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Genetic diversity characterization of Wood apple (*Feronia* spp.) for identification of superior genotypes

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Abstract

Wood apple fruit is a hard shelled many seeded berry with its pinkish brown aromatic sour – sweet pulp being the edible portion. Total eleven (11) numbers of wood apple 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 with the help of Biodiversity International Tropical Fruit Descriptor (NBPGR) and so for preliminary evaluation, fruit physical parameters and fruit biochemical parameters has been analyzed. During the course of survey the tree age of wood apple varied from 15yrs to above 40yrs in age at different germplasm in which the height and trunk girth varied from 10m to above 20m and 88.2 cm to 157.4 cm. Branching pattern and crown shape was observed to be irregular in all the accessions whereas, tree growth habit varied from erect to spreading type. Leaf characters in wood apple reveals obovate leaf blade shape, obtuse to retuse leaf apex, cuneate leaf base, odd pinnate leaf orientation and pinnate leaf venation. Length of the leaf blade ranges from 3.05 cm to 4.12 cm and breadth varies from 1.06 cm to 3.08 cm. Maximum fruit length was recorded in ACC-8 (8.04 cm) and breadth of 7.96 cm in ACC-6. Maximum fruit weight (306.02 g), peel weight (73.68 g) and number of seeds (28.42) was from the ACC-4. Maximum total sugar 28.57 mg/100 gm pulp (ACC-1), reducing sugar 13.88 mg/100 gm (ACC-4), acidity 1.77 mg/100 gm (ACC-5) and Vit. C 102.04 mg/100 gm (ACC-2) has been recorded. Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters, a total of 7 clusters were formed for fruit physical parameters and a total of 6 clusters were formed for fruit biochemical parameters.

Keywords: Wood apple, Branching pattern, Fruit parameters, Leaf characters

Introduction

The wood apple syns. *F. elephantum* Correa; *Limonia acidissima* L.; *Schinus limonia* L.) is the only species of its genus, in the family Rutaceae with 2n=18 chromosomes. Wood apple is native and common in the wild dry plains of India and Ceylon and cultivated along roads and edges of fields and occasionally in orchards. The fruit is a hard shelled many seeded berry with its pinkish brown aromatic sour – sweet pulp being the edible portion. Edible full ripe pulp contains, on an average, 69.5% moisture, 7.3% protein, 0.6% fat and 1.9% mineral matter. Total soluble solids content of pulp varies around 7.0, total acidity ranges between 3.0-2.3% and pectin content between 3-5% (Pal, S.L. *et al.*, 2018) [6] which adds up to nearly 140 calories. The wood apple is rich in Beta carotene (Vijayakumar, *et al.*, 2013) [10]. Wood apple fruit contains flavonoids, glycosides, saponins and tannins. There are reports that some coumarins and tyramine derivatives were also isolated from the fruits of *Limonia* (Ilango and Chitra, 2009) [4]. In India used as a liver and cardiac tonic, as an astringent means of halting diarrhea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, as is the powdered rind. Intensive work on germplasm collection, evaluation and improvement was done on lasora, karonda and khejri which resulted in identification of some high yielding genotypes in these crops (Meghwal, 2018) [5]. There has been a growing interest world over to explore, search and collect germplasm of plants which could have economical viability in near future (Rapoport *et al.* 1995) [7].

Genetic diversity is maintained in traditional agro-ecosystems by cultural intervention as well as by natural selection. To safeguard the existing diversity of wood apple and to achieve sustainable development based on use of available genetic wealth, promotion and conservation is of immense importance. Azad *et al.*, (2016) [1] 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 a) Morpho-taxonomic characterization of germplasm using Biodiversity International Tropical Fruit Descriptor and b) 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 Wood apple (*Feronia sps.*) 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 will be done following nearest neighborhood

method of hierarchical clusters analysis of squared Euclidean distance matrix on the basis of characters measured following Dillon and Goldstein (1984).

