Original article

Variability studies in avocado (*Persia americana* Mill.) using physico-chemical properties

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Summary

Introduction – **An attempt was made to analyze the morphological and molecular diversity in avocado (*Persia americana* Mill.) populations collected from different regions of India, using multivariate.** *Materials and methods* – **Morphological traits were recorded and evaluated for variance and mean comparisons. The top four traits contributing to economic value were used to arrive at selection of plus trees for further use. Sequence Related Amplified Polymorphism (SRAP) molecular markers were used for generating information on genetic variation and relationships among the accessions.** *Results and discussion* – **Significant variation was found in fruit-related morphological characters and fruit quality traits. The different avocado accessions were successfully categorized through combined analysis into various clusters based on genetic diversity and also established relatedness among them.** *Conclusion* – **This work would go a long way in characterization, identification and selection of parents in breeding programmes of this exotic crop.**

Keywords
avocado, morphological variability, SRAP markers, genetic diversity, elite trees

Significance of this study

What is already known on this subject?

- Avocado is said to be the world's most nutritious commercially grown fruit, rich in bio-compounds with many health benefits. It has high nutritional density as a source of fats, protein, fibre, major antioxidants, vitamins C, E, and minerals such as copper and iron. In India, avocado is grown in scattered areas in southern tropical states like Tamil Nadu, Kerala, Karnataka, and Maharashtra, and in the northeastern Himalayan state of Sikkim. Availability of high-quality nursery plants of select varieties and systematic planting would help make avocado popular in India.

What are the new findings?

- The study on genetic diversity and relatedness using morphological, biochemical and molecular markers was successful in categorizing avocado accessions into different groups based on geographical origin. Combination of all three systems of characterization provided evidence that significant diversity exists among the accessions.

What is the expected impact on horticulture?

- Currently avocado is not a commercial crop in India. Production and quality can be increased through breeding efforts. The analysis done in this study would be useful in selecting superior accessions which can be used as parents for development of promising varieties suitable for the desired region.

Introduction

Avocado (*Persia americana* Mill., $2n = 2x = 24$) belongs to the family Lauraceae and order Laurales. It is a polymorphic tree species growing in tropical countries. The world production exceeds 3.5 million tonnes of which 20% is traded. Mexico is the largest producer, with almost 1.9 million tonnes (60%) of the total production. Other major producers are Peru, Chile, California, South Africa, Colombia, Israel, Australia, and Spain. Guatemala, Morocco, the Philippines, China, and India are emerging as new production countries. It is a highly variable tree crop divided into three races, *viz.*, West Indian, Guatemalan, and Mexican, depending on factors such as harvest season, ability to withstand cold temperatures and fruit skin type. Avocado is said to be the world's most nutritious commercially grown fruit (Purseglove, 1968). The entire fruit is rich in biocompounds (pulp, seed, and peel) and has many health benefits, such as antimicrobial, antioxidant and anticancer activities, as well as dermatological uses besides others. The dietary value is largely contributed by monosaturated fatty acid, exceptional mineral (especially copper and iron), vitamin and beneficial anti-oxidant phytochemical content. Avocado fruit can be consumed directly as a high-energy food source because of the high content of lipids (up to 30%) and proteins (up to 4%), which is significantly higher than in other fruits. Besides, it is also a good source of oil (Quinones-Islas *et al.*, 2013) with the subtropical avocado having high oil content compared to other races.

In India, this crop was introduced by American missionaries and other foreign visitors during last century. They brought seedlings and a few varieties with them which were used for planting. Later, seeds of these seedlings and varieties were planted by growers in different parts of the country, particularly in Tamil Nadu, Kerala, and Karnataka. All

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Table 1. Geographical coordinates, altitude, climatic zone of the collection sites, MAT, MAP, and top soil texture of the studied areas.

