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Research Article

Genetic variability and interrelationship among biochemical traits of Indian clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) germplasm

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Abstract
Forty two Indian clusterbean genotypes were subjected to biochemical characterization. Biochemical profiling revealed the presence of ample amount of variation for crude protein, crude fibre, crude fat, ash, carbohydrate and guar gum content. Correlation analysis among the profiles expressed crude fibre positively and significantly correlated with ash content, but negatively and significantly correlated with crude protein. Carbohydrate showed significant and negative correlation with crude protein. Gum content showed positive and significant correlation with crude protein, but negative association with ash and carbohydrate content. High crude fat and ash content was found in the genotypes namely Amrit11, PNB, HVG2-30, T local and M local. These genotypes can be used for vegetable purpose as their length of the pod is higher than other genotypes. The genotype HFG119 has recorded high crude fat, ash and fibre content, which are the reasons attributed for the suitability as a fodder. Genotypes namely HGS16, RGC 1066, RGC1033, GAU 513, HGS870, MRSG6, RGM 1, RGC1002, SRG1008 suitable for gum extraction, after that they can be used for guar meal. Clustering based on proximate analysis distinguished the entire genotypes into gum producing and non gum producing genotypes.

Key words
Clusterbean, guar gum, biochemical profiling, correlation, cluster analysis

Introduction

Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) is a drought tolerant annual legume crop, known for its versatile usage like gum extraction, vegetable, fodder, green manure and medicinal purpose. Guar for gum extraction largely cultivated in *kharif* (rainy season) season across the Northern India especially states like Rajasthan, Haryana, Gujarat, Punjab, parts of Uttar Pradesh and Madhya Pradesh in India. The significantly higher prices of guar in recent time (after shale rush in India) triggered to expand the crop into non-traditional regions and seasons. The crop is now being cultivated in dry tracts of Chhattisgarh, Andhra Pradesh, Karnataka, Tamil Nadu and other parts in *kharif* as well as in summer season. The crop has also been able to compete with other *kharif* crops like groundnuts, pearl millet, sorghum, cotton, etc (NIAM 2014). Since the crop has deep root system and can able to grow very well with limited irrigation even as a rainfed crop. The crop is now grown on deep black, clay type of soils along with sandy and red soils, especially in South India, the crop has been now grown in all three seasons due to its conducive climate (NIAM 2014).

The dicot clusterbean seed from the outside to the interior consists of three major fractions, *viz.*, the germ (43-47%), endosperm (35-42%) and husk or hull (14-17%). The endosperm fraction of its seed is rich in galactomannan type of gum and is popularly known as *guar* gum or guaran, while the germ and hull portion is termed as *guar* meal which is obtained after the extraction of gum. It is rich in protein and used as animal and poultry feed (Rodge 2008).

Guar gum has been used in different industries *viz.*, shale industries (oil and gas well drilling - oil fracking), paper industries, textile industries, food industries, cosmetics, mining, pharmaceuticals, *etc.* Guar gum has emerged as the most important agro-chemical because of its non-toxic, eco-friendly nature among synthetic gums and Food and Drug Administration (FDA) designated it into Generally Recognized As Safe (GRAS)(Lee *et al.*2004). Foreign exchange earned from its export has increased from Rs 142 crores in 1994 to Rs 21,287 crores during 2013 in India (APEDA 2014); hence clusterbean is transited from neglected legume into industrial crop.

Limited work has been done on qualitative traits analysis and less information is available on genetic variability in clusterbean pertaining to qualitative traits. The present study was formulated to assess the extent of genetic variability and association of qualitative characters, *viz.*, crude protein, crude fat, crude fibre, ash, carbohydrate and gum contents among 42 elite genotypes of cluster bean.

Materials and Methods

A set of 42 elite genotypes collected across India, representing the different eco-geographical diversity for clusterbean, were subjected to various

biochemical analyses) at Department of Forage crops, Centre for Plant Breeding and Genetics, TNAU, Coimbatore(Table 1).

A set of two random sampling from each genotype was used for biochemical analysis. Harvested seeds were oven dried at 60°C for one week to eliminate the moisture content before analysis of proximate compositions. The seeds were ground and flour of 32 mesh size was used to assess for crude protein, crude fibre, crude fat, ash carbohydrate and gum.

Crude protein: The crude protein content of ground seed samples was determined by the micro Kjeldhal method (Stuart, 1936). The nitrogen content thus obtained was multiplied by a factor 6.25 (Dubetz and Welis 1968) to obtain the crude protein content.

