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SSR Marker Variability in a Set of Indian Cultivars from a Typical Cassava-Growing Area

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important tropical tuber crop grown in India. It is of South American origin that reached Indian shores through Portuguese travelers. Even though only a few varieties were introduced in the beginning, much variability developed by virtue of flowering and natural hybridisation. This variability, based on morphological and biometrical characters, has been occasionally studied. This paper is an attempt to assess this variability in a typical cassava growing area based on molecular markers and to analyse the factors promoting variability. SSR (Simple Sequence Repeat) markers are found to be ideal for analysing molecular variability in plant populations. Thirty six SSR markers developed at CIAT, Cali, Columbia and available in the public domain were chosen for the study. Thirty eight varieties of cassava were collected from a typical cassava-growing region, where high variability was noticed in tuber yield, starch content and cooking quality. Cassava yield in this area is comparatively high. DNA was extracted following standard protocols and was amplified using 36 SSR primers. Each primer produced one or two bands. The similarity between different varieties was quantified using the software package NTSYS-pc (Numerical Taxonomy Multivariate Analysis System). The similarity between varieties varied from 44 to 90%. A similarity matrix was used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Means (UPGMA), to study the grouping pattern. The set of 38 varieties was grouped into five clusters. This information will be useful in planning hybridisation between distantly related varieties so that wide variation can be realized in the hybrid population. The wider the variability, the better will be the chance of selection of superior varieties.

Keywords: accessions, dendrogram, genetic diversity, *Manihot esculenta*, natural inter-varietal hybrids

INTRODUCTION

The starchy root crop cassava (*Manihot esculenta* Crantz) is grown in many tropical countries of the world, ranking fourth in importance as a dietary staple and sixth on an overall basis (Fregene *et al.* 2003). It is an important tuber crop in the tropical region of India. Because of its high adaptability to rainfed conditions and low-fertility soils; it became a subsidiary food and a famine reserve crop. Cassava is an introduced crop in India. It is of South American origin. It is believed to have reached India in the 16th century through Portuguese travelers. The crop established itself in the southern part of India, which has agroclimatic conditions very similar to the center of origin. So, a planned introduction was made by local rulers in 19th century. Later more varieties were introduced through international organizations. Even though only a few varieties were introduced in the beginning, many natural recombinants have evolved over the years and good variability is available in the field. Studies were conducted earlier to assess the variability based on biometrical characters as well as RAPD markers (Pillai *et al*. 2002, 2004). The present study is an attempt to measure the variability based on Simple Sequence Repeat (SSR) markers. SSR or microsatellites have been widely recognized as powerful and informative genetic markers in both animals and plants (Yasodha *et al.* 2008). SSRs consist of tandem repeated units of short nucleotide motifs that are 1-6 bp long. Di-, tri-, and tetranucleotide repeats are the most common and widely distributed throughout genomes (Jarne and Lagoda 1996). Their great utility as genetic markers comes from their inherent variability that is derived from unusually high mutation rates for nucleotide sequences within SSR loci (Peakall *et al*. 1998). SSR markers have been widely used for analyzing molecular variability in different tuber crops like yam (Tostain *et al.* 2007), taro (Mace *et al.* 2002), potato (Gillen and Novy 2007) and vegetable soybean (Makiko *et. al.* 2007). In cassava, SSR markers have been used to search for duplicates in the cassava collection at CIAT, Cali, Colombia (Chavarriaga-Aquirre *et al*. 1999) and to analyze variation in natural populations of putative progenitors of cassava (Olsen and Schaal 2001). This study is to quantify the variability and also diversity available in the land races of cassava in a typical cassava-growing area in India.

MATERIALS AND METHODS

Plant material

Cassava landraces were collected from the district of Idukki (9° 51′ 01.60″ N; 76° 57′ 58.84″ E) and adjoining areas. This region is important as cassava is a staple food crop in this hilly region, where rice can not be cultivated. Twelve villages in the area were visited. The total sample collection area was around 60 km^2. The varieties were planted at the CTCRI farm and were evaluated for tuber yield, taste, disease incidence, etc. Data on yield, starch, taste and disease incidence were collected. Yield per plant ranged from 1.73 to 5 kg. Starch percent ranged from 14.40 (ID-37) to 34.60 (ID-15). Taste was good in 6 out of 38 varieties. No variety was completely free of Cassava Mosaic Disease (detailed morphological data is given in **Table 1**).

