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Comparative evaluation of red and white aril genotypes of Manila tamarind for fruit physicochemical and bioactive attributes

D. S. Mishra[1]✉, Anshuman Singh[2], V. V. Appa Rao[1], Vikas Yadav[1], M. K. Berwal[3], Prakashbhai Ravat[1], Deepak Kumar Sarolia[3], Jagadish Rane[3], Yazgan Tunç[4] & Ali Khadivi[5]✉

The cultivation and trade of underutilized fruits are gaining prominence worldwide, largely on account of their capacity to contribute to a nutritious diet. Manila tamarind [*Pithecellobium dulce* (Roxb.) Benth] is a fast-growing, nitrogen-fixing tree, with a fairly high tolerance to abiotic stresses. Despite significant potential in terms of food, fodder, timber, and medicine, it has largely remained an orphan crop. There is a dearth of systematic research on the exploration, conservation, and genetic improvement of Manila tamarind. Our study aimed to assess the genetic variability for commercially important fruit, aril, and leaf attributes in 22 diverse accessions of Manila tamarind comprising both white and red aril genotypes. Precise characterization of the existing genetic resources is a requisite for the commercial cultivation of Manila tamarind. The study was conducted with 15 white and 7 red accessions of *P. dulce*, which were planted in a square system of planting between and within row distances of 5 m each. One of the major contributions of the present study was that we examined genotypic variations in biochemical attributes, such as TSS, acidity, TSS: acidity ratio, total sugars, ascorbic acid, protein, mineral contents, and bioactive compounds; these factors significantly improve the nutritional value and eating quality of Manila tamarind arils. Most of the traits examined by us differed remarkably ($p < 0.001$) among the accessions. Some economically relevant traits, such as pulp weight, aril weight, aril total phenols, aril flavonoids, aril total antioxidant activity, and leaf flavonoids exhibited a high degree of variability, indicating the scope for the selection of elite genotypes and divergent parents for future hybridization programs. The highly variable values of total soluble solids (17.33–26.46 °Brix), acidity (0.54–1.07%), ascorbic acid (82.54-138.49 mg 100 g− 1), total sugars (12.45–18.81%), and aril protein (3.15–6.32%) recorded in this study broadly meet fresh consumption and aril processing standards for Manila tamarind. A significant finding was that Manila tamarind accessions differed greatly in aril mineral contents (mg/100 g FW), including potassium (220.44-334.33), phosphorus (21.63–62.34), and calcium (14.06–39.12). Overall, two red aril genotypes (CHESM-27 and CHESM-33), and three white aril genotypes (CHESM-4, CHESM-20, and CHESM-24) were found to be particularly promising in terms of pod and aril quality attributes. Our findings are expected to pay the way for commercial cultivation of elite Manila tamarind genotypes, and their applications in pharmaceutical applications. Future studies should aim to elucidate the molecular basis of genetic diversity and relationships in Manila tamarind.

Keywords Bioactive components, Genotypic variation, *P. dulce*, Fruit quality

The production and trade of underutilized fruits are gaining importance globally, mainly in recognition of their contribution to a healthy diet[24,35,56,58,69]and significant medicinal potential[14,17,23,59,61]. Compared with many

[1]ICAR-Central Horticultural Experiment Station, 389340 Vejalpur, Panchmahals, Gujarat, India. [2]ICAR-Central Institute for Subtropical Horticulture, 226101 Rehmankhera, Lucknow, India. [3]ICAR-Central Institute for Arid Horticulture, 334006 Beechwal,Bikaner ,Rajasthan, India. [4]Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, Hatay Olive Research Institute Directorate, Hassa Station, 31700 Hassa, Hatay, Türkiye. [5]Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349 Arak, Iran. ✉email: dsmhort@gmail.com; a-khadivi@araku.ac.ir

other commercially grown fruit crops, the cultivation of underutilized fruit crops, such as Manila tamarind [*Pithecellobium dulce*(Roxb.) Benth; family Leguminosae; subfamily Mimosoideae], has numerous advantages in terms of ease of management, hardiness, and resilience to climatic change[35,63,64]. Manila tamarind is a fast-growing, evergreen, nitrogen-fixing, and salt-tolerant tree or shrub found throughout the plains of India and in the Andaman Islands[5,53,66]. It can tolerate degraded soil conditions and is resistant to fire and drought[8,48]. It is also widely distributed in many semi-arid regions of tropical Africa and America[9,28]. The *P. dulce* tree has multiple uses as a source of timber, firewood, honey, food, fodder, and medicinal ingredients[12,29,47,48,67]. It is also recommended for soil biological restoration[8].