Crude fibre: One gram of air dried seed sample was boiled and digested with 1.25 % sulphuric acid and 2.5% NaOH and then filtered with muslin cloth. The residues on muslin cloth were washed with double distilled water, to remove dissolved solid. The residue was dried at 100ºC for overnight and weighed (Georing and Van Soest 1970).

Crude fat: The crude fat was estimated by subjecting the two gram weight of ground seed sample to continuous extraction with petroleum ether in a Soxhlet apparatus. Once the extraction was completed, the petroleum ether was evaporated and the residue was dried and weighed. The fat content was recorded on per cent basis (AOAC 1970).

Ash: The ash content of the sample was determined by the method described by Hart and Fisher (1971). Five gram of seed sample (fine form) was weighed into a porcelain dish that had previously been weighed. This was dried at 100ºC for three hours in an oven. The dish with content was transferred to a muffle furnace and ignited at 500ºC until free from carbon. This was removed from the oven and the ash moistened with a few drops of water. The ash was re-dried in the oven at 100ºC for 3 hours and re-ashed in the furnace at 500ºC for another one hour. This was removed from the muffle furnace, allowed to cool for a moment, placed in a desiccators until it cooled, and was then weighed. The percentage ash was calculated as follows: % of Ash = {(B-C)/A } × 100. where, A = sample weight prior to drying B = weight of dish and contents after ashing C = weight of empty dish.

Carbohydrate: Total carbohydrate percentage was calculated by difference method as nitrogen free extractive (McDonald *et al.* 1973). The percentage carbohydrate content of seed was determined by summing up the percentages of moisture, ash, crude protein, fat (ether extract) and subtracting from 100. The difference in value was taken as the percentage total carbohydrate content of seed.

Gum: The most reliable and accurate method of gum estimation involve the extraction and purification of galactomannan (gum), which is then alcohol precipitated, dried and weighted. A rapid and accurate colorimetric method developed by Das *et al.* (1977) and improved by Joshi (2004) was used.

Seed samples were ground using Cyclotec Grinding Mill (0.2mm screen size) and 0.1g of ground sample was weighed in a 100 ml conical flask to which of 40 ml of $HgCl_2$ (0.01M) was added. The sample was autoclaved for 1 hr at 15 psi after which the samples cooled and made up the volume up to 100 ml using HgCl2(0.01M). After vigorous shaking the sample was centrifuged at 5000 RPM at room temperature for 10 minute and 0.5 ml supernatant was taken and further 4.5ml of ethyl alcohol was added to make up into 90 per cent alcohol and kept overnight. The solution is centrifuged for 15 min at 5000 rpm, discarding the supernatant and the residues dissolved in 0.1 M $HgCl_2$ (5ml) by keeping in boiling water bath for 1 hour. Finally the volume was made upto 5ml with distilled water. The sample was shaken vigorously and the estimation of sugar was done as per the method by Das *et al.* (1977).

One ml of extract was added with 2 ml phenol(2%) and to this, 5 ml concentrated sulphuric acid (GR) was mixed. The mixture was shaken vigorously, allowed to cool for half an hour and reading was taken on spectro-photometer at 490 nm. Standard and blank was run simultaneously. Blank was run with mercuric chloride solution 0.01M. Standard curve was prepared by using galactose and mannose in 1:2 ratio.

The method as detailed above *i.e.* preparation of gum by alcohol precipitation and estimation by colorimetric method was followed.

Correlation analysis: Phenotypic correlation revealed association between various proximate compounds with each other as per the standard method

*Cluster analysis:*Cluster analysis was performed as per the Ward's minimum variance method (Ward 1963).

Results and Discussion

Analysis of variance for the biochemical profiles revealed that the genotypes significantly differed from each other (Table 2).

Crude Protein: The crude protein showed an average of 30.68%. The genotype HGS832 showed

higher mean value (34.6%) and the genotype Amrit 11 was lower (21.9%). Thirteen genotypes namely RGC1055, HGS3-2, HGS2-1, GAU513, HGS870, HGS2-20, HGS365, HGS16, RGC1033, RGC1038, HGS2-4, HGS3-52 and HGS832 well performed above the average (Table 3).

Crude fat: An average of 3.57% was observed for crude fat. The genotype HGS870 showed higher mean value (4.9%) and the genotype HGS2-20 was lower (2.29%). Nineteen genotypes namely MRG1786, HVG2-30, Amrit11, RGC471, PNB, MRSG6, SRG1058, RGC197, M local, GAU512, T local, HGS16, RGM1, RGM2, HFG119, RGC1066, RGC1055, GAU513 and HGS870 well performed above the average (Table 3).