State	Location	Lat. N	Long. E	Altitude (m)	Zone	MAT (°C)	MAP (mm)	Soil
Karnataka	Tumkur	13°20'	77°6'	825	Steppe climate	24.4	630	Laterite
	Bangalore	12.97°	77.56°	900	Tropical savanna	23.4	859	Laterite
	Kodagu	12.37°	75.83°	1,050	Humid tropical	21.6	2,553	Laterite
Tamil Nadu	Kallar	11.28°	76.94°	372	Humid tropical	26.3	1,200	Laterite
	Burliar	11.34°	76.79°	830	Humid tropical	26.0	1,400	Laterite

the three races of avocado, *i.e.*, West Indian, Guatemalan and Mexican have been tried in different regions of India. In the tropical and near-tropical areas of Kerala, Tamil Nadu, and Karnataka, the West Indian race has adapted successfully and its cultivation is gaining popularity in coffee-based cropping system of these states. The hybrids of this race with Guatemalan race are performing well and helping to extend the harvest season in these regions. Some of the popular varieties in Tamil Nadu are Long, Round, Fuerte, Pollock, Peradeniya Purple Hybrid, Shambaganur, and Trapp. In colder regions such as Sikkim, the Guatemalan and Mexican race, along with their hybrids, is being cultivated. The hybrids have proven to be valuable since they combine the cold tolerance of the latter with the superior fruit traits of both. The varieties grown in Sikkim are Fuerte, Pinkerton, Green, and Hass (Ghosh, 2000). The wide range of benefits to human health, nutrition and livelihood security associated with avocado is increasing its demand in domestic and international markets. India is projected as one of the emerging production countries. However, lack of availability of certified planting materials, agronomic practices and diversity in soil and climatic conditions in different production areas is affecting the fruit quality and production. Research focused on this crop in other production countries has contributed to improve cultivars and rootstocks, higher and regular yields of quality fruit, better post-harvest and on tree life, increased tolerance to pests and diseases. Work in these areas and other aspects such as fluctuations caused by periodic colds, droughts, winds, or other factors, including alternate bearing habit of the crop, needs attention for increasing its productivity in India.

Taking into account the above, this study was aimed to evaluate the effect of locality on the physico-chemical characteristics of thirty-three avocado accessions and to develop a quality-index-score to select the superior ones for various desired parameters.

Materials and methods

Morphological evaluation

The study was carried out with thirty-two avocado accessions growing at different locations, *viz.*, Tumkur, Bangalore, and Kodagu in Karnataka, Kallar and Burliar in Tamil Nadu (Table 1). Ten randomly selected leaves were taken for measurement of length, width, and other morphological traits. Five fruits of each accession were individually analysed for different physico-chemical characteristics based on avocado descriptor from Plant Genetic Resources Institute (IPGRI, 1995). Fruit size (length and girth) was measured with the help of digital Vernier calipers while weight with digital top pan balance. The total soluble solids (TSS) were determined with Erma Hand Refractometer (0–32 °Brix).

Biochemical analysis

Total protein and sugar content was estimated in four accessions following Lowry's method (Lowry *et al.*, 1951) while the modified method of Lu *et al.* (2009) was followed for fat content in nine accessions. Avocado pulp was separated and homogenized in a blender. 10 g of dried avocado pulp powder was extracted for 5 h in a soxhlet apparatus using petroleum ether. Petroleum ether extract was filtered and evaporated under reduced pressure at 60 °C and the residue was dried at 95–100 °C for 30 min. Weight of crude residue was recorded as fat content of the sample.

DNA extraction and SRAP markers

For SRAP analysis genomic DNA was extracted from young leaves and its quality was checked and adjusted to 50 ng μL^{-1} for PCR reactions. Good quality DNA was obtained from 30 accessions and these were subjected to molecular analysis. Initial screening was carried out on a panel of 10 morphologically diverse genotypes with 45 primer combi-

Figure 4. Amplification of 29 avocado accessions with primer 3F-15R. L is a 100 bp DNA ladder used as the fragment size marker.

nations (PC), which resulted in selection of seven PCs giving good and reproducible amplification. Standard PCR reaction and conditions were used as suggested by Li and Quiros (2001). The PCR product was analyzed on 2% agarose gel with ethidium bromide staining in 1× TBE (Tris-borate-EDTA) buffer. A 100 bp DNA ladder as was used as the fragment size marker (Figure 4).

Scoring was done manually for intense and clearly resolved PCR amplified bands. SRAP matrix data sheet was prepared with presence (1) or absence of band (0). In order to estimate the band size a medium range DNA ruler (100 bp) was run along with amplified products. Jaccard's coefficient of similarity was used for assessing genetic relatedness among the accessions. Using the binary data and R software (R Core Team, 2013) a dendrogram was constructed based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Relationship among morphological, biochemical, and genetic distances

Similarity between the observations was measured using Gower distance and distance matrix was computed. The complete linkage method of hierarchical cluster analysis was followed for plotting dendrogram based on the distance matrix computed in the earlier step. PCA analysis was done based on numeric variables in the study. A web diagram was drawn using these traits (Simbo *et al.*, 2013) to identify superior trees with a combination of multiple desirable traits.