Result and Discussion

Total 11 numbers of accessions were taken from different locations according to convenience of the study Table 1. Branching pattern and crown shape was observed to be irregular in all the accessions whereas tree growth habit varied from erect to spreading type Table 2. Leaf characters in wood apple revealed that obovate leaf blade shape, obtuse to retuse leaf apex, cuneate leaf base, odd pinnate leaf orientation and pinnate leaf venation Table 3. A report has been documented where age of the wood apple plants varied between 13 and 70 years with yield potential in mother plants (Ghosh *et al.* 2012) [3].

The proximity value 11.96 between ACC-3 and ACC-6 denoted maximum similarity among accessions for plant physical parameters followed by 14.64 between ACC-2 and ACC-5. The maximum value 4959.61 denoted minimum similarity between ACC-9 and ACC-10 Table 4. Out of twelve germplasm accessions of Lasora (*Cordia myxa*) it was noted that there was not much variation in different accessions with respect to vegetative growth such as plant height and canopy area but significant variation was recorded in fruit yield per plant and fruit characteristics (Meghwal, 2018) [5].

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 clusters 1 to 3 comprised of homogeneous types having similarity in characterization attributes. Cluster 4, 5 and 6 comprised of single member namely ACC-8, ACC-9 and ACC-7. ACC-3 and ACC-6 formed the first cluster at the distance of 11.96 followed by second cluster between ACC-2 and ACC-5 at 14.64. ACC-1 and ACC-4 combines in third stage at the distance 26.83 which later combines at the distance 42.30 with first cluster formed between ACC-3 and ACC-6.

Table 1: General experimental site details of Wood apple accessions

Accession no.	Name of owner	Address	Site of characterization	
ACC-1	Mintu Roy	Salugara (Jalpaiguri)	26°74'88.54"	088°43'82.91"
ACC-2	Shariful Mondal	Fatepur (Nadia)	22°56'17.40"	088°35'16.87"
ACC-3	Subhajeet Ghosh	Kanchrapara (North 24 paraganas)	22°94'91.90"	088°42'09.91"
ACC-4	Gulam Rahaman	Anantpore (North 24 paraganas)	22°39'49.83"	088°51'28.45"
ACC-5	B.C.K.V.	Mandouri farm (Nadia)	22°56'51.09"	088°31'1.58"
ACC-6	Masihar Rahaman	Anantpore (North 24 paraganas)	22°39'49.83"	088°51'28.45"
ACC-7	Chakka Tula Mondal	Anantpore (North 24 paraganas)	22°39'49.83"	088°51'28.45"
ACC-8	Kazi Abdul Gani	Fatepur (Nadia)	22°56'17.40"	088°35'16.87"
ACC-9	Arun Biswas	Baikara (Nadia)	22°54'52.60"	088°36'58.84"
ACC-10	Ashish koley	Haripal (Hoogly)	22°86'77.69"	088°36'76.32"
ACC-11	Sahuji Bandyopadyay	Canning (South 24 paraganas)	22°31'12.58"	088°65'79.42"

Table 2: General tree characteristics of Wood apple accessions

Accession no.	Branching pattern	Tree growth habit	Crown shape
ACC-1	Irregular	Errect	Irregular
ACC-2	Irregular	Errect	Irregular
ACC-3	Irregular	Errect	Irregular
ACC-4	Irregular	Errect	Irregular
ACC-5	Irregular	Errect	Irregular
ACC-6	Irregular	Spreading	Irregular

ACC-7	Irregular	Errect	Irregular
ACC-8	Irregular	Errect	Irregular
ACC-9	Irregular	Spreading	Irregular
ACC-10	Irregular	Errect	Irregular
ACC-11	Irregular	Errect	Irregular

Table 3: Leaf characters of Wood apple accessions

Accession no.	Leaf blade shape	Leaf apex shape	Leaf base shape	Leaf Orientation	Leaf Venation
ACC-1	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-2	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-3	Obovate	Obtuse Retuse	Cuneate	Odd pinnate	Reticulate
ACC-4	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-5	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-6	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-7	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-8	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-9	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-10	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate
ACC-11	Obovate	Obtuse-Retuse	Cuneate	Odd pinnate	Reticulate