The fruit of Manila tamarind is a pod consisting of sweet edible arils covering black seeds; depending on genotype, the arils are white or red[50,73]. Aril is a fleshy and sweet pulp that covers the seed partially or completely[9]. Pods are tightly coiled, irregularly shaped, and turn greenish brown to reddish at the time of maturity. Each pod has about 5–12 seeds which are shiny black and attached to the pods by a red funicle[43,65]. *P. dulce* aril is a good source of vitamins, minerals, dietary fiber, protein, and carbohydrates[50,52,56,74], and shows antioxidant and hepatoprotective properties[33,36]. The level and composition of nutraceutical and physicochemical parameters in *P. dulce* fruit vary greatly with genotype[50,56], and the stage of fruit maturity[74]. The aril is consumed raw, roasted, or used in the preparation of a beverage similar to lemonade[7,42,53], and various value-added products like RTS, squash, and syrup[37,40,56]. It has numerous medicinal uses, and exhibits strong analgesic, anti-inflammatory, antibacterial, antidiarrheal, antiulcer, antioxidant, hypoglycemic, and hepatoprotective properties[13,25,30,52,66,73,75].

Limited efforts have been made to methodically explore, assess, and improve the genetic resources of Manila tamarind[16,19,40]. Until recently, the Manila tamarind germplasm resources were mostly sourced from the landraces adapted to certain niche areas, resulting in a very narrow genetic base[63]. To our knowledge, some selections have been carried out only in the Philippines for big pods with tiny seeds, and red, sweet, and less astringent arils[18]. There is a need to identify prolific-bearing and high-yielding genotypes of Manila tamarind with large and sweet pods[32]. Gaining a deeper comprehension of the fruit's physicochemical and biochemical characteristics is crucial for creating potential cultivars that are appropriate for various end uses[38]. Precise characterization of the existing genetic resources is a requisite for the commercial cultivation of Manila tamarind in India and elsewhere. Selecting the parents for genetic gains can be facilitated by having a thorough understanding of the gene pool that can be used in genetic improvement programs as well as morpho-genetic characterization of each genotype[38]. Plant trait determination and genetic characterization studies are important in establishing plant breeding programs[80,81].

Until recently, genetic diversity analysis in *P. dulce* has mainly focused on variations in aril, leaf, and bark phytochemical traits in one or two genotypes i.e. either white aril or red aril types. To the best of our knowledge, this is the first study where a fairly large number of red-aril and white-aril *P. dulce* accessions were characterized using a range of economically important traits. To address the aforementioned research gaps, the present study was carried out with the objectives of examining the diversity in pod physicochemical traits, and bioactive compounds in the white and red accessions of *P. dulce*, and identify promising *P. dulce* accessions for commercial cultivation and for use as parents in the genetic improvement programs.

Materials and methods

Study site

The study was conducted at the Central Horticultural Experiment Station (CHES) of the Indian Council of Agricultural Research- Central Institute for Arid Horticulture located in Vejalpur, Panchmahal, Gujarat, India (22°41′N, 73°33′E with an altitude of 113 m above sea level) during 2023 and 2024 fruiting seasons. The experimental location has a hot, semi-arid climate with an average annual precipitation of about 750 mm. The soils of the experimental farm are mostly shallow, and sandy loam in texture. The soil pH is approximately 6.65, and the organic carbon content ranges from 0.35 to 0.45%. The formal identification of the samples was performed by Dr. Mishra. A voucher specimen of this material has been deposited in the publicly available herbarium of ICAR-Central Horticultural Experiment Station, Vejalpur with deposition number PD-3425.

Experimental material

The study was conducted with 15 white and 7 red accessions of *P. dulce* planted in the field gene repository at CHES, Panchmahal, Gujarat, India (Table 1; Fig. 1). The trees of each *P. dulce* accession, aged 8–9 years, were planted in a square system of planting with between and within row distances of 5 m each. Recommended crop management practices were adopted for healthy tree growth.

Fruit physical properties

At the commercial maturity stage (March-April), when pods begin to exhibit distinctive colors with some noticeable splitting, ten pods from different directions of three trees of each accession were randomly collected to record various observations. The pod weight (PWt, g), aril weight (ArWt, g), peel weight (PlWt, g), and seed weight (SWt, g) were recorded using a precision balance (0.01 g accuracy). Pod width (PW, mm), seed length (SL, mm), and seed width (SW, mm) were measured using a Vernier caliper (Mitutoyo, Japan). Number of seeds/pod (NSP) were manually counted.

Fruit chemical properties

The filtered aril juice was used for determining titrable acidity (Acid, %) and total soluble solids (°Brix). Total soluble solids were estimated using an Erma Hand Refractometer (0–32 °Brix). Titrable acidity (% of citric acid) was determined using N/10 NaOH and phenolphthalein as indicators as described in AOAC[1]. The TSS: acidity ratio (TA) was calculated as the ratio between TSS and acidity. Ascorbic acid content (mg 100 g^{-1}) was determined using 2,6-dichlorophenol indophenol dye[1]. Total sugars (%) were estimated by Lane and Eynon's