Results and discussion

Variability among the accessions

Appreciating the nature and extent of variability among avocado genotypes for traits of economic importance are essential to plan effective breeding and germplasm conservation programs. The germplasm lines showed high variation in economically important traits, *viz.*, fruit weight (g plant^{-1}) which ranged from 220.40 to 871.00 with mean value of 340.2 and 13 to 27% fat with a mean of 22.62, respectively. The accessions also exhibited diversity in fruit length, fruit width, seed weight per fruit and TSS showing large differences between maximum and minimum values. The coefficient of variation for all the 12 quantitative characters studied (Tables 2 and 3) showed a wide variability ranging between 5.8% and 115.91%, and thus can be considered as traits of interest in breeding programs of avocado. The maximum variability (115.91%) was recorded for yield followed by fruits per plant (105.96) and fruit weight (37.64%). Seed weight and TSS also showed high variation (32.74 and 20.57%). These traits are influenced by several factors such as parentage, climate, soil, *etc.* and thus bound to vary. The correlation of fruit weight with different parameters was worked out (Table 4). The correlation matrix data showed that fruit weight was positively correlated with fruit diameter (0.86), fruit length (0.66), seed weight (0.61), and leaf width (0.52). Yield was positively correlated with number of fruits per plant (0.96).

In a previous study by Magdalita and Valencia (2002), significant difference among the genotypes was reported for traits like fruit weight, fruit length, peel weight, seed weight and edible portion, while narrower variation was observed for fruit width and total soluble solids. Correlation analysis showed that fruit weight was correlated with fruit width (0.77), while fruit length was positively and highly correlated with flesh thickness (r = 0.83) at the bottom of the fruit. In another study by the same workers in 2004, it was noted that fruit weight was correlated with fruit width and seed weight with respect to quality parameters such as titratable acidity, reducing sugar, non-reducing sugar and total sugar

Table 3. Range, mean, standard deviation and coefficient of variation for biochemical parameters of fruits of different avocado accessions.

Name	Fat (%)	Protein (%)	Sugar (%)
Avocado-1	23.6		
Avocado-3	26.0		
Avocado-4	24.0		
Avocado-5	22.0		
Avocado-6	21.0		
Avocado-18	13.0	3.1	3.9
Avocado-26	27.0	2.3	4.0
Avocado-27	22.0	3.2	3.9
Avocado-28	25.0	3.1	3.5
Max.	27	3.2	4
Min.	13	2.3	3.5
Range	14	0.9	0.5
Mean	22.62	2.93	3.83
Median	23.6	3.1	3.9
SD	4.12	0.42	0.22
SE	1.37	0.21	0.11
CV (%)	18.16	14.34	5.8

Table 4. Correlation matrix for different fruit traits for 32 avocado accessions.

	Leaf length	Leaf width	Fruits plant^{-1}	Fruit weight	Yield	Fruit length	Fruit diameter	Seed weight	TSS
Leaf length	1	0.33	0.49	0.04	0.48	0.04	0.01	-0.03	0.12
Leaf width		1	-0.01	0.52	0.02	0.41	0.29	0.32	0.07
Fruits plant^{-1}			1	0.12	0.96	-0.04	-0.01	-0.05	0.06
Fruit weight				1	0.29	0.66	0.86	0.61	0.21
Yield					1	0.08	0.19	0.11	0.15
Fruit length						1	0.50	0.48	0.24
Fruit diameter							1	0.63	0.15
Seed weight								1	0.36
TSS									1

which was attributed to the genetic makeup of genotypes and the prevailing climatic conditions. Nkansah (2013) reported that in accessions maintained at University of Ghana Forest and Horticultural Crops Research Centre significant genetic diversity was observed among the avocado lines and cultivars in terms of leaf, tree, fruit, and yield characteristics. Spearman's rank correlation coefficient revealed significant positive correlations among plant height, canopy width, stem girth, and number of branches.