Crude fibre: The crude fibre showed an average of 6.16%. The genotype MRSG6 showed higher mean value (7.91%) and the genotype HGS2-4 was lower (3.84%). Six genotypes namely SRG1058, HFG119, RGC1002, RGC1066, R local and MRSG6 performed above the average (Table 3).

Ash: The ash was recorded with an average of 4.25%. The genotype M local showed higher mean value (6.1%) and the genotype HGS2-20 was lower (3.25%). Eight genotypes namely PNB, RGC1002, RGC1033, HFG119, Amrit 11, T local, HVG2-30 and M local performed above the average (Table 3).

Carbohydrate: The total carbohydrate showed an average of 47.52%. The genotype Amrit 11 showed higher mean value (54.35%) and the genotype HGS16 was lower (42.75%). Five genotypes namely SRG1058, RGM1, FS277, PNB and Amrit11 performed above the average (Table 3).

Gum Percentage: The gum content showed an average of 26.88%. The genotype RGC1002 showed higher mean value (35.01%) and the genotype showed lower mean value (12.84%). Twenty four genotypes namely HGS2-1, GAU512, HGS16, HGS2-4, HGS182, HGS75, HGS870, HGS365, HGS3-2, HGS258, RGC1055, HGS3-52, RGC1031, RGM1, RGC936, MRG1786, RGC197, RGM2, RGC986, RGC1066, HGS884, RGC471, MRSG6 and RGC1002 performed above the average (Table 3).

Biochemical profiling: Biochemical profiling categorized the genotypes for crude protein, crude fat, crude fibre, ash, carbohydrate and gum content. High crude fat and ash content was found in the genotypes namely Amrit11, PNB, HVG2-30, T local and M local. In case of biochemical profiling, the genotypes namely Amrit11, PNB, HVG2-30, T local and M local performed above the average for the traits namely crude fat and crude ash content. Ash gives us an idea of the mineral matter contained in a plant. Among pulses, clusterbean seeds appear to have the highest ash content and mineral concentrations (Pathak *et al.* 2011). These genotypes are having the rich source for ash; green pods also source for Fe and Zn in the form of delicious green vegetable(Rodge 2008), hence, these five genotypes are much suitable as vegetable clusterbean.

Total carbohydrate content was more in the genotypes namely Amrit 11 and PNB. These two genotypes Amrit 11 and PNB are having the quality of edible pod and they are very much preferred as green vegetable.

The genotype HFG119 has recorded high crude fat, ash and fibre content, which are the reasons attributed for the suitability as a fodder. Rich source of fibre and ash mineral along with crude fat yields higher level of milk yield among cattle, and these kinds of genotype exhibits the glabrous and broad green leaves can be highly utilized as a fodder value, hence, the genotype HFG119 is very much preferred as fodder type. Clusterbean is a rich source of soluble fibre and known for their cholesterol lowering effect. The beneficial anti-hyper cholesterolaemic effect of whole dietary clusterbean as a source of dietary fibre was evaluated in high cholesterol diet induced hyper cholesterolaemia in experimental rats (Pande *et al.*2012).

The genotype HGS16 showed higher level of crude fat and crude protein content. High crude protein and ash content was recorded in the genotype RGC1033. While the crude protein, ash and gum content are considered together, the genotypes namely GAU513 and HGS870 are found suitable. The crude fat, ash and gum content are very high in RGC1066 and MRSG6. The crude fat, total carbohydrate and gum content were found together higher level in the genotype RGM1 and can be exploited for these characters. However, the genotype RGC1002 possesses high crude fibre, ash and gum content. As the only genotype having more than 35% of gum, this has been categorized under high gum group. The crude fat, crude fibre and total carbohydrate were found to be higher in the genotype SRG1058. The germ and outer seed-coat of guar seed together constitute guar meal. Removal of gum from guar seeds increases the protein content of the residual byproduct, *i.e.* guar meal. The guar seeds result in 62-68% of guar meal having a rich source of protein content by about 35-46%. It contains about 1.5 times more protein than guar seed and is compared well and other vegetable protein sources like oilseed cake used in poultry diets. The proximate composition and nutritive value of defatted guar meal, protein isolates and protein concentrate are beneficial for monogastric animals (Kumar and Rodge 2012). Hence, these genotypes namely HGS16, RGC 1066, RGC1033, GAU 513, HGS870, MRSG6, RGM 1, RGC1002, SRG1008 suitable for gum

extraction , after that they can be used as good source for guar meal industries. These genotypes may be used for dual purpose as gum and guar meal in further breeding programmes.