Sr. No	Genotype	Pedigree	Source	Pod coiling pattern	Peel color	Aril color
1	CHESM-1	Landrace	Bhedia	Tightly coiled	Light green	Greenish white
2	CHESM-2	Landrace	Vejalpur	Tightly coiled	Light green	Creamy white
3	CHESM-3	Open selection	Alindra	Curved	Green	Whitish green
4	CHESM-4	Selection	CHES, Vejalpur	Spiraled	Bright pink	Milky white
5	CHESM-5	Open selection	Halol	Tightly coiled	Maroon	Creamy white
6	CHESM-6	Open selection	Kandach	Coiled	Greenish brown	Creamy white
7	CHESM-7	Open selection	Kandach	Tightly coiled	Light maroon	Milky white
8	CHESM-10	Open selection	CHES, Vejalpur	Curved	Brownish green	Creamy white
9	CHESM-12	Open selection	Vejalpur	Tightly coiled	Light maroon	Creamy white
10	CHESM-17	Open selection	Por	Tightly coiled	Light pink	Milky white
11	CHESM-20	Open selection	Kanod	Lightly coiled	Pink	Milky white
12	CHESM-22	Open selection	Por	Lightly coiled	Brownish	white
13	CHESM-24	Open selection	Waghodia	Tightly coiled	Pink	Off white
14	CHESM-26	Open selection	Rampur	Spiraled	Light pink	Milky white
15	CHESM-27	Selection	CHES, Vejalpur	Lightly coiled	Dark maroon	Dark red
16	CHESM-28	Open selection	Halol	Tightly coiled	Light pink	Light pink
17	CHESM-29	Open selection	CHES, Vejalpur	Lightly coiled	Light maroon	Whitish green
18	CHESM-30	Open selection	CHES, Vejalpur	Curved	Light pink	Light red
19	CHESM-31	Open selection	Kandach	Lightly coiled	Light maroon	Light pink
20	CHESM-32	Open selection	Rabod	Lightly coiled	Light green	Light pink
21	CHESM-33	Open selection	Rabod	Lightly coiled	Red	Red
22	CHESM-34	Selection	Valiya	Lightly coiled	Light red	Light red

Table 1. List of *Pithecellobium dulce* genotypes used in the study.

Fig. 1. Variability in pod characters of 22 *Pithecellobium dulce* genotypes.

method using Fehling's solutions as described in AOAC[1]. Protein determination was carried out by the micro-Kjeldahl method using the factor 6.25. The sample (0.50 g) was digested in H_2SO_4 (10 ml) in the presence of 1 g catalyst K_2SO_4 (10 g) + $CuSO_4$ (1 g). Subsequently, the volume was made up to 100 ml. 10 ml of sodium hydroxide (NaOH, 40%) was added to 10 ml of digest, followed by steam distillation; the distillate was collected in 10 ml of boric acid (4%). Then nitrogen content was determined by using titration with 0.005 N H_2SO_4 (Bharghava and Raghupathi, 1993). All results are expressed on a fresh weight basis (FW).

Bioactive compounds and antioxidants

Leaf biochemical parameters were determined on recently mature leaves. Total phenols (TP) present in the arils (ArTP) and leaf extracts (LTP), expressed as mg of gallic acid equivalent per g (mg GAE g^{-1}), were estimated following the Folin-Ciocalteu reagent[4,78]. The reaction mixture containing an appropriate aliquot of ethanolic extract, 0.5 ml of 1 N phenol reagent (Folin- Ciocalteu), and 6% sodium carbonate solution (2 ml) (w/v) was incubated at a boiling water bath for 2 min. Then, the tubes were kept at room temperature to cool the solution and the absorbance was recorded at 650 nm against a reagent blank. The total flavonoid content in leaves (LFlav) and arils (ArFlav) was determined by the aluminum chloride-based colorimetric method[79]. A volume of extracts (0.50 ml) was mixed with 0.3 ml each of 5% $NaNO_2$ and 10% $AlCl_3$ and 3.4 ml of 1 M NaOH. The resultant reaction mixtures were incubated for 15 min at room temperature and the OD was measured at 510 nm against the reagent blank. The total flavonoid content was expressed as mg of catechol equivalent per g (mg CE g^{-1}). In the case of total antioxidant activity (TAA), the reducing capacity of *P. dulce* aril (ArTAA) and leaf (LTAA) extracts were assayed by the DPPH method[3]. Different concentrations (100 to 500 µg/ml) of aqueous methanolic extract of aril and leaves of *P. dulce* (100 µl) were allowed to react with 2.9 ml of 0.006% methanolic DPPH for 10 min under dark conditions. A control was also run simultaneously with 100 µl distilled water instead of extract and absorbance was taken at 450 nm using a UV-VIS spectrophotometer (UV-2550, SHIMADZU). Ascorbic acid was used as a reference standard (10–50 µg) and the results were expressed as mg of ascorbic acid equivalent per g (mg AAE g^{-1}).

Aril mineral contents

The calcium (ArCa), phosphorus (ArP), and potassium (ArK) contents in arils were estimated on a fresh weight basis using diacid-digested samples[38]. One gram of fresh aril was digested in 25 ml of diacid mixture (concentrated nitric acid and perchloric acid in a 9:4 ratio), and distilled water was added to achieve the final volume (100 ml). ArCa was determined by EDTA titration[38], ArP was estimated colorimetrically using an atomic absorption spectrophotometer (ELICO SL 164, India), and ArK using a flame photometer (ELICO CL 361, India)[5].