Cluster analysis and phenetic relationships

Based on the distance matrix calculated, the most similar accessions are A-8 and A-13 which belong to cluster 2, while the most dissimilar pair is A-10 (cluster 2) and A-26 (cluster 1). A dendrogram was generated for highlighting relationships among the studied avocado accessions (Figure 1). The dendrogram tree consisted of three main groups. A-26 (belonging to Kodagu) has been placed in cluster 1 and has been separated from the rest of the accessions. Accessions from

TABLE 2. Range, mean, standard deviation and coefficient of variation for physico-chemical parameters of fruits of 32 different avocado accessions.

Name	Leaf length (cm)	Leaf width (cm)	Fruits plant^{-1} (no)	Fruit weight (g)	Yield (kg)	Fruit length (cm)	Fruit diameter (cm)	Seed weight (g)	TSS (°Brix)	Location	Fruit color	Flesh color
Avocado-1	14.25	7.75	23	294.2	6.77	8.01	9	82	5.4	Tu	G	CW
Avocado-3	12.4	7.1	13	250.4	3.26	7.46	8.78	53	5.2	Tu	G	CW
Avocado-4	13.63	9.19	16	245.8	3.93	9.41	7.67	48.67	4.9	Tu	G	CW
Avocado-5	12.72	7.81	22	351.4	7.73	9.86	8.41	70.33	5.1	Tu	G	CW
Avocado-6	11.48	6.98	27	339.4	9.16	8.96	8.87	67.6	5.3	Tu	G	CW
Avocado-7	16.88	9.31	86	293	25.2	9.89	8.86	76.25	5.6	Ba	G	CW
Avocado-8	16.88	7.45	25	274	6.85	9.66	8.41	71.25	5.1	Ba	G	CW
Avocado-9	10.7	5.99	21	278	5.84	9.51	8.39	72.01	5.8	Ba	G	CW
Avocado-10	7.1	5.3	18	250	4.5	9.38	8.31	72.56	5.3	Ba	G	CW
Avocado-11	14.14	7.04	15	231	3.47	9.12	8.21	65	6.1	Ba	G	CW
Avocado-12	10.9	6.32	21	243	5.1	9.15	8.23	68	4.8	Ba	G	CW
Avocado-13	15.38	7.36	21	265	5.57	9.21	8.41	68.5	5.2	Ba	G	CW
Avocado-14	14.23	7.11	57	253	14.42	9.23	8.33	62.5	5	Ba	R	CW
Avacado-15	12.42	6.68	11	231	2.54	9.11	8.12	65	4.6	Ba	G	CW
Avocado-16	13.23	7.19	61	274.4	16.74	8.96	8.46	36.6	3.6	Ka	G	WY
Avocado-17	14.11	7.26	73	545.8	39.84	9.98	9.91	106.8	5	Bu	G	WY
Avocado-18	13.05	7.38	6	269.6	9.71	8.66	8.51	42.2	4.6	Ko	G	WY
Avocado-19	14.43	6.46	95	393.4	37.37	9.61	9.21	35.8	4.7	Ko	G	WY
Avocado-20	12.2	5.47	81	449.6	36.42	9.71	9.571	76	8	Ko	G	WY
Avocado-21	13.08	5.62	20	356.8	7.14	8.95	9.1	68	5.7	Ko	G	WY
Avocado-22	14.96	7.86	55	220.4	12.12	9.3	7	42.8	8.4	Ko	G	WY
Avocado-23	13.39	7.01	14	439.4	6.15	9.63	9.53	46.4	5.3	Ko	G	WY
Avocado-24	12.97	6.62	22	261.5	5.75	8.32	8.39	76.8	6.8	Ko	G	WY
Avocado-25	13.67	5.92	120	386.3	46.36	9.41	8.41	68	4.8	Ko	G	WY
Avocado-26	12.68	13.68	16	871	13.94	11.31	10.89	126.8	6.6	Ko	G	WY
Avocado-27	14.92	8.39	125	442.4	55.3	9.35	9.46	115.6	5.8	Ko	G	WY
Avocado-28	13.28	7.74	19	445.4	8.46	9.53	9.35	49.8	4.5	Ko	G	WY
Avocado-29	12.13	5.94	13	416.2	5.41	9.36	9.11	81.6	4.5	Ko	G	WY
Avocado-30	17.86	7.5	244	423.6	103.36	9.11	8.98	67.3	7	Ko	G	WY
Avocado-31	12.89	7.12	51	342.6	17.47	8.61	7.96	61.2	5	Ko	G	WY
Avocado-32	13.78	6.75	192	274.4	52.68	8.41	7.8	36.6	3.6	Ko	G	WY
Avocado-33	14.19	9.37	80	274.4	21.95	8.43	7.79	36.6	3.6	Ko	G	WY
Min.	7.10	5.30	6.00	220.40	2.54	7.46	7.00	35.80	3.60			
Max.	17.86	13.68	244.00	871.00	103.36	11.31	10.89	126.80	8.40			
Range	10.76	8.38	238.00	650.60	100.82	3.85	3.89	91.00	4.80			
Median	13.34	7.12	22.50	285.50	8.81	9.27	8.44	67.80	5.15			
Average	13.44	7.33	51.97	340.20	18.77	9.21	8.67	66.17	5.34			
SD	1.97	1.54	55.06	128.04	21.75	0.68	0.75	21.67	1.10			
SE	0.35	0.27	9.73	22.63	3.85	0.12	0.13	3.83	0.19			
CV (%)	14.69	21.02	105.96	37.64	115.91	7.42	8.63	32.74	20.57			