DNA extraction

DNA isolation was carried out according to Dellaporta *et al*. (1983) using 2 g of fresh young leaf tissue obtained from 3 to 4 week old plants. Between 500 µg and 1 mg of high quality DNA was obtained from each extraction and quantified by spectrophoto-

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Table 1 List of cassava germplasm used in the present study and their distinctive morphological characters.

Name	Stem color	Apical leaf color	Petiole color	Shape of central leaf	Apical pubescence	Tuber skin color	Rind color	Flesh color	Neck	Flowering	Periderm
ID1	R	R	G	LA	A	LB	C	C	S	-	LG
ID2	R	P	DR	E	P	B	PI	W	L	-	G
ID3	LB	P	R	L	A	LB	W	W	L	-	LG
ID4	GR	G	G	LE	A	LB	LPI	C	S	-	DG
ID5	G	G	PI	L	A	LB	LPI	C	S	-	LG
ID6	LB	G	BR	E	A	LB	W	W	S	-	LG
ID7	DB	G	G	L	A	LB	C	Y	S	-	LG
ID8	LG	PI	PI	E	A	LB	C	W	S	-	LG
ID9	LB	G	DR	L	A	LB	C	W	S	-	LG
ID10	DB	LP	P	E	P	DB	PI	W	L	F	DG
ID11	LB	G	G	L	A	LB	PI	C	S	F	DG
ID12	LB	LP	P	E	P	LB	C	W	L	-	DG
ID13	LB	P	DP	L	P	LB	C/PI	W	S	-	G
ID14	LB	R	R	L	P	LB	PI	W	S	-	G
ID15	LB	G	P	E	P	DB	C	W	L	-	DG
ID16	LB	G	G	E	A	LB	C	W	S	-	LG
ID17	GR	G	P	LE	A	LB	C/PI	W	S	-	DG
1D18	GR	G	G	L	A	LB	C	W	S	-	LG
ID19	DB	G	P	LE	P	LB	C	W	L	-	DG
ID20	LB	G	G	L	A	LB	C	W	S	-	LG
ID21	DB	LP	P	E	A	LB	C	W	S	-	G
ID22	DB	LP	P	L	A	LB	C	W	S	-	DG
ID23	LB	G	P	LE	A	LB	LP	C	L	-	DG
ID24	GR	LP	P	E	P	LB	LP	W	S	-	LG
ID25	LB	LP	P	L	A	B	PI	W	S	-	G
ID26	B	LP	R	L	A	B	PI	W	L	-	LG
ID27	B	LP	R	E	A	LB	R	W	S	-	LG
ID28	LB	LP	P	L	P	LB	PI	W	S	-	G
ID29	LB	GP	G	E	P	LB	C	W	S	-	G
ID30	LB	G	G	LA	P	C	PI	W	S	-	G
ID31	LG	P	RP	L	A	LB	C	W	S	-	DG
ID32	LB	PG	G	L	A	LB	C	C	L	-	LG
ID33	SG	G	G	LA	A	C	C	W	L	-	LG
ID34	LB	R	R	E	A	C	C	W	L	-	LG
ID35	LB	GP	P	L	A	LB	PI	W	S	-	LG
ID36	LB	GP	P	E	P	B	R	W	S	-	LG
ID37	LB	G	PI	LA	A	LB	LP	C	S	-	LG
ID38	B	G	PI	E	P	DB	PI	W	L	F	DG

B-Brown, G-Green, LG-Light green, DG-Dark green, P-Purple, PI-Pink, C-Cream, W-White, R-Red, LP-Light purple, S-Short, L-Long, F-Flower, A-Absent, P-Present

meter. A set of 36 SSR markers developed at CIAT (Chavarriaga-Aguirre *et al*. 1998; Mba *et al*. 2001) was used for amplification (**Table 2**).

PCR parameters and gel analysis

The 36 combinations of forward and reverse SSR primers were used for amplification of 25 µl reaction mixture consisting of 10 mM Tris, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mM dNTP, 20 ng of genomic DNA, 0.2 µm of each forward and reverse primer and 1 unit of *Taq* polymerase was prepared. Samples were subjected to one step of 2 min at 95°C and 40 steps of 30 sec at 94°C, 1 min at 55°C, 1 min at 72°C and then a final step of 5 min at 72°C. PCR products were separated on a 2% agarose gel in TBE buffer at 90 V for 2 or 3 h and detected by staining with ethidium bromide (10 µg/ml), following Pinto *et al*. (2005) with suitable modifications (instead of 29 steps 40 steps were used).