Statistical analyses

The experiment was laid out in a randomized complete block design. Observations related to pod (fruit) physical parameters were recorded from randomly sampled trees ($n=3$) of each accession. Fruit physical and chemical properties, biochemical, antioxidants, and mineral contents were determined on randomly sampled firm-ripe fruits ($n=10$). One-way Analysis of Variance (ANOVA) was used to test the significant differences ($p<0.05$) (JASP v. 0.17.3). Means were compared using the Tukey test. Data are presented as mean ± standard deviation. Principal Component Analysis (PCA) was carried out to discern the key trends in data. Pearson's bivariate correlations between variables and the associated significance levels were calculated. Hierarchical cluster analysis was carried out using the Ward's distance[20].

Results

Genotypic differences for pod and peel-related traits

The results for the analysis of variance (ANOVA) and mean comparisons revealed strong differences among the studied genotypes for most of the pod and peel-related traits. The highly significant *F* values for all the traits ($p<0.001$) suggested that genotype accounted for a significant proportion of variance in the dependent variables (Table 2). The pod weight (PWt) varied between 10.22 g (CHESM-29) and 32.92 g (CHESM-6), while pod width (PW) ranged between 12.24 (CHESM-30) and 19.65 mm (CHESM-10). Compared with the average value of 19.27 g, the PWt was considerably higher in genotypes CHESM-6 and CHESM-24. The peel weight (PlWt) varied between 1.26 g (CHESM-31) and 5.12 g (CHESM-24), while peel percent (PlP) ranged between 7.05% (CHESM-6) and 34.32% (CHESM-30). Notably, genotypes CHESM-6 and CHESM-31 had very low peel content (< 10.0%) when compared with most other genotypes. Of these traits, PWt was the most variable with a coefficient of variation of ~ 20.0% (Table 2).

Genotypic differences for seed-related traits

Table 3 shows the variation among the tested genotypes for seed physical properties. The *F* values were highly significant for all the traits ($p<0.001$) except seed width (SW) ($F=2.40$, $p=0.039$) and number of seeds per pod (NSP) ($F=3.98$, $p=0.003$). It was interesting to note that both lower (1.19 g) and higher (3.40 g) values of seed weight (SWt) values were recorded in red aril genotypes (CHESM-31 and CHESM-33, respectively) while it ranged between 1.66 g (CHESM-10) and 2.62 g (CHESM-24) in white aril genotypes. White aril genotype CHESM-6 recorded the lowest seed percent (SP) (6.04%) while red aril genotype CHESM-30 recorded the highest SP (24.85%). The seed length (SL) ranged between 10.13 mm (CHESM-28) and 12.96 mm (CHESM-33), SW between 6.57 mm (CHESM-22) and 9.68 mm (CHESM-20), and NSP between 6.66 (CHESM-31) and 14.0 (CHESM-30). In general, seed-related traits varied more among red aril genotypes than among white aril genotypes (Table 3).

Genotypic differences for aril-related traits

The genotypic differences for aril physical attributes were invariably highly significant ($p<0.001$) except for ArWt ($F=4.47$, $p=0.002$). Both aril weight (ArWt) and aril percent (ArP) were the highest in CHESM-6 (28.45 g and 86.31%, respectively). Comparably, CHESM-29 recorded the lowest ArWt (5.60 g) and CHESM-30 recorded the lowest ArP (40.59%). Genotype CHESM-6 had the highest aril length (ArL) (25.20 mm) and aril width (ArW) (28.45 mm), while these two traits were the lowest (14.20 and 10.60 mm, respectively) in CHESM-22. In comparison with other traits, ArP exhibited a very high degree of variability (CV = 69.29%) (Table 4).