Based on Gower's distance, minimum distance found between A-13 and A-8 and maximum distance between A-10 and A-26.
Tu: Tumkur; Ba: Bangalore; Ko: Kodagu; Bu: Burliar; Ka: Kallar; G: Green; R: Red; CW: Creamy White; WY: Whitish Yellow.

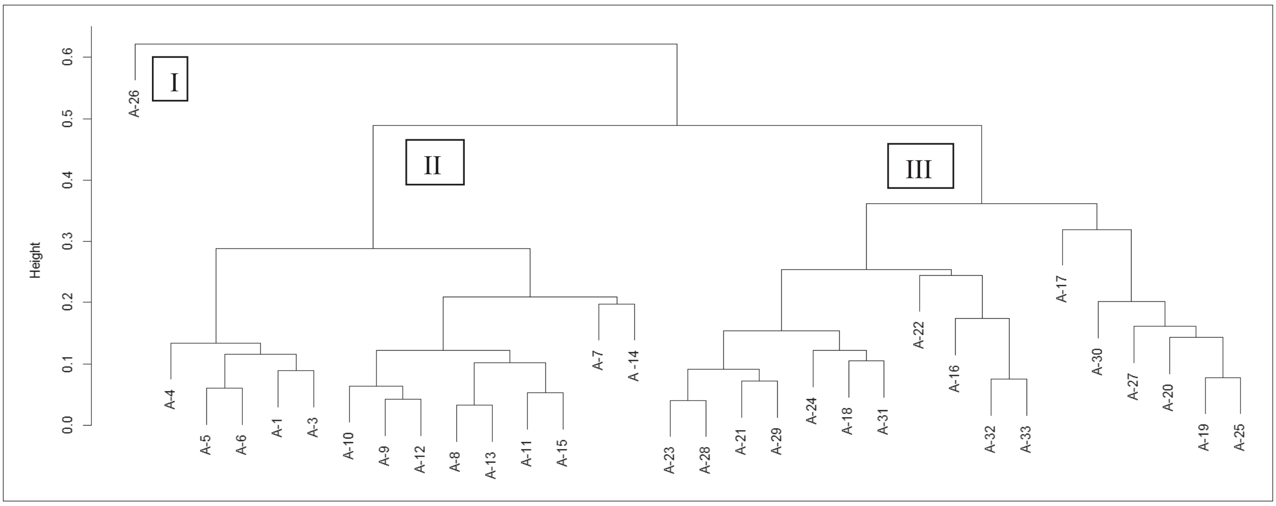

FIGURE 1. Dendrogram depicting relationships among 32 avocado populations based on morphological characterization of the accessions.

Tumkur (A1-A6) and Bangalore (A7-A15) have been placed in cluster 2. The rest of the accessions belonging to Kodagu, Kallar, and Burliar have grouped together in cluster 3. The accessions have been effectively separated based on location.

Principal components analysis

The first two principal components together explained 62.4% of the total variation of morphological traits. It is depicted as two-dimensional biplot arrangement (Figure 2). The first principal component explained 37.4% of the whole variation. Fruit-related traits, such as seed weight, fruit weight, fruit length, and fruit diameter, largely contributed to variation for this principal component. The second principal component explained 25% of total variation. Vegetative traits such as leaf length, yield and fruits per plant contributed to the dissimilarity of this principal component.

