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Identification of superior genotypes of Chalta (*Dillenia indica*) through germplasm characterization

Anant Tamang, Venkata Satish Kuchi, BC Das and Soumik Ray

Abstract
Dillenia indica belongs to genus Dillenia among 60 other species. Total ten (10) numbers of Chalta plants were stratified from different agroclimatic zones of West Bengal depending on availability of fruit crop and convenience of the study at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during the years 2013-2015. Under the observation for characterization, the plant physical parameters has been analyzed and so for preliminary evaluation fruit physical parameters and fruit biochemical parameters has been analyzed. Tree age of Chalta accessions varied from 7yrs to 30yrs with height of 10 m to above 20 m and 106.8 cm to 164 cm trunk girth. Branching pattern was observed to be irregular in different accession. Irregular crown shape and erect to spreading type tree growth habit is present in different accessions. Leaf characters in Chalta reveals elliptic leaf blade shape, apiculate leaf apex, cuneate leaf base, alternate spiral phyllotaxy (leaf orientation) and pinnate type leaf venation. Length of the leaf blade ranges from 32.28 cm to 52.64 cm and breadth varies from 10.62 cm (ACC-4) to 14.54 cm (ACC-2). Maximum fruit length was recorded in ACC-5 (8.32 cm), fruit breadth of 11.04 cm (ACC-10), maximum fruit weight (calyx and seed) 612.08 g (ACC-10) and non-edible weight of 102.00g (ACC-4). TSS % ranged from 4.48° Brix (ACC-9) to 6.86° Brix (ACC-10). Maximum total sugar of 3.42 mg/100 gm fruit pulp (ACC-4), reducing sugar 2.29 mg/100 gm (ACC-3) has been recorded along with 2.33 mg/100 gm fruit pulp acidity (ACC-2) and 34.32 mg/100 gm fruit pulp Vit.C (ACC-8). Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters, 5 clusters were formed for fruit physical parameters and 3 clusters were formed for fruit biochemical parameters.

Keywords: Chalta, physical parameters, total soluble solids, total sugar

Introduction
The genus Dillenia has 60 species, *Dillenia indica* is originated from southeastern Asia i.e. India, Sri Lanka east to southwestern China (Yunnan) and Vietnam, and south through Thailand to Malaysia and Indonesia (Lim, 2012) [11]. In India, it is distributed in Sub Himalayan tract, Assam, North Bengal, Bihar, Orissa, Madhya Pradesh, and Gujarat.

Fruits of chalta are globose, 10–15 cm in diameter, indehiscent, persistent sepals, fleshy and slightly swollen (Dipal and Priti, 2013) [6]. The fruit pulp is sour and used in Indian cuisine in curries, jam (Ouu khatta) and jellies. The fruits of *Dillenia indica* as well as *Dillenia pentagyna* were also eaten raw but not very much well known by people (Dubey *et al.*, 2009; Pradhan and Badola, 2008; Sharma and Pegu, 2011) [8, 13, 18]. A study in the Buxa Tiger Reserve by ecologists Sekar & Sukumar has shown that Asian elephants appear to have a particular fondness for the fruits of *Dillenia indica* (elephant apple) and are hence an important seed disperser for this tree.

Abdille *et al.*, (2005) [1] recorded 34% of total phenolics in methanolic extract and polysaccharide like anarabino galactan in fruits of *Dillenia indica*. The stem bark contains flavanoids like kaempferol, quercetin, isorhamnatin and rhamnetin3glucoside; terpenoids like lupeol, betunaldehyde, betulin, betulunic acid, mallic acid, asitosterol, stigmasterol and phenolics (Khanum *et al.*, 2007; Khare, 2007). *Dillenia indica* fruit extract most likely participates in the wound healing process as a result of its anti-inflammatory properties (Domenico *et al.*, 2011) [7]. There is no standard cultivar of *Dillenia indica*. Only one desi variety is found to grow in our country (Das and Das, 2003) [5].