Scoring gels and data analyses

Each band was scored as present (1) or absent (0) and cluster analysis of the SSR 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 SSR data and resulting dendrogram was compared.

RESULTS AND DISCUSSION

All 38 varieties showed clear banding pattern for SSR markers (**Fig. 1**). Each primer showed 1 to 2 bands. A total of 1273 DNA bands were scored, of which 15 bands were found to be polymorphic. Similarity Index based on presence or absence of a specific band showed that the genetic similarity between varieties in this region varied from 44 to 90% (**Table 3a, 3b**). This is a wide variability considering the fact that only very few varieties were introduced to the area. Similar results were reported in African cultivars by Marmey *et al.* (1994), using RAPD markers. Though only a limited number of genotypes were introduced to Africa from Latin America, the intraspecific analysis showed very high variability. The dendrogram constructed, based on the Similarity Index is given in **Fig. 2**. Thirty eight varieties of cassava collected from Idukki district and adjoining areas were grouped into 5 clusters. At 0.45 coefficient 2 clusters A and B are distinguished. At 0.51 coefficient 2 subclusters of B are evident. They are B1 and B2. Again at 0.56 coefficient 2 subclusters of B1 are evident. They are B1.1 and B1.2. At coefficient 0.54 two subclusters of B2 are seen, which is represented by B2.1 and B2.2. The number of varieties in a cluster ranged from 4 to 13 (**Table 4**). The varieties which come under cluster A have some common characters like stem colour (LB), periderm colour (G), flesh colour (white), small neck, presence of apical pubescence and absence of flower. However, the varieties which come under cluster B do not have many common characters except, in cluster B2.1, where all the 4 varieties have brown

Table 2 Sequence of SSR primers used for amplification.

№	Sequence (5′-3′): top, forward; bottom, reverse
1	GGTAGATCTGGATCGAGGAGG
	CAATCGAAACCGACGATACA
2	CGACAAGTCGTATATGTAGTATTCACG
	GCAGAGGTGGCTAACGAGAC
3	ACTGTGCCAAAATAGCCAAATAGT
	TCATGAGTGTGGGATGTTTTTATG
4	AGTGGAAATAAGCCATGTGATG
	CCCATAATTGATGCCAGGTT
5	AACTGTCAAACCATTCTACTTGC
	GCCAGCAAGGTTTGCTACAT
6	TGTCCAATGTCTTCCTTTCCTT
	CTTTTTGCCAGTCTTCCTGC
7	TGTGACAATTTTCAGATAGCTTCA
	CACCATCGGCATTAAACTTTG
8	CAACAATTGGACTAAGCAGCA
	CCTGCCACAATATTGAAATGG
9	AGGTTGGATGCTTGAAGGAA
	GGATGCAGGAGTGCTCAACT
10	CATTGGACTTCCTACAAATATGAAT
	TGATGGAAAGTGGTTATGTCCTT
11	GGAAACTGCTTGCACAAAGA
	CAGCAAGACCATCACCAGTTT
12	AGTGCCACCTTGAAAGAGCA
	TTGAGTGGTGAATGCGAAAG
13	CGTTGATAAAGTGGAAAGAGCA
	ACTCCACTCCCGATGCTCGC
14	CAGGCTCAGGTGAAGTAAAGG
	GCGAAAGTAAGTCTACAACTTTTCTAA
15	AAGGAACACCTCTCCTAGAATCA
	CCAGCTGTATGTTGAGTGAGC
16	GTACATCACCACCAACGGGC
	AGAGCGGTGGGGCGAAGAGC
17	AAGACAATCATTTTGTGCTCCA
	TCAGAATCATCTACCTTGGCA
18	ACCACAAACATAGGCACGAG
	CACCCAATTCACCAATTACCA
19	AACGTAGGCCCTAACTAACCC
	ACAGCTCTAAAAACTGCAGCC
20	TCGAGTGGCTTCTGGTCTTC
	CAAACATCTGCACTTTTGGC
21	TCAAACAAGAATTAGCAGAACTGG
	TGAGATTTCGTAATATTCATTTCACTT
22	GCAATGCAGTGAACCATCTTT
	CGTTTGTCCTTTCTGATGTTC
23	GGCTGTTCGTGATCCTTATTAAC
	GTAGTTGAGAAAACTTTGCATGAG
24	ATAGAGCAGAAGTGCAGGCG
	CTAACGCACACGACTACGGA
25	TCTCCTGTGAAAAGTGCATGA
	TGTAAGGCATTCCAAGAATTATCA
26	CATGCCACATAGTTCGTGCT
	ACGCTATGATGTCCAAAGGC
27	ACAATTCATCATGAGTCATCAACT
	CCGTTATTGTTCCTGGTCCT
28	TTCCAGACCTGTTCCACCAT
	ATTGCAGGGATTATTGCTCG
29	CGATCTCAGTCGATACCCAAG
	CACTCCGTTGCAGGCATTA
30	CCAGAAACTGAAATGCATCG
	AACATGTGCGACAGTGATTG
31	GCTGAACTGCTTTGCCAACT
	CTTCGGCCTCTACAAAAGGA
32	TGAGAAGGAAACTGCTTGCAC
	CAGCAAGACCATCACCAGTTT
33	TTGGCTGCTTTCACTAATGC
	TTGAACACGTTGAACAACCA
34	CCTTGGCAGAGATGAATTAGAG
	GGGGCATTCTACATGATCAATAA
35	ATCCTTGCCTGACATTTTGC
	TTCGCAGAGTCCAATTGTTG
36	ACAATGTCCCAATTGGAGGA
	ACCATGGATAGAGCTCACCG