However, genotypes namely Amrit11, PNB, T local, HVG2-30 and M local possessed lengthy pods and higher 100 seed weight. Since these genotypes possess such a high pod length with poor single plant yield (grains), is highly preferred for vegetable purpose. Even though they have higher 100 seed weight, the pods are picked up before maturity; it can be used as vegetable. However, the higher seed weight is useful in quick germination of the genotypes an above this, may be found adaptable among farmers.

Crude fibre positively and significantly correlated with ash content, but negatively and significantly correlated with crude protein (Table 4 and Fig 1). Gum content showed positive and significant correlation with crude protein, but negative association with ash and carbohydrate content. As the vegetable clusterbean were rich in crude fibre and ash content in contrast they were low in gum content. Pathak *et al.* (2011) also observed the similar trend of association between gum with crude fibre and ash. Since, there is a possibility to combine the selection of fibre and ash together. Cluster analysis based on Ward's minimum variance method (Ward 1963) revealed that two major clusters among the 42 genotypes. In this all gum producing genotypes were grouped with one cluster and vegetable type clusterbean genotypes were on other clusters (Fig 2).

The wide variation in the chemical compositions of clusterbean genotypes from different bio-geographical regions shows that there is rich diversity found. Twenty four genotypes identified for having high gum content among 42 accessions. Genotypes namely PNB, T local, M local, HVG2-30 and Amrit 11 found with high crude fibre and ash content are suitable for vegetable purpose. HFG119 is identified for fodder purpose as it rich in crude fibre, crude fat and ash content. Genotypes namely HGS16, RGC 1066, RGC1033, GAU 513, HGS870, MRSG6, RGM 1, RGC1002 and SRG1008 are higher in gum, crude protein and crude fat, hence it is suitable for gum extraction, after that they can be used as good source for guar meal industries. These genotypes may be used for dual purpose as gum and guar meal in further breeding programmes. Gum content showed positive and significant correlation with crude protein, but negative association with ash and carbohydrate. Crude fibre and ash content exhibited positive and significant correlation, as evident that they are suitable for vegetable purpose. Based on cluster analysis, two clusters were formed; one consisted of 35 genotypes and another of seven genotypes. Cluster I occupied by the vegetable and fodder genotypes namely T local, M local, PNB, Amrit 11, HFG 119 and HVG2-30, in contrast cluster II possessed with gum genotypes only. It is clearly evident that proximate anlaysis distinguish the entire genotypes into gum producing and non gum producing genotypes.

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Table 1. Details of clusterbean germplam used in this study

S. No	Genotypes	Source
1	RGC197,RGC471, RGC936, RGC986, RGC1002, RGC1003, RGC1017, RGC1031, RGC1033, RGC1038, RGC1055,RGC1066, RGM1 and RGM2,	Rajasthan Agricultural Research Institute, SK Rajasthan Agricultural University, Durgapur, Rajasthan
2	HGS2-1,HGS2-4, HGS2-20, HGS3-2,HGS3-52,HGS16, HGS75, HGS182, HGS258,HGS365, HGS563, HGS832, HGS870,HGS884,HFG119, FS277 and HVG2-30	CCS Haryana Agricultural University, Hisar, Haryana
3	SRG1058, CAZG10-2,MRG1786 and MRSG6	Central Arid Zone Research Institute, Jodhpur, Rajasthan
4	GAU512 and GAU 513	Sardarkrushinagar Dantiwada Agricultural University, Krushinagar, Gujarat
5	PNB	Indian Agricultural Research Institute, Pusa, New Delhi
6	T local	Local landrace from Thiruvanamalai, Tamil Nadu
7	M local	Local landrace from Mettur, Tamil Nadu
8	Amrit 11	Local variety from Gujarat
9	R local	Local landrace from Rajasthan

Table 2. Analysis of variance for biochemical profile

Source	df	Mean Sum of Square (MSS)					
		Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate	Gum
Genotype	41	18.77[a]	1.18 [a]	1.76 [a]	1.14 [a]	12.63 [a]	19.33 [a]