To safeguard the existing diversity of this fruit and to achieve sustainable development based on use of available genetic wealth, promotion and conservation of this species is of immense importance. Morphological variation of a crop indicates the genetic diversity and effect from environment. Both environmental and genetic effects contribute to phenotypic variation within and among populations (Andrew *et al.*, 2010) [2].

Beside the importance for nutritional value and a source of income, diversity of this fruit also has a cultural and social value and contributes to the stability of ecosystems. A couple of researches on growth of minor fruits such as in cowphal (*Garciniacowa*) (Roy *et al.,* 2010) [16], china cherry (*Muntingia calabura*) (Rahman *et al.,* 2010) [14], deshi and bilati gab (Hasan *et al.,* 2014), report on chalta in Bangladesh and its fruit growth (Hasan *et al.,* 2015 & 2016) [9, 10] is available. Hence, the current study was conducted for

1. Morpho-taxonomic characterization of germplasms using Biodiversity International Tropical Fruit Descriptor.
2. To perform preliminary evaluation of the varieties/types of under-utilized fruit crops for their productivity and nutritional quality.

Materials and Methods

Different agroclimatic zones of West Bengal were stratified depending on availability of fruit crop and on convenience of the study. The study has been carried out in Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during the years 2013-2015.

Data for this study has been generated through key informant survey and individual household survey from respondents by using semi-structured questionnaire. *In-situ* characterization and preliminary evaluation of under-utilised/minor fruit Chalta (*Dillenia indica*) are performed following Biodiversity International Tropical Fruit Descriptor.

The general information like age of the plant, behaviour of the plant was documented by making a questionnaire at the time of the survey. The genotypes were selected randomly with their varying age and location, which were further given accession numbers considering each accession as a treatment and replicating it for five times for all the quantitative parameters.

The analysis has been carried in three parts according to the variation in age of the plants and observations to be recorded. Under the observation for characterization, the plant physical parameters, physical and biochemical parameters of fruit were analyzed according to NBPGR tropical fruit descriptors.

Multivariate analysis of characterization and evaluation parameters was done following nearest neighborhood method of hierarchical clusters analysis of squared Euclidean distance matrix on the basis of characters measured following the guidelines by Dillon and Goldstein (1984).

Result and Discussion

Total 10 numbers of Chalta plants were taken under the study as per availability from different locations. Ownership and location of the accessions were recorded as mentioned in Table 1.

Branching pattern of the plants was observed to be irregular in all accessions. Irregular crown shape and erect to spreading type tree growth habit was present in different accessions Table 2. Leaf characters in Chalta revealed elliptic leaf blade shape, apiculate leaf apex, cuneate leaf base, alternate spiral leaf orientation and pinnate leaf venation in all the observed accessions Table 3. Sunil *et al*., (2011) [19] reported the tree growing tall to 15m with fascicled leaves at the end of the branches, oblong-lanceolate, acuminate, 2030 cm long and sharply serrate.

The minimum proximity value 56.76 between ACC-2 and ACC-7 denoted the maximum similarity with respect to plant physical parameters followed by 144.04 between ACC-6 and ACC-10. The maximum value 4257.65 between ACC-3 and ACC-8 denoted the minimum similarity between the accessions Table 4. A study had been conducted in which different types of jack fruit, bael, ber, jamun and water apple have been identified with significant variation in leaf size and shape, weight, fruit shape and bio-chemical qualities Chakraborti *et al*., (2008) [13].

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters. The data presented in the Table 5 and Fig. 1 showed that 3 clusters (1, 3 and 4) comprised of homogeneous types having similarity in characterization attributes. Cluster 2, 5 and 6 compromised single accessions namely ACC-1, ACC-4 and ACC-8. The first accessions to form cluster with minimum distance was ACC-2 and ACC-7 at 56.76, later ACC-3 paired them at the distance of 158.20. Second cluster was formed with single accession ACC-1 whereas, ACC-6 and ACC-10 pairs at the distance of 144.04 to form the third cluster. Likewise, ACC-5 and ACC-9 forms the fourth cluster at the maximum allowed distance of co-efficient. Cluster fifth and sixth were formed with single accession each ACC-4 and ACC-8.