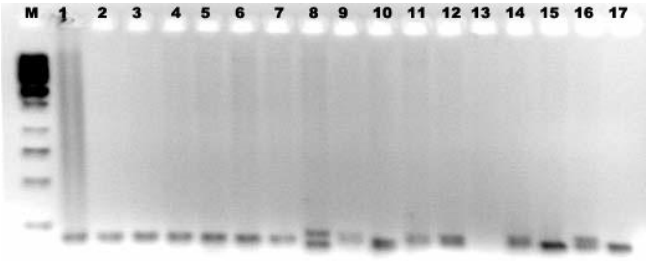

Fig. 1 Representative gel showing SSR marker profile of 17 accessions. Lane M showing 1 Kb molecular weight marker; Lane 1-17 showing SSR pattern of cassava accessions.

tuber skin. It is quite possible that if the DNA analysis is done using PAGE method, more resolution could have been obtained and more morphological variability could have been reflected in the DNA polymorphism (Ispizua *et al*. 2007). In this group of varieties taken for the study, 90% similarity is observed only between two varieties, ID-1 and ID-2. Both these varieties have a remarkably red stem. ID-1 is a newly evolved variety, with a sturdier stem and higher yield. It is quite possible that ID-1 has evolved from ID-2, and may be a natural seedling, selected and multiplied by farmers. The varieties ID-13, ID-28, ID-29, ID-30, ID-14 and ID-15 make a distinct cluster. Within the cluster also, two pairs ID-29 and 30 as well as ID-14 and 15 are closely related with 80 and 70% similarity, respectively. Morphological analysis of the data shows that they have some common features with a popular edible variety M4, introduced earlier. Most of them have a few leaves with three leaflets, a wild character which appears in segregating hybrids. Similarly, ID10 a high yielding variety which was reported earlier in local newspapers also resemble the introduced variety in certain characters, but it is branching type. ID38 a variety publicized recently in visual media also resemble the released variety, but it is highly branching, like the wild cassava, and its fertilizer responsiveness is very high. It is quite possible that they are chance seedling progenies of the same variety. The broad variability of cassava landraces found in this region reveals a valuable germplasm resource for cassava improvement. The high variability suggests that the species might have genes, in high frequencies, for adaptation to the area, and a high amount of additive genetic variance, upon which progress in plant breeding depends. Similar results were reported by Fregene *et al.* (2003) from Tanzania. It may also represent a heterotic gene pool and provide an opportunity for the systematic exploitation of hybrid vigour in cassava. The allogamous nature of cassava can produce a large pool of volunteer seedlings that natural and human selections can act on, to produce new varieties. Spontaneous recombination and farmer selection from the volunteer seedlings of new varieties is also occurring in this area. The overall effect of spontaneous recombination in the farmers' fields is able to maintain high levels of genetic variability. The production of new varieties not only maintains a high level of genetic variability but also serves as insurance against crop failure due to biotic and a biotic stresses. Many farmers in the area grow more than one variety. Hybridization between genetically distant varieties will give rise to a wide spectrum of variation in the progeny. This is essential for making selection from the hybrid population.