Genotype	PWt	PW	PlWt	PlP
CHESM-1	18.88 ± 3.72b-d	15.87 ± 2.42a-d	2.64 ± 0.37ab	14.19 ± 1.84b-d
CHESM-2	17.51 ± 3.21b-d	15.04 ± 1.87a-d	4.04 ± 0.66ab	23.21 ± 2.02ab
CHESM-3	20.89 ± 3.59a-d	16.57 ± 2.23a-d	3.16 ± 0.49ab	15.55 ± 4.09b-d
CHESM-4	19.52 ± 1.09b-d	18.68 ± 1.63ab	3.32 ± 0.98ab	17.04 ± 5.28b-d
CHESM-5	26.06 ± 5.46a-c	19.44 ± 0.96ab	4.27 ± 1.87ab	16.63 ± 6.87b-d
CHESM-6	32.92 ± 7.87a	19.42 ± 1.03ab	2.33 ± 0.71ab	7.05 ± 0.95d
CHESM-7	21.63 ± 2.98a-d	16.55 ± 0.33a-d	2.54 ± 0.32ab	11.74 ± 0.34b-d
CHESM-10	22.01 ± 2.71a-d	19.65 ± 0.75a	2.71 ± 0.82ab	12.21 ± 2.65b-d
CHESM-12	15.15 ± 2.97b-d	14.93 ± 0.44a-d	2.38 ± 0.54ab	15.64 ± 0.60b-d
CHESM-17	18.57 ± 6.21b-d	17.04 ± 2.17a-d	3.06 ± 1.11ab	17.71 ± 7.34b-d
CHESM-20	20.58 ± 1.56a-d	16.68 ± 1.05a-d	4.28 ± 0.99ab	20.79 ± 4.53a-d
CHESM-22	15.16 ± 3.11b-d	15.35 ± 0.41a-d	3.01 ± 0.98ab	20.30 ± 7.64b-d
CHESM-24	27.05 ± 8.68ab	19.11 ± 2.95ab	5.12 ± 3.85a	17.67 ± 7.69b-d
CHESM-26	13.55 ± 1.88d	14.68 ± 2.42b-d	3.28 ± 0.36ab	24.69 ± 5.45ab
CHESM-27	22.21 ± 2.69a-d	16.10 ± 0.45a-d	3.53 ± 1.09ab	15.77 ± 4.02b-d
CHESM-28	16.78 ± 1.88b-d	15.30 ± 0.09a-d	2.88 ± 1.27ab	16.79 ± 5.35b-d
CHESM-29	10.22 ± 1.57d	13.19 ± 0.48 cd	2.07 ± 0.31ab	20.90 ± 6.76a-c
CHESM-30	13.68 ± 6.30 cd	12.24 ± 1.78d	4.54 ± 1.51ab	34.32 ± 3.95a
CHESM-31	13.54 ± 1.33d	17.33 ± 1.99a-c	1.26 ± 0.06b	9.37 ± 1.02 cd
CHESM-32	17.69 ± 3.06b-d	16.41 ± 1.78a-d	3.83 ± 0.23ab	21.91 ± 2.43a-c
CHESM-33	21.62 ± 1.22a-d	15.63 ± 0.84a-d	4.28 ± 0.89ab	19.74 ± 3.54b-d
CHESM-34	18.75 ± 3.09b-d	16.21 ± 0.57a-d	3.82 ± 0.91ab	20.22 ± 1.61b-d
Mean	19.27	16.43	3.29	17.86
CV	26.55	11.94	28.17	31.98
F	5.56	9.00	18.14	14.70
p	< 0.001	< 0.001	< 0.001	< 0.001

Table 2. Analysis of variance (ANOVA) and mean comparisons for pod and peel-related traits in *Pithecellobium dulce* genotypes. The differences between the means indicated by different letters in the same column are significant at the $p < 0.001$ level. PWt- pod weight (g), PW- pod width (mm), PlWt- peel weight (g), and PlP- peel percentage (%).

Genotypic differences for fruit and leaf biochemical attributes

Table 5 shows the genotypic differences for fruit biochemical parameters. The *F* values were mostly highly significant ($p < 0.001$), except for aril protein (ArPt) ($F = 2.87$, $p = 0.002$). The TSS ranged between 17.33 °Brix (CHESM-10) and 26.46 °Brix (CHESM-28) with an average of 21.32 °Brix. Both acidity [0.54 (CHESM-6) and 1.07 (CHESM-24)] and TSS: acidity ratio (TA) differed remarkably among genotypes [20.65 (CHESM-1) and 42.79 (CHESM-29)], and were found to be more variable in terms of coefficient of variation (≥ 20.0%) when compared with TSS (CV = 12.51%). The ascorbic acid (AA) content ranged between 82.54 mg/100 g (CHESM-22) and 138.49 mg/100 g (CHESM-4) with an average of 107.26 mg/100 g. Of the tested genotypes, CHESM-28 exhibited the highest content of total sugars (TS) (18.81%) while it was the lowest (12.45%) in CHESM-10. Similarly, ArPt ranged between 3.15% (CHESM-24) and 6.32% (CHESM-27). There were highly significant differences among the genotypes for aril minerals as well as aril and leaf bioactive compounds (Table 6). The aril total phenols (ArTP) were the lowest (2.13 GAE mg g^{-1} FW) in CHESM-22 and the highest (11.18 GAE mg g^{-1} FW) in CHESM-29. Compared with the average value of 0.53 CE mg g^{-1} FW, some genotypes, including CHESM-29, CHESM-32, CHESM-33, and CHESM-34 had much higher aril flavonoids (ArFlav) contents (> 0.80 CE mg g^{-1} FW) while it was considerably lower (< 0.20 CE mg g^{-1} FW) in some genotypes, such as CHESM-2, CHESM-6, and CHESM-7.