Gandhi (2013) reported *Dillenia indica* to posses medium sized evergreen trees up to 30 m tall, leaf blade oblong or obovate-oblong, fruits are aggregate and globose, 10–15 cm in diameter. *Dillenia pentagyna* trees up to 15 m tall, leaves are petiolate 2–5cm, shape is oblong to obovate-oblong, fruits are globose in shape, 0.5–1 cm in diameter.

Table 1: General experimental site details of Chalta accessions

Accession no.	Name of owner	Address	Site of characterization	
ACC-1	B.C.K.V.	Mohanpur (Nadia)	22°56'44.59"	088°32'60.56"
ACC-2	Animesh Roy	Salugara (Jalpaiguri)	26°42'45.54"	088°27'2.91"
ACC-3	Mukul Roy	Kalyani (Nadia)	22°54'52.60"	088°36'58.84"
ACC-4	B.C.K.V.	Jaguli farm (Nadia)	22°56'50.84"	088°32'23.26"
ACC-5	B.C.K.V.	Staff quarter (Nadia)	22°56'46.49"	088°32'10.53"
ACC-6	B.C.K.V.	Ladies hostel (Nadia)	22°56'44.59"	088°32'60.56"
ACC-7	Shefali Das	Fatepur, Jaguli (Nadia)	22°56'17.40"	088°35'16.87"
ACC-8	Nikhil Mondal	Bamanpara (Nadia)	22°55'7.11"	088°35'16.87"
ACC-9	Gulam Rahman	Anantpore (North 24 Paraganas)	22°39'49.83"	088°51'28.45"
ACC-10	Shariful mandol	Fatepur (Nadia)	22°56'17.40"	088°35'16.87"

Table 2: General tree characteristics of Chalta accessions

Accession no.	Branching pattern	Tree growth habit	Crown shape
ACC-1	Irregular	Spreading	Irregular
ACC-2	Irregular	Spreading	Irregular

ACC-3	Irregular	Spreading	Irregular
ACC-4	Irregular	Erect	Irregular
ACC-5	Irregular	Erect	Irregular
ACC-6	Irregular	Spreading	Irregular
ACC-7	Irregular	Erect	Irregular
ACC-8	Irregular	Spreading	Irregular
ACC-9	Irregular	Spreading	Irregular
ACC-10	Irregular	Spreading	Irregular

Table 3: Leaf characters of Chalta accessions

Accession no.	Leaf blade shape	Leaf apex shape	Leaf base shape	Leaf Orientation	Leaf Venation
ACC-1	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-2	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-3	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-4	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-5	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-6	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-7	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-8	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-9	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate
ACC-10	Elliptic	Apiculate	Cuneate	Alternate spiral	Pinnate

Table 4: Proximity Matrix for Chalta plant physical attributes

	Squared Euclidean Distance								
	1:ACC-1	2:ACC-2	3:ACC-3	4:ACC-4	5:ACC-5	6:ACC-6	7:ACC-7	8:ACC-8	9:ACC-9
2:ACC-2	504.41								
3:ACC-3	227.28	200.77							
4:ACC-4	2066.81	1757.68	2494.33						
5:ACC-5	3229.69	2228.63	3390.98	272.28					
6:ACC-6	1163.48	369.04	1020.80	844.82	932.12				
7:ACC-7	339.78	56.76	115.62	2090.83	2728.76	550.09			
8:ACC-8	3882.47	3247.62	4257.65	454.10	375.06	1827.99	3749.14		
9:ACC-9	2309.41	1347.04	2250.23	321.71	186.47	481.19	1768.16	529.30	
10:ACC-10	1151.98	565.50	1175.68	369.17	581.45	144.04	824.99	1210.89	245.14
This is a dissimilarity matrix									

Table 5: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for Chalta plant physical characters

No. of clusters	Cluster members (Allowed distance co-efficient 186.47)
1	Acc-2, Acc-7, Acc-3
2	Acc-1
3	Acc-6, Acc-10
4	Acc-5, Acc-9
5	Acc-4
6	Acc-8