Selection of plus trees with superior fruit traits

The values of selected economic traits *viz.* fruit weight, ratio of fruit to seed weight, fruit length and TSS were normalized and a combined score for all the genotypes was assigned by giving the weights 4, 3, 2 and 1 to the normalized values of fruit weight, ratio of fruit to seed weight, fruit length and TSS, respectively (Table 5). The genotype A-26 scored highest (8.0), followed by A-19 (5.9), A-23 (5.6), and A-28 (5.1). Four trees (A-26, A-19, A-23, and A-28) could be identified with the best combination of the most desirable fruit traits. These may be selected for further multiplication (Figure 3). These trees performed better than the average for at least three of the four fruit traits, *viz.*, fruit weight, ratio of fruit to seed weight, fruit length, and TSS studied. The trees with superior desirable traits may be multiplied vegetatively since such a method of propagation assures the conservation of the traits of interest (Tchoundjeu *et al.*, 2006). For avocado, such vegetative multiplication has been standardized and can be accomplished by grafting or cutting. The practice of grafting is common in avocado due to the beneficial effect of rootstocks in imparting salinity tolerance (Mikelbart *et al.*, 2002). In contrast to concerns highlighted by several authors that strategies to produce genetic gain by selecting for certain traits may decrease genetic variation within a population, Ugese *et al.* (2010) demonstrated for *V. paradoxa* that such a selection instead, resulted in higher genetic diversity. Recent trends indicate the global potential for cultivating avocado, Guatemala, Morocco, the Philippines, China, and India have been projected as potential areas where the species can be cultivated based on annual rainfall and temperature, thus bringing benefits of the species to a larger population, and simultaneously alleviating poverty and preserving the environment.

Molecular characterization of the accessions

UPGMA algorithm was used to generate a dendrogram using the SRAP data (Figure 5). The 29 avocado accessions got clustered into three groups (Group I, Group II, and Group III). These results on the grouping of accessions on the basis of genetic distance were generally consistent with the results from morphological analysis. Group I contained 5 accessions collected from the Tumkur region. Group II contained accessions from Kodagu region, while group III comprised of 5 accessions of which one was from Bangalore region and the remaining from Kodagu region. This group also included the accession with maximum fruit weight (A-26). In general, it was found that genotypes derived from identical or neighboring areas clustered into the same group or subgroup. The accessions from Tamil Nadu (A-16 and A-17) clustered into different groups, I and II, respectively. The four elite trees identified fall into group II (A-19 and A-23) and group III (A-26

TABLE 5. Normalized values of selected economic fruit traits for scoring the accessions.

Genotype	Normalized values				Combined score
	Fruit weight	Fruit weight/seed weight	Fruit length	TSS	
A-19	0.3	1.0	0.8	0.2	0.59
A-23	0.3	0.8	0.7	0.4	0.56
A-26	1.0	0.5	1.0	0.6	0.80
A-28	0.3	0.7	0.7	0.2	0.51