Table 4: Proximity Matrix for Wood apple 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	10:ACC-10
2:ACC-2	87.39									
3:ACC-3	29.41	184.42								
4:ACC-4	26.83	94.19	24.58							
5:ACC-5	52.22	14.64	109.15	41.09						
6:ACC-6	52.85	262.27	11.96	62.36	179.97					
7:ACC-7	1052.42	565.93	1387.15	1112.48	746.36	1565.86				
8:ACC-8	2489.47	1685.60	2968.64	2522.65	1977.51	3224.50	332.14			
9:ACC-9	3209.82	2305.45	3779.08	3316.38	2645.74	4076.01	592.79	146.12		
10:ACC-10	250.41	533.63	131.60	191.36	405.62	94.45	2129.02	3880.04	4959.61	
11:ACC-11	204.79	383.42	124.39	125.47	286.01	115.34	1742.36	3279.54	4336.59	35.77

This is a dissimilarity matrix

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

No. of clusters	Cluster members (Allowed distance co-efficient 42.30)
1	Acc-3, Acc-6, Acc-1, Acc-4
2	Acc-2, Acc-5
3	Acc-10, Acc-11
4	Acc-8
5	Acc-9
6	Acc-7

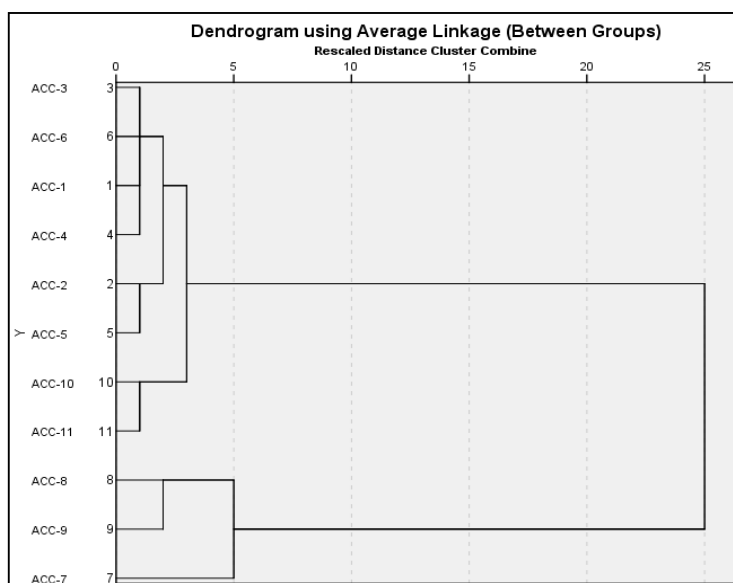


Fig. 1: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Wood apple plant physical attributes.

The value 47.53 denoted maximum similarity between ACC-4 and ACC-6 followed by 49.36 between ACC-7 and ACC-9 for fruit physical parameters. The maximum value 42812.52 between ACC-1 and ACC-8 denoted minimum similarity Table 6.

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 7 clusters were formed for fruit physical parameters. The data presented in the Table 7 and Fig. 2 shows that cluster 1, 3, 4 and 5 comprised of homogeneous types having similarity in characterization attributes. On the other hand cluster 2, 6 and 7 comprised single member namely ACC-8, ACC-1 and ACC-2. The first cluster was formed in between ACC-4 and ACC-6 at the coefficient distance 47.53. The third cluster was formed in between ACC-3 and ACC-10 at the distance 87.41. Similarly, fourth cluster was formed between ACC-5 and ACC-11 at the distance 123.93 and fifth cluster comprised ACC-7 and ACC-9 paired at the distance 49.36.

ACC-7 and ACC-9 with minimum value 0.00 denoted the maximum similarity followed by 6.25 between ACC-8 and ACC-9. The maximum value 3870.38 between ACC-1 and

ACC-8 denoted minimum accessions similarities for fruit biochemical parameters Table 8. The study of wood apple (*Feronia limonia*) in crop improvement programme showed great variability in physico-chemical characters among the 15 genotypes (Shete *et al.* 1999). Another work in improvement of some minor fruits through selection recorded fruit physical and chemical characteristics with much variability (Singh *et al.* 1999) [9].

Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for fruit biochemical parameters. The data presented in the Table 9 and Fig. 3 shows that cluster 1, 2 and 3 comprised of homogeneous types having similarity in characterization attributes. On the other hand cluster 4, 5 and 6 comprised single member namely ACC-4, ACC-1 and ACC-11. The first cluster comprised ACC-7, ACC-9, ACC-8 and ACC-10 paired at the coefficient distance 0.00, 6.26 and 83.62. Second cluster was formed between ACC-5 and ACC-6 at the coefficient distance 77.22. Likewise third cluster comprised ACC-2 and ACC-3 paired at the coefficient distance 97.36 respectively.

Table 6: Proximity matrix for Wood apple 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	10:ACC-10
2:ACC-2	1305.92									
3:ACC-3	9208.60	3673.77								
4:ACC-4	38765.07	26022.97	10189.98							
5:ACC-5	16888.61	8911.67	1159.30	4487.14						
6:ACC-6	38205.23	25604.69	9919.52	47.53	4314.06					
7:ACC-7	615.54	626.71	6111.18	31785.12	12538.73	31401.62				
8:ACC-8	42812.52	29401.18	14142.53	3658.13	8286.24	4343.86	35922.13			
9:ACC-9	724.90	808.63	6368.62	32171.20	12858.89	31828.26	49.36	36145.17		
10:ACC-10	9417.88	3768.03	87.41	10137.44	1165.17	9815.44	6575.62	13833.00	6897.46	
11:ACC-11	16582.46	8750.40	1147.92	4762.01	123.93	4684.62	12025.82	8278.83	12207.34	1317.20

This is a dissimilarity matrix

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

No. of clusters	Cluster members (Allowed distance co-efficient 123.93)
1	Acc-4, Acc-6
2	Acc-8
3	Acc-3, Acc-10
4	Acc-5, Acc-11
5	Acc-7, Acc-9
6	Acc-1
7	Acc-2

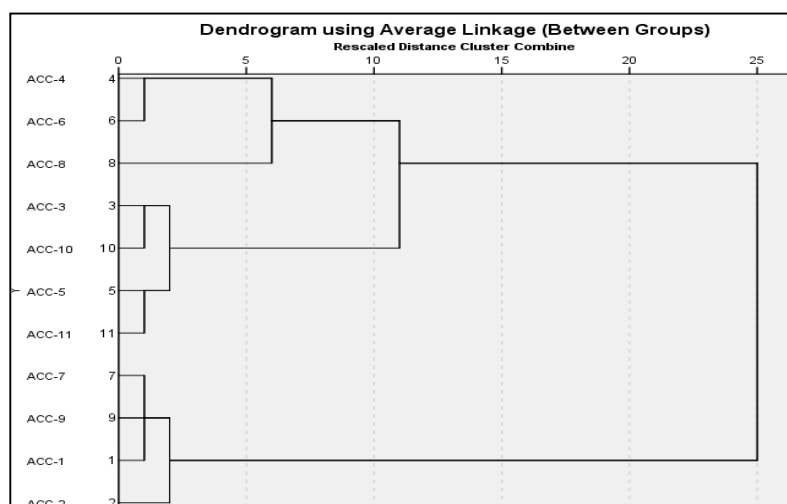


Fig 2: Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Wood apple fruit physical characters.

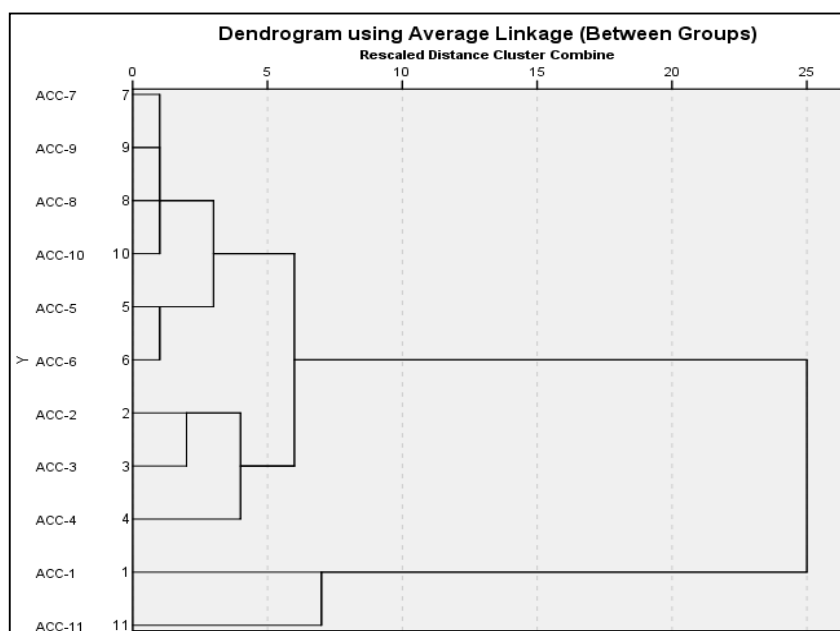
Table 8: Proximity matrix for Wood apple fruit biochemical attributes