Our study showed that high variability exists in this collection. This region is important in terms of genetic diversity as cassava remained an important food crop in the hilly area, where low land for paddy cultivation was less. The forces affecting genetic differentiation in this area over the years could be analyzed. It is found that this region offers ideal climate for flowering and seed setting of cassava. Progressive farmers have the ingenuity to select healthy seedlings from the field, evaluate them and supply the promising varieties to others. This study showed good variabi-

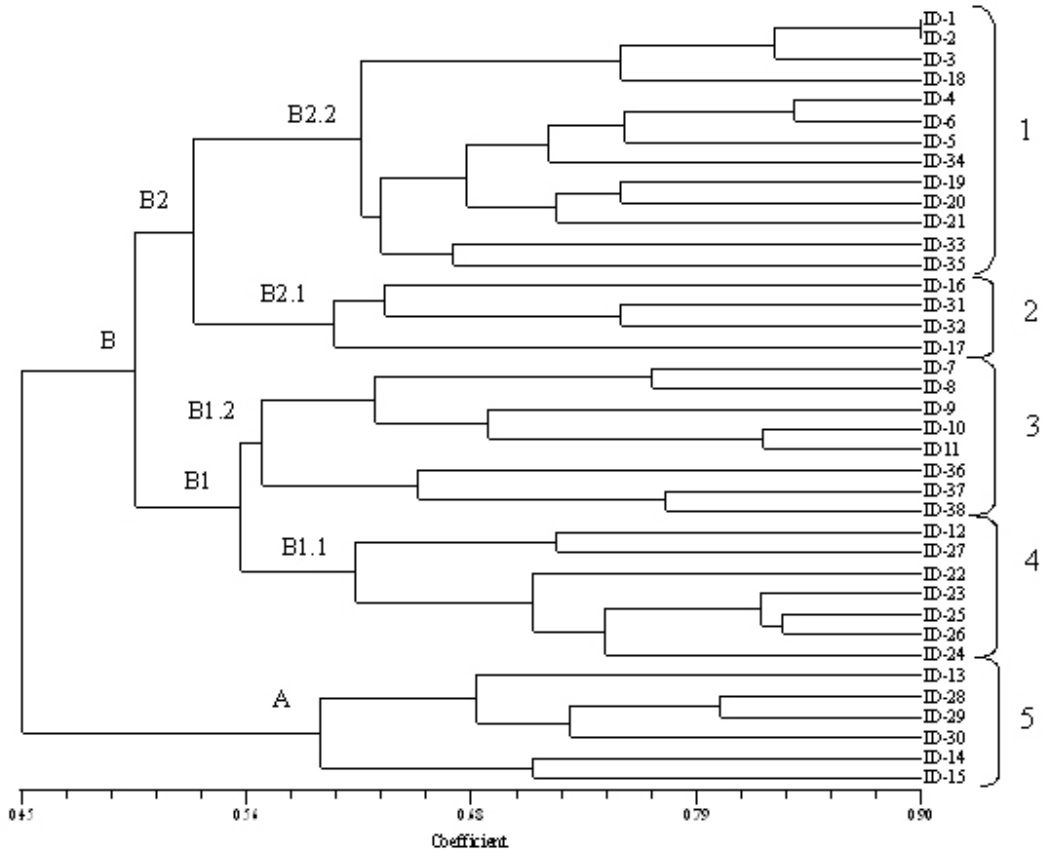

Fig. 2 UPGMA dendrogram based on similarity matrix constructed from the 38 varieties of cassava collected from Idukki district and adjoining areas.

lity in the cassava varieties collected from a typical cassava growing area. Information on genetic distance between varieties will be helpful in planning crossing program, between distantly related varieties from the collection, so that a wide spectrum of variation can be obtained in the hybrid progeny, for the breeders to make selection.