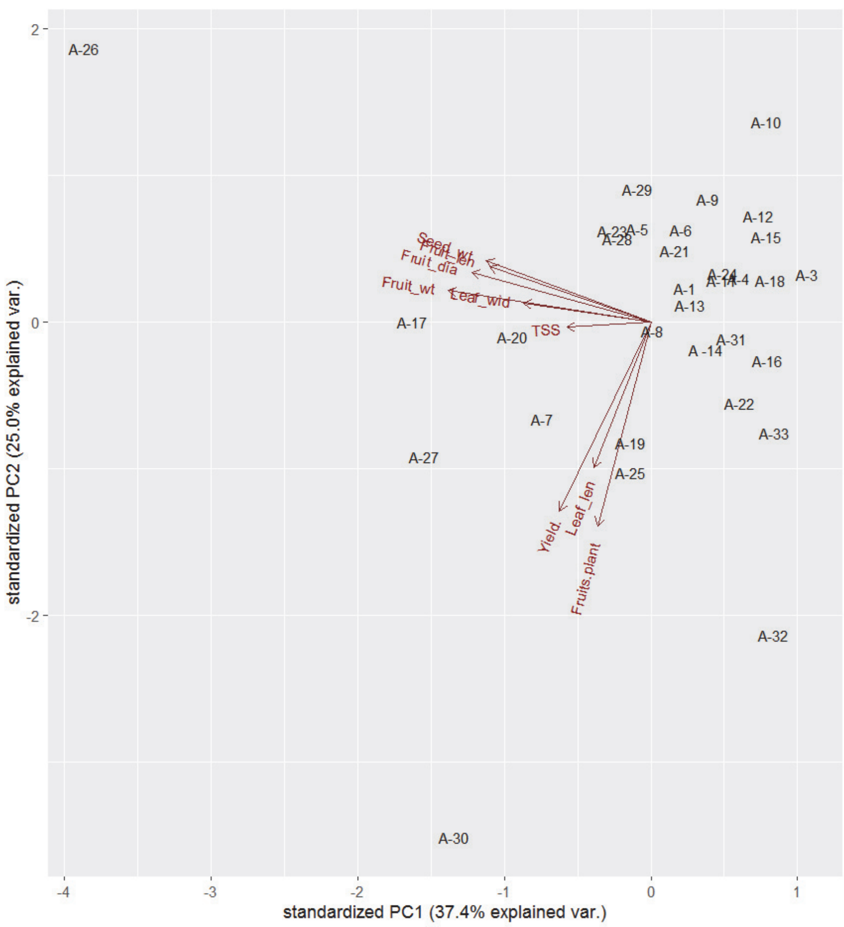

FIGURE 2. A two-dimensional scatter plot depicting phenetic relationships among avocado accessions using the first two principal components from morphological data.

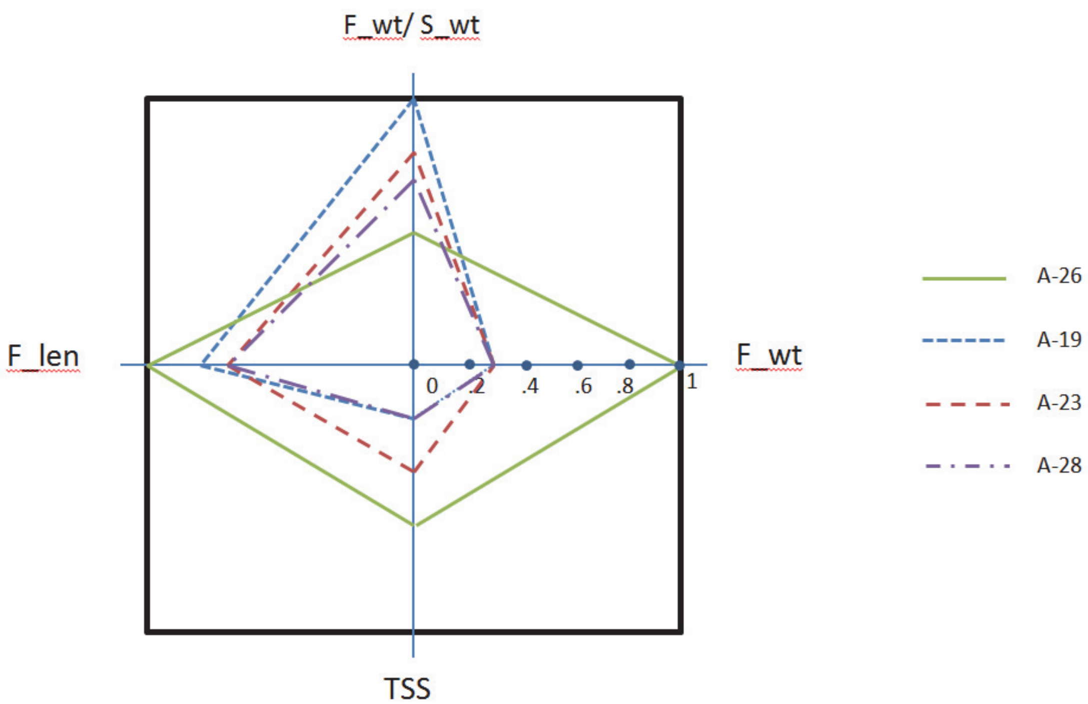

FIGURE 3. Multi-trait web diagram of tree-to-tree variation in fruit traits. Trees superior in the fruit traits studied are shown here.

and A-28). Since most of the accessions are of seedling origin and have been derived from a limited gene pool, SRAP marker has proven to be more informative than other PCR-based techniques in detecting genetic diversity (Budak *et al.*, 2004) and has been successfully used to study the genetic diversity, and relationships among several species (Cai *et al.*, 2011; Abedian *et al.*, 2012). The narrow genetic base in investigated materials suggested broadening the genetic base by introduction of new genes into existing breeding materials.