[a] Significant at the 0.01 level

Table 3. Mean of various biochemical profiles of Indian clusterbean genotypes

S.No	Genotype	Crude protein (%)	Crude fat (%)	Crude fibre(%)	Ash (%)	Carbohydrate (%)	Gum (%)
1	RGC1002	30.55	2.48	7.27	5.00	46.7	35.01
2	HFG119	26.95	4.38	7.04	5.35	48.3	21.71
3	HGS884	30.25	3.09	6.22	4.05	48.4	32.80
4	HGS16	33.90	4.33	6.61	4.40	42.8	27.80
5	GAU512	31.35	4.24	5.22	4.05	47.2	27.58
6	HGS365	33.75	2.69	5.70	4.40	45.5	29.66
7	RGC1066	31.00	4.43	7.46	4.40	44.8	32.37
8	HGS75	30.90	2.95	5.07	4.30	48.8	29.35
9	HVG2-30	29.60	4.02	6.79	6.05	45.6	15.69
10	HGS2-4	34.40	2.47	3.84	3.45	47.9	28.12
11	RGC471	31.55	4.10	6.64	4.35	45.4	32.84
12	T local	25.40	4.29	6.52	6.00	49.8	18.96
13	RGC1017	31.35	2.64	5.57	4.05	48.4	22.37
14	HGS3-52	34.45	3.03	5.48	4.40	44.7	29.90
15	HGS563	31.40	2.80	5.59	3.45	48.8	26.80
16	RGM2	31.30	4.37	5.45	3.40	47.5	30.69
17	SRG1058	26.65	4.16	6.89	4.10	50.3	26.52
18	HGS2-20	33.05	2.29	6.18	3.25	47.3	27.15
19	RGC936	32.00	3.31	6.02	4.10	46.6	30.11
20	HGS258	31.85	3.48	6.59	3.55	46.6	29.78
21	RGC1055	32.35	4.78	6.33	3.55	45.0	29.85
22	HGS2-1	32.60	3.44	5.51	4.30	46.2	27.47
23	FS277	26.20	3.91	4.64	4.70	52.6	18.91
24	RGC197	30.65	4.16	5.27	3.45	48.5	30.57
25	Amrit11	21.90	4.07	6.25	5.45	54.4	15.32
26	HGS870	32.90	4.90	6.13	4.65	43.4	29.63
27	CAZG10-2	30.75	3.28	6.16	4.40	47.4	25.02
28	MRG1786	31.45	3.98	4.26	3.45	48.9	30.15
29	HGS182	32.05	2.48	4.20	3.60	49.7	28.14
30	RGC1038	34.35	2.38	4.72	4.20	46.4	26.07
31	M local	25.20	4.21	6.63	6.10	49.9	12.84
32	MRSG6	27.45	4.13	7.91	4.45	48.1	32.84
33	RGC1003	30.80	2.90	6.56	4.10	47.7	27.19
34	RGM1	28.10	4.34	4.93	3.25	51.4	30.08
35	R local	32.10	3.57	7.55	3.40	45.4	25.70
36	RGC986	30.51	3.64	6.75	4.15	47.0	31.80
37	PNB	23.00	4.12	6.57	4.90	53.4	18.21
38	GAU513	32.60	4.80	5.53	3.45	45.6	26.25
39	RGC1031	30.80	3.06	6.20	3.80	48.2	29.96
40	HGS3-2	32.35	3.24	5.50	4.35	46.6	29.72
41	HGS832	34.60	2.99	5.01	3.65	45.8	22.13
42	RGC1033	34.10	2.29	6.57	5.10	44.0	26.02
	Mean	30.68	3.57	6.16	4.25	47.5	26.88
	SEd	1.62	0.35	0.67	0.58	2.62	0.49
	CD(5%)	3.29	0.71	1.34	1.18	5.29	1.89

Table 4. Inter relationship among biochemical traits

	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate	Gum
Crude protein	1.000	-0.435[b]	-0.315[b]	-0.531[b]	-0.808[b]	0.556[b]
Crude fat		1.000	0.276	0.178	0.064	-0.146
Crude fibre			1.000	0.418[b]	-0.200	-0.009
Ash				1.000	0.136	-0.595[b]
Carbohydrate					1.000	-0.452[b]
Gum						1.000