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Table 3a Genetic similarity matrix of 38 cassava genotypes based on SSR markers.

№	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.0																		
2	0.92	1.0																	
3	0.83	0.89	1.0																
4	0.75	0.77	0.82	1.0															
5	0.68	0.68	0.71	0.79	1.0														
6	0.77	0.75	0.76	0.86	0.79	1.0													
7	0.66	0.66	0.65	0.75	0.74	0.81	1.0												
8	0.59	0.61	0.56	0.68	0.69	0.74	0.81	1.0											
9	0.59	0.59	0.60	0.70	0.73	0.74	0.71	0.72	1.0										
10	0.58	0.60	0.61	0.69	0.68	0.73	0.74	0.71	0.77	1.0									
11	0.61	0.65	0.64	0.68	0.61	0.72	0.71	0.66	0.74	0.87	1.0								
12	0.51	0.53	0.54	0.58	0.57	0.58	0.67	0.58	0.58	0.71	0.68	1.0							
13	0.50	0.52	0.53	0.49	0.56	0.53	0.64	0.59	0.57	0.62	0.57	0.77	1.0						
14	0.50	0.54	0.49	0.49	0.54	0.51	0.60	0.61	0.55	0.56	0.51	0.67	0.76	1.0					
15	0.44	0.46	0.47	0.49	0.52	0.51	0.56	0.49	0.55	0.58	0.51	0.65	0.76	0.80	1.0				
16	0.67	0.67	0.68	0.62	0.61	0.64	0.65	0.60	0.56	0.59	0.50	0.60	0.57	0.59	0.57	1.0			
17	0.66	0.68	0.67	0.59	0.58	0.61	0.58	0.53	0.57	0.52	0.53	0.53	0.56	0.54	0.60	0.71	1.0		
18	0.78	0.80	0.85	0.71	0.68	0.67	0.58	0.53	0.59	0.62	0.61	0.57	0.50	0.50	0.50	0.65	0.72	1.0	
19	0.71	0.69	0.72	0.78	0.69	0.74	0.59	0.54	0.62	0.59	0.62	0.58	0.53	0.51	0.51	0.62	0.67	0.77	1.0

Table 3b Genetic similarity matrix of 38 cassava genotypes based on SSR markers.

