explore the sequence differences between them and to develop functional markers which may be used in marker based screening of genotypes in molecular breeding programme.

Table-4
Cluster composition of cassava accessions for root morphological traits and amylose content

Sl.No.	Clusters	No. of Genotypes	Mean amylose content (%)	Code No.
1	I	10	23.1	CL1, CL3, CL4, CL5, CL6, CL8, CL11, CL12, CL31, CL36
2	II	26	23.1	CL24, CL25, CL26, CL27, CL28, CL29, CL30, CL32, CL33, CL34, CL35, CL37, CL38, CL39, CL40, CL41, CL42, CL43, CL44, CL45, CL46, CL47, CL48, CL49, CL50, CL51
3	III	4	17.5	CL2, CL15, CL16, CL20
4	IV	10	21.0	CL7, CL9, CL10, CL13, CL14, CL17, CL19, CL21, CL22, CL52
5	V	2	16.1	CL18, CL23

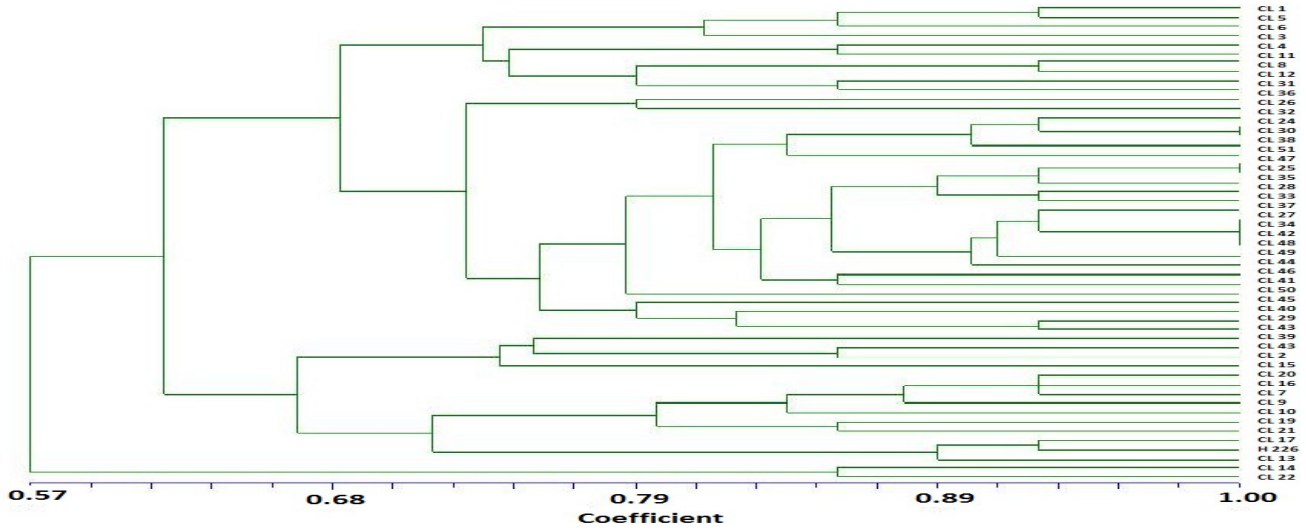

Figure-3
Dendrogram showing relationship among 52 cassava genotypes based on seven morphological data

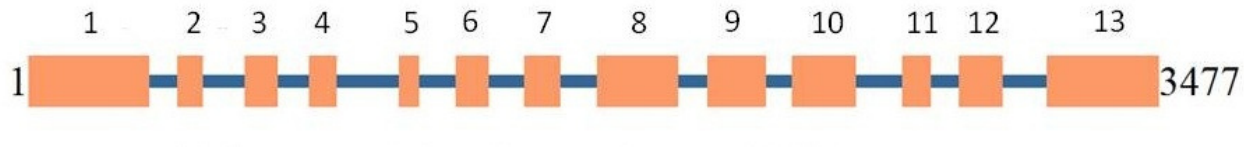

Figure-4
Gene model for the candidate gene *GBSS I*

Conclusion

Farmers are the saviours of cassava on farm diversity in Western Ghats region of Tamil Nadu by way of acquiring and maintaining local landraces which are productive even under unfavourable conditions. Cassava farmers enjoy diversity and eagerly acquire new landraces. They rarely discard unproductive landraces, retaining them, even if at low frequencies, arguing that they can become productive under different climatic conditions. The other reason for diversity is the occurrence of natural introgression through gene flow and growing volunteer seedlings. We also argue that recombination and gene flow play a major role in the dynamics of genetic diversity of cassava in traditional farming systems. Diversity analysis and clustering of genotypes for morphological and biochemical traits helps in developing core collections which makes handling of large germplasms easy for plant breeders. Knowledge on the variability in amylose content may help in finding appropriate industrial utility and to formulate efficient quality improvement programme. Developing knowledge on the sequence diversity for *GBSS 1* gene will help in formulating molecular breeding programme to meet industrial needs.

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