The tested genotypes differed remarkably from one another in aril total antioxidant activity (ArTAA) with CHESM-6 exhibiting its lowest value (4.11 AAE mg g^{-1}) while CHESM-27 showed the highest ArTAA (27.09 AAE mg g^{-1}). Considerable genotypic differences were also noted for the aril minerals, including K (220.44–334.33 mg 100 g^{-1} FW), P (21.63–62.34 mg 100 g^{-1} FW), and Ca (14.06–39.74 mg 100 g^{-1} FW). However, aril P content was found to be more variable (CV = 34.18%) than both K (CV = 13.86%) and Ca (CV = 25.63%) (Table 6). The leaf total phenols (LTP) ranged between 7.25 GAE mg g^{-1} FW (CHESM-2), and 16.95 GAE mg g^{-1} FW (CHESM-27). Similarly, the leaf flavonoid (LFlav) content varied between 0.34 CE mg g^{-1} FW (CHESM-33), and 1.21 CE mg g^{-1} FW (CHESM-27) and LTAA differed between 8.61 AAE mg g^{-1} (CHESM-31)–30.54 AAE mg g^{-1} (CHESM-27). In general leaf samples had more bioactive compounds than aril and amongst white and red genotypes, red accessions contained a higher quantity in leaf and aril both (Table 6).

Genotype	SWt	SP	SL	SW	NSP
CHESM-1	2.46 ± 0.79ab	12.77 ± 1.82b-e	11.36 ± 0.69ab	9.10 ± 0.51a	9.67 ± 0.58ab
CHESM-2	1.89 ± 0.34ab	10.91 ± 1.92b-e	10.56 ± 0.59ab	8.44 ± 0.95ab	8.67 ± 0.58a
CHESM-3	2.37 ± 0.62ab	11.81 ± 4.48b-e	10.92 ± 0.85ab	9.03 ± 0.75a	9.68 ± 2.09ab
CHESM-4	2.06 ± 0.22ab	10.56 ± 1.08b-e	11.23 ± 0.49ab	9.29 ± 0.22a	9.32 ± 3.05ab
CHESM-5	1.96 ± 0.61ab	7.46 ± 1.50de	10.18 ± 0.48b	8.77 ± 0.32ab	8.65 ± 2.31b
CHESM-6	1.97 ± 0.43ab	6.04 ± 0.78e	11.98 ± 0.82ab	8.48 ± 0.38ab	8.66 ± 1.53b
CHESM-7	2.41 ± 0.20ab	11.21 ± 0.78b-e	11.17 ± 0.14ab	9.03 ± 1.12a	9.34 ± 0.58ab
CHESM-10	1.66 ± 0.21ab	7.56 ± 0.63de	11.32 ± 0.41ab	8.04 ± 0.52ab	7.33 ± 0.58b
CHESM-12	2.20 ± 0.59ab	14.35 ± 1.40	11.11 ± 0.24ab	8.70 ± 0.27ab	9.67 ± 2.31ab
CHESM-17	2.06 ± 0.29ab	11.64 ± 2.52b-e	11.26 ± 1.04ab	9.03 ± 0.76a	9.01 ± 0.98ab
CHESM-20	2.53 ± 0.60ab	12.24 ± 2.22b-e	12.25 ± 0.27ab	9.68 ± 0.35a	10.33 ± 0.58ab
CHESM-22	1.68 ± 0.61ab	10.99 ± 2.48b-e	10.25 ± 2.53ab	6.57 ± 1.23b	6.67 ± 0.58b
CHESM-24	2.62 ± 0.08ab	10.25 ± 2.72b-e	11.90 ± 0.67ab	8.87 ± 0.59a	10.00 ± 2.01ab
CHESM-26	1.99 ± 0.43ab	14.93 ± 4.04bc	12.03 ± 0.71ab	9.45 ± 0.67a	9.33 ± 2.52ab
CHESM-27	2.77 ± 0.42ab	12.65 ± 2.57b-e	11.81 ± 0.23ab	8.72 ± 0.68ab	10.33 ± 1.16ab
CHESM-28	1.34 ± 0.02b	8.05 ± 0.75c-e	10.13 ± 0.05b	8.12 ± 0.59ab	8.67 ± 0.58b
CHESM-29	2.52 ± 0.49ab	24.66 ± 2.63a	11.52 ± 1.07ab	9.64 ± 0.55a	7.67 ± 2.08b
CHESM-30	3.34 ± 1.34a	24.85 ± 2.22a	11.14 ± 1.15ab	8.50 ± 0.37ab	14.00 ± 3.46a
CHESM-31	1.19 ± 0.21b	8.86 ± 1.70b-e	10.29 ± 0.20ab	8.20 ± 0.25ab	6.66 ± 0.58b
CHESM-32	2.38 ± 0.70ab	13.28 ± 1.54b-d	11.39 ± 0.96ab	9.11 ± 1.11a	7.68 ± 1.53b
CHESM-33	3.40 ± 0.49a	15.71 ± 1.75b	12.96 ± 0.99a	9.01 ± 1.52a	10.66 ± 1.16ab
CHESM-34	2.64 ± 0.79ab	13.83 ± 2.16b-d	11.73 ± 0.79ab	9.42 ± 0.56a	8.67 ± 1.53b
Mean	2.25	12.48	11.30	8.78	9.12
CV	24.53	37.65	6.42	7.71	17.16
F	27.054	13.614	15.03	2.40	3.98
p	< 0.001	< 0.001	< 0.001	0.039	0.003

Table 3. Analysis of variance (ANOVA) and mean comparisons for seed-related traits in *Pithecellobium dulce* genotypes. The differences between the means indicated by different letters in the same column are significant at the $p < 0.001$ level. SWt- seed weight (g), SP, seed percentage (%), SL- seed length (mm), SW- seed width (mm), and NSP- number of seed pod.