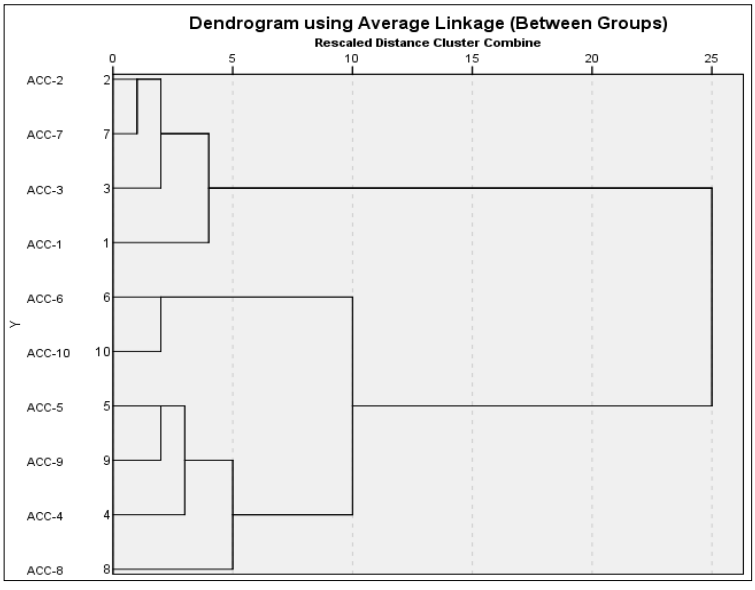

Fig 1: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Chalta plant physical attributes.

The value 15.04 between ACC-1 and ACC-9 denoted the maximum similarity followed by 101.50 between ACC-4 and ACC-5 with respect to fruit physical parameters. The value 95541.01 between ACC-2 and ACC-10 denoted the minimum similarity Table 6.

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 5 clusters were formed for fruit physical parameters. The data presented in the Table 7 and Fig. 2 shows that cluster 1 and 2 comprised of homogeneous types having similarity in characterization attributes. On the other hand cluster 3, 4 and 5 comprised single member namely ACC-10, ACC-2 and ACC-8. The first cluster comprised of ACC-1 and ACC-9 which paired at the distance of 15.04. Later ACC-3 pairs with the first formed cluster at the coefficient distance of 420.93. Similarly, the second cluster comprised of ACC-4 and ACC-5 which paired at the distance of 101.50 followed by ACC-6 and ACC-7 at the distance 187.42 and 448.19 respectively. Azad *et al*., (2016) working on morphological diversity among accessions of cinnamon stated that the genetic diversity could be used for exploitation of economically important traits in the future.

The minimum value 0.76 between ACC-4 and ACC-5 denoted the maximum similarity followed by 0.80 between ACC-5 and ACC-6 for fruit bio chemical characters. The maximum value 557.66 between ACC-3 and ACC-8 denoted the maximum dissimilarity among two accessions Table 8.

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 3 clusters were formed for fruit biochemical parameters. The data presented in the Table 9 and Fig. 3 shows that cluster 1 and 2 comprised of homogeneous types having similarity in characterization attributes. On the other hand cluster 3 comprised single member namely ACC-3. The first cluster was formed between ACC-4 and ACC-5 at the coefficient distance of 0.76 later followed by ACC-6 and ACC-10 at the distance 1.02 and 1.65. ACC-1 and ACC-2 pairs at the distance 3.01 and later ACC-7 pairs with ACC-1 and ACC-2 at the distance 4.20. Accessions ACC-1, ACC-2 and ACC-7 pairs with ACC-4, ACC-5, ACC-6 and ACC-10 at the coefficient distance of 6.09 respectively. Second cluster comprised of ACC-8 and ACC-9 which paired at the coefficient distance of 3.93.