№	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20
1	0.64	0.59	0.62	0.62	0.69	0.67	0.66	0.62	0.56	0.54	0.52	0.59	0.54	0.55	0.51	0.54	0.59	0.64	0.65
2	0.68	0.61	0.62	0.62	0.71	0.67	0.64	0.60	0.54	0.54	0.54	0.59	0.52	0.51	0.53	0.52	0.53	0.60	0.61
3	0.65	0.62	0.69	0.69	0.72	0.76	0.63	0.57	0.53	0.53	0.53	0.58	0.47	0.48	0.52	0.55	0.50	0.63	0.64
4	0.69	0.68	0.75	0.71	0.78	0.68	0.61	0.57	0.49	0.51	0.53	0.60	0.53	0.56	0.56	0.59	0.56	0.71	0.74
5	0.64	0.67	0.66	0.70	0.71	0.69	0.66	0.62	0.58	0.52	0.52	0.55	0.60	0.65	0.61	0.62	0.59	0.76	0.79
6	0.67	0.62	0.65	0.67	0.76	0.72	0.61	0.57	0.53	0.53	0.47	0.66	0.57	0.64	0.60	0.63	0.64	0.73	0.68
7	0.66	0.67	0.60	0.58	0.65	0.65	0.60	0.58	0.56	0.60	0.56	0.67	0.62	0.65	0.63	0.66	0.73	0.64	0.63
8	0.71	0.66	0.59	0.55	0.62	0.62	0.61	0.59	0.59	0.65	0.59	0.64	0.63	0.68	0.68	0.67	0.66	0.59	0.58
9	0.69	0.70	0.69	0.61	0.66	0.64	0.63	0.57	0.59	0.61	0.55	0.62	0.65	0.68	0.68	0.67	0.62	0.67	0.62
10	0.70	0.69	0.72	0.64	0.67	0.59	0.56	0.60	0.66	0.62	0.62	0.69	0.72	0.75	0.69	0.68	0.63	0.70	0.63
11	0.71	0.64	065	0.67	0.68	0.58	0.49	0.51	0.53	0.62	0.65	0.66	0.65	0.66	0.62	0.61	0.64	0.61	0.60
12	0.67	0.62	0.61	0.51	0.58	0.52	0.57	0.61	0.65	0.67	0.71	0.78	0.71	0.60	0.64	0.63	0.64	0.63	0.60
13	0.64	0.67	0.58	0.54	0.57	0.53	0.68	0.60	0.78	0.72	0.74	0.67	0.68	0.65	0.61	0.64	0.61	0.54	0.55
14	0.62	0.63	0.52	0.42	0.49	0.53	0.62	0.66	0.74	0.70	0.68	0.65	0.62	0.63	0.61	0.60	0.61	0.52	0.51
15	0.56	0.59	0.54	0.44	0.49	0.53	0.60	0.70	0.76	0.66	0.60	0.55	0.62	0.65	0.51	0.58	0.53	0.54	0.53
16	0.57	0.56	0.61	0.55	0.66	0.70	0.71	0.71	0.61	0.53	0.49	0.56	0.51	0.50	0.64	0.55	0.52	0.59	0.54
17	0.58	0.57	0.60	0.64	0.71	0.73	0.72	0.68	0.54	0.56	0.52	0.59	0.56	0.55	0.59	0.64	0.63	0.62	0.63
18	0.66	0.59	0.70	0.66	0.67	0.77	0.66	0.66	0.58	0.56	0.56	0.57	0.52	0.49	0.59	0.56	0.57	0.66	0.73
19	0.63	0.60	0.69	0.71	0.76	0.68	0.65	0.57	0.53	0.53	0.57	0.62	0.61	0.56	0.66	0.67	0.70	0.77	0.80
20	0.63	0.64	0.71	0.75	0.70	0.70	0.63	0.61	0.55	0.57	0.59	0.62	0.67	0.66	0.68	0.67	0.70	0.79	1.0
21	0.64	0.67	0.70	0.68	0.69	0.63	0.64	0.64	0.58	0.54	0.58	0.67	0.76	0.75	0.75	0.80	0.73	1.0	
22	0.67	0.64	0.59	0.63	0.64	0.66	0.59	0.79	0.59	0.65	0.67	0.78	0.77	0.74	0.78	0.83	1.0		
23	0.70	0.69	0.62	0.64	0.65	0.63	0.64	0.62	0.68	0.68	0.68	0.75	0.86	0.85	0.85	1.0			
24	0.71	0.62	0.67	0.61	0.66	0.62	0.61	0.61	0.61	0.69	0.65	0.72	0.77	0.76	1.0				
25	0.67	0.66	0.63	0.61	0.62	0.58	0.61	0.65	0.71	0.67	0.65	0.74	0.87	1.0					
26	0.72	0.71	0.62	0.62	0.59	0.55	0.60	0.60	0.72	0.72	0.74	0.81	1.0						
27	0.77	0.66	0.65	0.59	0.66	0.58	0.55	0.57	0.67	0.71	0.77	1.0							
28	0.72	0.67	0.62	0.54	0.59	0.53	0.58	0.60	0.76	0.84	1.0								
29	0.66	0.67	0.58	0.50	0.53	0.57	0.62	0.64	0.82	1.0									
30	0.64	0.63	0.62	0.50	0.55	0.55	0.68	0.70	1.0										
31	0.62	0.57	0.62	0.52	0.57	0.69	0.80	1.0											
32	0.62	0.63	0.62	0.56	0.69	0.37	1.0												
33	0.65	0.60	0.62	0.73	0.72	1.0													
34	0.67	0.62	0.71	0.73	1.0														
35	0.68	0.67	0.74	1.0															
36	0.76	0.71	1.0																
37	0.83	1.0																	
38	1.0																		

Table 4 List of varieties in different clusters.

Cluster №	№ of varieties
1 (B2.2)	13 (ID-1, 2, 3, 18, 4, 6, 5, 34, 19, 20, 21, 33, 35)
2 (B2.1)	4 (ID- 16, 31, 32, 17)
3 (B1.2)	8 (ID-7, 8, 9, 10, 11, 36, 37, 38)
4 (B1.1)	7 (ID-12, 27, 22, 23, 25, 26, 24)
5 (A)	6 (ID-13, 28, 29, 30, 14, 15)

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