Principal component analysis

The results of principal component analysis (PCA) are shown in Supplementary 1. The first four Principal Components with Eigenvalue > 1.0 accounted for 62.30% of the cumulative variance in data. The PC1 accounted for 27.60% of the total variation; it was largely a linear combination of ArTP, ArFlav, ArTAA, LFlav, and LTP. The PC2 explained 14.90% of the total variance in data and was loaded heavily on PWt, Arwt, ArL, ArW, TSS, AA, TS, ArK, and ArCa. The PC3 summarized about 12.20% of the variance in data and was mainly a construct of PlWt, SWt, SL, and NSP. As expected, the Eigenvalues and the proportion of variance explained by the subsequent Principal Components declined with PC4 accounting for 7.70% of the total variation in data (Supplementary 1). The variable loadings on the first two principal components are illustrated as the PCA biplot in Fig. 2. It was found that the PC1 separated most of the pod edible components (aril physical attributes) from non-edible components (PlP and SP) as well as the nutritional and bioactive compounds. Given its strong association with the traits accounting for antioxidant value (e.g., ArTP, ArTAA, ArFlav, LFlav, and LTP), the PC1 was labeled as the 'bioactive component'. The PC2 was well represented by the mixed variables (e.g., PWt, ArL, ArWt, SW, AA, TSS, TS, ArK, and ArCa) that directly or indirectly influence the pod edible quality and mineral contents; it may thus be termed as the 'pod quality component' (Fig. 2).

Correlation analysis

The Pearson's bivariate correlations and the associated *p*-values between the measured traits are shown in Supplementary 2 and Fig. 3. Pod physical traits of *P. dulce* showed significant positive correlations with each other; PWt mostly exhibited strong positive correlations with ArWt ($r = 0.950$, $p = 0.000$), ArW ($r = 0.782$, $p = 0.000$), PW ($r = 0.699$, $p = 0.000$), ArL ($r = 0.647$, $p = 0.000$) and ArP ($r = 0.491$, $p = 0.000$). Similarly, PWt had significant negative correlations with PlP ($r = -0.384$, $p = 0.001$), ArTP ($r = -0.359$, $p = 0.003$), ArPt ($r = -0.328$, $p = 0.007$), ArTAA ($r = -0.282$ $p = 0.022$), and SP ($r = -0.535$, $p = 0.000$). The PW had strong positive correlations with ArL ($r = 0.736$, $p = 0.000$), ArWt ($r = 0.734$, $p = 0.071$), ArW ($r = 0.677$, $p = 0.000$), ArP ($r = 0.634$, $p = 0.000$), and strong negative correlations with SP ($r = -0.712$, $p = 0.000$), PlP ($r = -0.475$, $p = 0.000$) and ArTAA ($r = -331$, $p = 0.000$). The PlWt was correlated positively with PlP ($r = 0.604$, $p = 0.000$), SWt ($r = 0.530$, $p = 0.000$), NSP ($r = 0.500$, $p = 0.000$), and inversely with ArP ($r = -0.437$, $p = 0.000$) (Fig. 3). The PlP and SP were strongly positively correlated ($r = 0.626$, $p = 0.000$), and had inverse relationships with edible components, such as ArWt, ArL and ArW ($p = 0.000$). However, both of them had positive correlations with most of the bioactive components