Table 6: Proximity matrix for Chalta fruit physical attributes

	Squared Euclidean Distance								
	1:ACC-1	**2:ACC-2**	**3:ACC-3**	**4:ACC-4**	**5:ACC-5**	**6:ACC-6**	**7:ACC-7**	**8:ACC-8**	**9:ACC-9**
2:ACC-2	22476.41								
3:ACC-3	356.36	24154.92							
4:ACC-4	10964.80	63136.65	9199.41						
5:ACC-5	10490.21	62825.01	9088.98	101.50					
6:ACC-6	8261.14	57045.80	6973.30	233.79	141.04				
7:ACC-7	12111.38	67355.65	11028.56	548.56	215.67	580.36			
8:ACC-8	6295.78	4981.26	7337.49	32950.09	32569.42	28462.06	35739.20		
9:ACC-9	15.04	21662.70	485.49	11693.10	11171.82	8877.17	12792.31	5873.81	
10:ACC-10	25741.54	95541.01	23647.49	3483.26	3417.30	4938.44	2608.94	57060.34	26769.30
This is a dissimilarity matrix									

Table 7: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for Chalta fruit physical attributes.

No. of clusters	Cluster members (Allowed distance co-efficient 448.19)
1	Acc-1, Acc-9, Acc-3
2	Acc-4, Acc-5, Acc-6, Acc-7
3	Acc-10
4	Acc-2
5	Acc-8

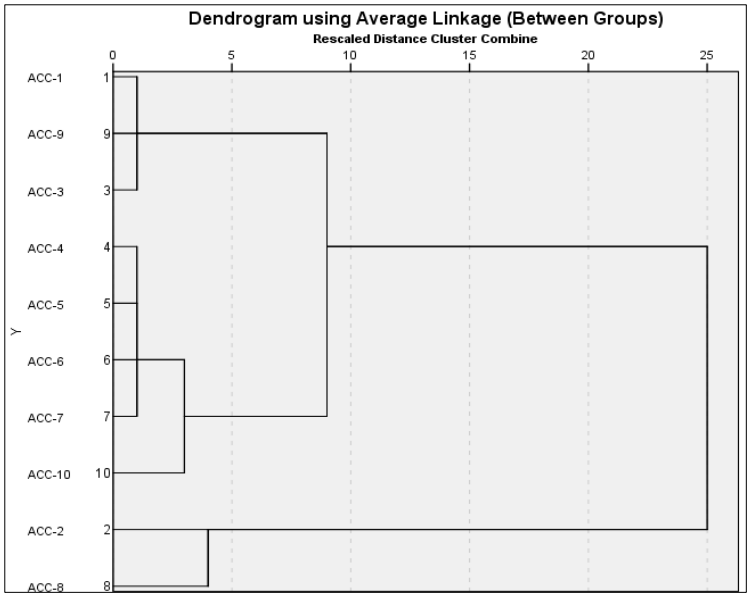

Fig 2: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Chalta fruit physical attributes.

Table 8: Proximity matrix for Chalta fruit biochemical attributes

	Squared Euclidean Distance								
	1:ACC-1	**2:ACC-2**	**3:ACC-3**	**4:ACC-4**	**5:ACC-5**	**6:ACC-6**	**7:ACC-7**	**8:ACC-8**	**9:ACC-9**
2:ACC-2	3.01								
3:ACC-3	175.44	139.98							
4:ACC-4	4.52	7.85	168.87						
5:ACC-5	2.72	5.65	174.39	.76					
6:ACC-6	3.78	4.84	155.34	1.25	.80				
7:ACC-7	3.91	4.49	170.43	8.26	4.54	4.73			
8:ACC-8	109.98	142.19	557.66	119.62	111.02	126.59	114.79		
9:ACC-9	82.36	109.04	489.43	94.96	85.45	98.66	83.42	3.93	
10:ACC-10	7.47	10.07	177.09	1.70	1.43	1.81	8.69	113.88	90.09
This is a dissimilarity matrix									

Table 9: Details of clusters for characterization variables using average linkage clustering methods on squared Euclidean distance matrix for Chalta fruit bio-chemical attributes.