	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	10:ACC-10
2:ACC-2	1433.02									
3:ACC-3	1833.26	97.36								
4:ACC-4	1826.90	203.28	477.25							
5:ACC-5	3363.31	438.36	421.71	413.14						
6:ACC-6	2626.69	251.61	371.01	141.51	77.22					
7:ACC-7	3596.53	545.65	472.36	652.73	108.17	262.43				
8:ACC-8	3870.38	652.65	544.67	772.29	133.34	330.32	6.25			
9:ACC-9	3596.54	545.58	472.28	652.50	108.01	262.23	.00	6.26		
10:ACC-10	3380.77	453.61	482.11	507.96	133.55	210.73	75.63	99.63	75.61	
11:ACC-11	638.30	1008.95	1671.86	884.16	2291.31	1586.61	2522.32	2771.15	2522.32	2163.42

This is a dissimilarity matrix

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

No. of clusters	Cluster members (Allowed distance co-efficient 97.36)
1	Acc-7, Acc-9, Acc-8, Acc-10
2	Acc-5, Acc-6
3	Acc-2, Acc-3
4	Acc-4
5	Acc-1
6	Acc-11

**Fig. 3:** Dendrogram using average linkage hierarchical clustering of squared Euclidean distance matrix for Wood apple fruit bio-chemical characters.

Conclusion

Branching pattern and crown shape was observed to be irregular in all the accessions whereas, tree growth habit varied from erect to spreading type. Leaf characters in wood apple reveals obovate leaf blade shape, obtuse to retuse leaf apex, cuneate leaf base, odd pinnate leaf orientation and pinnate leaf venation. During the course of survey the tree age of wood apple varied from 15yrs to above 40yrs in age at different germplasm. Height and trunk girth varied from 10m to above 20m and 88.2 cm to 157.4 cm. Length of the leaf blade ranges from 3.05 cm to 4.12 cm and breadth varies from 1.06 cm to 3.08 cm. Maximum fruit length was recorded in ACC-8 (8.04 cm) and breadth of 7.96 cm in ACC-6. Maximum fruit weight (306.02 g), peel weight (73.68 g) and number of seeds (28.42) was from the ACC-4. TSS ranged from 6.08° Brix (ACC-1) to 15.12° Brix (ACC-5). The present result is in partial conformity with the report of Ghosh

et al. (2012) [3] with fruit weight of wood apple between 130 to 225 g, pulp percentage 42.9 to 60.6%, T.S.S. range between 15 and 18.4° brix and acidity 1.7 to 4.6%. Maximum total sugar 28.57 mg/100 gm pulp (ACC-1), reducing sugar 13.88 mg/100 gm (ACC-4), acidity 1.77 mg/100 gm (ACC-5) and Vit. C 102.04 mg/100 gm (ACC-2) has been recorded. The pulp contains 18.1% carbohydrate, 7.1% protein, 3.7% fat, 5.0% fiber and 1.9% mineral matter. The pulp is a rich source of calcium, phosphorus, iron and vitamins like carotene, riboflavin, niacin, thiamine and vitamin C (Diengngan, S. and Hasan, M.A. 2015) [2]. Average linkage technique clustering when applied on squared Euclidean distance matrix a total of 6 clusters were formed for plant physical parameters, a total of 7 clusters were formed for fruit physical parameters and a total of 6 clusters were formed for fruit biochemical parameters.

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