[a] Significant at the 0.01 level; [b] Significant at the 0.05 level

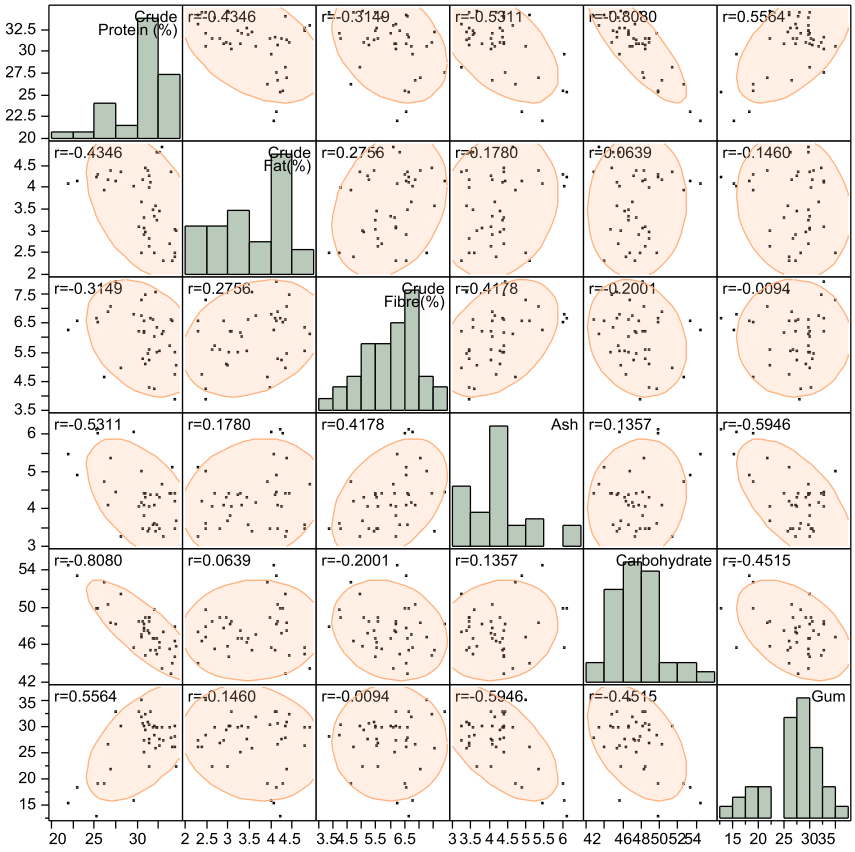

Fig.1 Scatter plot among the genotypes based on correlation

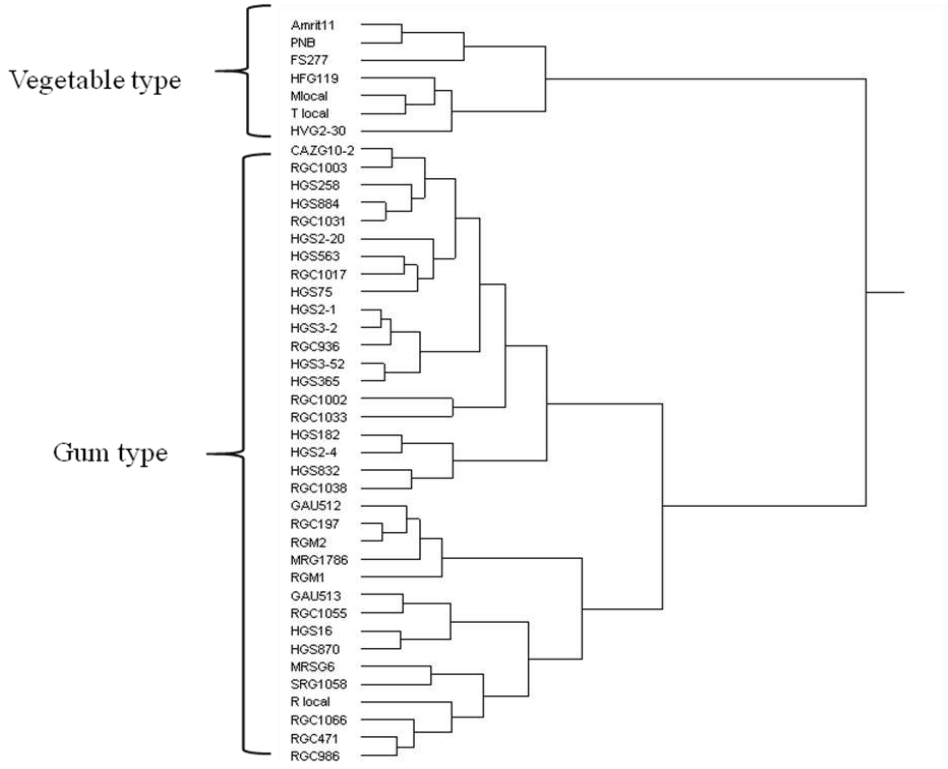

Fig.2 Dendrogram based on Ward's minimum variance method