Conclusion

The present study shows extensive genetic variability in avocado in India which can be further enhanced by introducing commercial cultivars from other parts of the world. The potential of avocado germplasm can be further improved by studying the correlations between various traits determining productivity for breeding. Studies focused on genetic, physiological, biochemical, and molecular aspects would help in classifying the collections and removing duplicates to strengthen the breeding efforts and spread of this crop in India.

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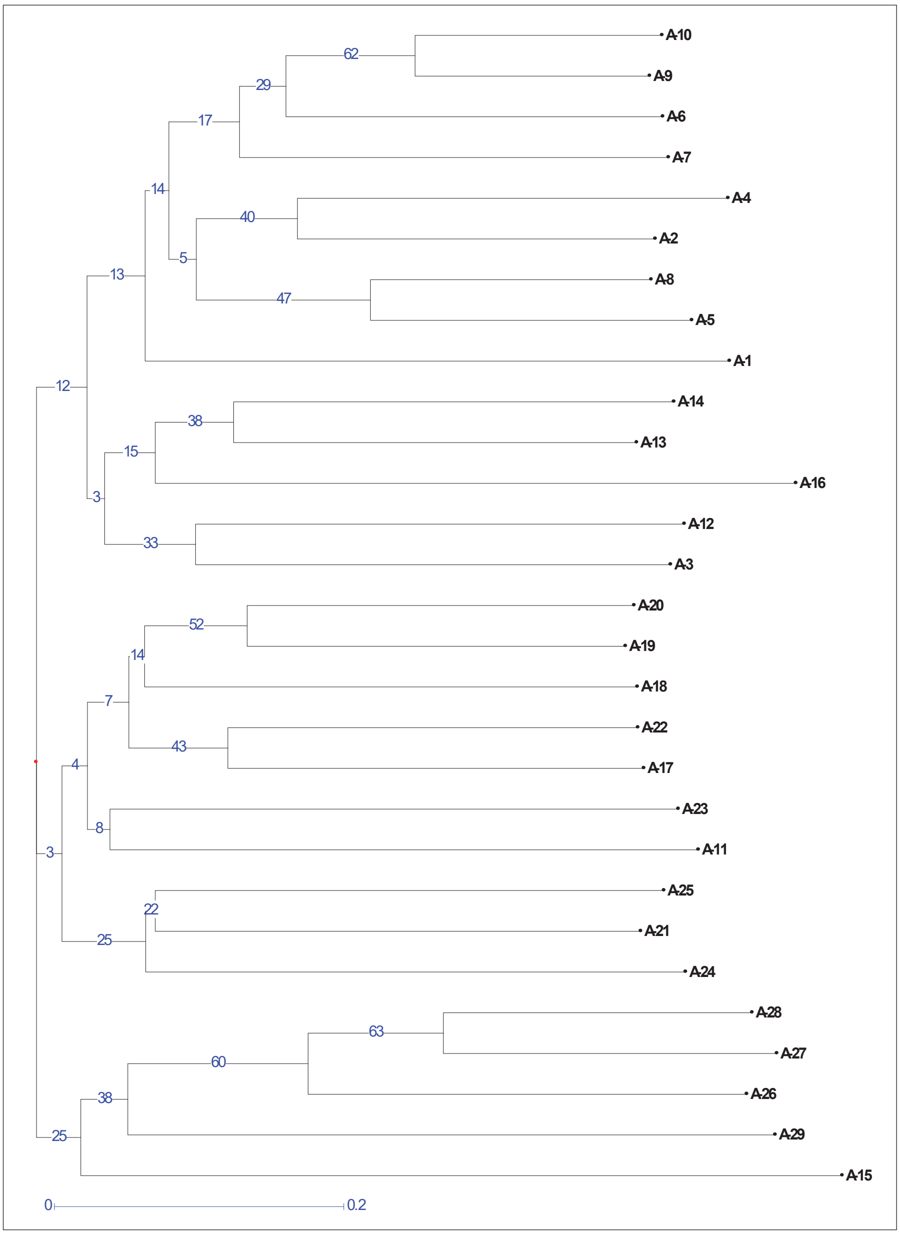

FIGURE 5. Dendrogram generated from SRAP data showing relationships of 29 avocado accessions based on Unweighted Pair-Group Method of Arithmetic Averages (UPGMA).

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