No. of clusters	Cluster members (Allowed distance co-efficient 6.09)
1	Acc-4, Acc-5, Acc-6, Acc-10, Acc-1, Acc-2, Acc-7
2	Acc-8, Acc-9
3	Acc-3

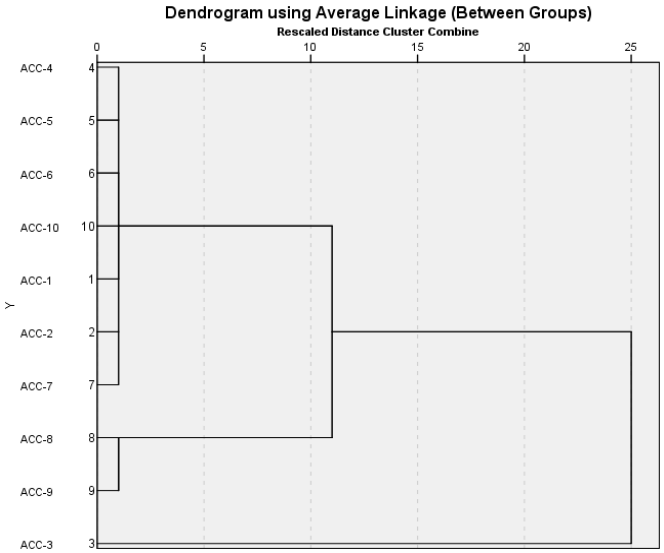

Fig 3: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Chalta fruit bio-chemical attributes.

Conclusion

Total ten (10) numbers of Chalta plants were taken under the study as per availability. Tree age of Chalta accessions varied from 7yrs to 30yrs with height of 10 m to above 20 m and 106.8 cm to 164 cm trunk girth. Branching pattern was observed to be irregular in different accession. Irregular crown shape and erect to spreading type tree growth habit is present in different accessions. Leaf characters in Chalta reveals elliptic leaf blade shape, apiculate leaf apex, cuneate leaf base, alternate spiral phyllotaxy (leaf orientation) and pinnate type leaf venation. Length of the leaf blade ranges from 32.28 cm to 52.64 cm and breadth varies from 10.62 cm (ACC-4) to 14.54 cm (ACC-2). According to Ravindran *et al.*, (2004) [15] leaf shape of cinnamon also varies from oval or elliptic to lanceolate-oval or narrowly elliptic, 3×7–8×25cm, leaf apex shortly or broadly acuminate and leaf base acutish or cuneate. A study by Wijesinghe and Gunarathna, (2001) [20] showed correlation between leaf size and shape with yield in seven different types of true cinnamon. Maximum fruit length was recorded in ACC-5 (8.32 cm), fruit breadth of 11.04 cm (ACC-10), maximum fruit weight (calyx and seed) 612.08 g (ACC-10) and non-edible weight of 102.00g (ACC-4). The present result regarding fruit weight is in partial conformity with the report of Bose *et al.*, (2002) [4] who reported that single fruit weight of *Dillenia* ranged between 400 and 600g. Hasan *et al.*, (2016) [10] has also reported the fruit weight attained maximum at 77 DAF with the value of 523 g. TSS % ranged from 4.48° Brix (ACC-9) to 6.86° Brix (ACC-10). Maximum total sugar 3.42 mg/100 gm fruit pulp (ACC-4), reducing sugar 2.29 mg/100 gm (ACC-3). Acidity 2.33 mg/100 gm fruit pulp (ACC-2) and Vit.C 34.32 mg/100 gm fruit pulp (ACC-8). (Mandal and Mazumdar 1995) [12], the average weight per fruit is 592 g, specific gravity as 0.98, stalk stylar length as 8.8cm, total sugars 0.61%, reducing sugars 0.43%, non reducing sugars 0.17%, total titratable acidity (equivalence of citric acid) 0.69% and the vitamin C 20 mg per 100 g of pulp.

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters, 5 clusters were formed for fruit physical parameters and 3 clusters were formed for fruit biochemical parameters.

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