Genotype	ArWt	ArL	ArW	ArP
CHESM-1	13.69 ± 2.57b-d	20.10 ± 1.48a-d	16.03 ± 0.57b-d	72.63 ± 1.74a-d
CHESM-2	11.47 ± 2.53b-d	18.22 ± 2.61b-e	14.94 ± 1.86b-d	65.19 ± 4.25b-e
CHESM-3	15.22 ± 4.11b-d	18.96 ± 2.28b-e	15.53 ± 2.78b-d	72.01 ± 8.15a-d
CHESM-4	14.08 ± 1.64b-d	19.91 ± 0.46a-d	15.98 ± 0.29b-d	72.11 ± 6.60a-d
CHESM-5	19.78 ± 5.24ab	20.78 ± 3.23a-c	17.64 ± 0.89bc	75.69 ± 8.19a-d
CHESM-6	28.45 ± 6.94a	25.20 ± 1.52a	28.45 ± 6.94a	86.31 ± 1.53a
CHESM-7	16.57 ± 2.52bc	20.69 ± 2.01a-c	15.78 ± 1.38b-d	76.47 ± 1.23a-d
CHESM-10	17.62 ± 2.01bc	20.88 ± 0.91a-c	19.56 ± 0.76b	80.12 ± 1.88a-c
CHESM-12	10.45 ± 1.87b-d	18.01 ± 0.52b-e	13.93 ± 0.51b-d	69.15 ± 1.86a-e
CHESM-17	13.31 ± 6.06b-d	18.03 ± 0.68b-e	14.51 ± 2.29b-d	69.92 ± 9.33a-e
CHESM-20	13.77 ± 1.42b-d	19.27 ± 1.41b-e	15.35 ± 0.75b-d	66.89 ± 6.39b-e
CHESM-22	10.46 ± 2.76b-d	14.20 ± 2.08e	10.61 ± 1.61d	68.61 ± 10.19b-e
CHESM-24	19.22 ± 4.52ab	23.24 ± 2.49ab	17.14 ± 1.58bc	72.27 ± 5.72a-d
CHESM-26	8.15 ± 2.38 cd	18.49 ± 2.58b-e	13.34 ± 2.68b-d	60.11 ± 8.66de
CHESM-27	15.72 ± 2.41bc	19.75 ± 0.68b-d	16.04 ± 1.81b-d	70.75 ± 4.93a-e
CHESM-28	12.52 ± 0.59b-d	18.98 ± 0.78b-e	14.60 ± 0.24b-d	75.01 ± 4.54a-d
CHESM-29	5.60 ± 1.52d	16.94 ± 0.46c-e	12.71 ± 0.82 cd	54.25 ± 7.51ef
CHESM-30	5.72 ± 3.22d	15.04 ± 1.74de	10.58 ± 0.29d	40.59 ± 4.34f
CHESM-31	10.98 ± 1.21b-d	19.18 ± 0.96b-e	15.82 ± 1.99b-d	81.04 ± 1.65ab
CHESM-32	11.22 ± 2.03b-d	18.62 ± 0.72b-e	15.07 ± 1.02b-d	63.39 ± 0.61c-e
CHESM-33	13.83 ± 0.87b-d	17.96 ± 1.47b-e	14.73 ± 0.57b-d	64.06 ± 4.96b-e
CHESM-34	12.20 ± 1.34b-d	19.15 ± 2.37b-e	14.46 ± 0.78b-d	65.52 ± 3.96b-e
Mean	13.64	19.16	15.58	69.19
CV	36.43	12.22	22.55	13.90
F	4.47	5.14	22.84	30.59
p	0.002	< 0.001	< 0.001	< 0.001

Table 4. Analysis of variance (ANOVA) and mean comparisons for aril-related traits in *Pithecellobium dulce* genotypes. The differences between the means indicated by different letters in the same column are significant at the $p < 0.001$ level. ArWt- aril weight (g), ArL- aril length (mm), ArW- aril width (mm), and ArP- aril percentage (%).

of aril (ArFlav and ArTAA) and leaves (LFlav and LTAA). The SWt showed strong positive correlations with NSP, SP, SL, and SW, and a strong negative correlation with ArP ($r = -0.488$, $p = 0.000$). The SP had significant negative correlations with fruit aril physical, including ArP, ArWt, ArW, and ArL ($p = 0.000$). The ArWt had strong positive correlations with ArP, ArL, and ArW ($p = 0.000$), and moderate negative correlations with ArFlav, ArTAA, and ArTP. The TSS showed remarkably strong positive correlations with TS ($r = 0.963$, $p = 0.000$), and AA ($r = 0.630$, $p = 0.000$). Likewise, ArTP had strong positive correlations with ArTAA ($r = 0.831$, $p = 0.000$), ArFlav ($r = 0.632$, $p = 0.000$), and ArK ($r = 0.656$, $p = 0.000$), and ArTAA exhibited strong positive correlations with LTP ($r = 0.611$, $p = 0.000$), and ArK ($r = 0.574$, $p = 0.000$). There were strong positive correlations between AA and TS ($r = 0.613$, $p = 0.000$), and ArFlav and ArTAA ($r = 0.738$, $p = 0.000$) (Supplementary 2; Fig. 3).

Hierarchical cluster analysis

The hierarchical cluster analysis using Ward's linkage method grouped the *P. dulce* genotypes into two broad clusters (Fig. 4). The first cluster (cluster I) was further divided into subclusters IA and IB, both comprising five genotypes each. The sub-cluster IA had three white (CHESM-12, CHESM-26, and CHESM-29) and two red genotypes (CHESM-27 and CHESM-30). Similarly, sub-cluster IB had three red (CHESM-32, CHESM-33, and CHESM-34), and two white genotypes (CHESM-2 and CHESM-22). The Cluster II was also divided into sub-clusters IIA and IIB. While IIA consisted exclusively of 7 white genotypes (CHESM-10, CHESM-6, CHESM-24, CHESM-5, CHESM-4, CHESM-3, and CHESM-7), cluster IIB comprised two red aril (CHESM-28 and CHESM-31) and three white aril genotypes (CHESM-17, CHESM-20, and CHESM-1). A perusal of the heat map also revealed that genotypes in Cluster I were mostly low in pod width (PW) and aril-related attributes, such as ArP, ArL, ArW, and ArWt. Likewise, the genotypes in Cluster II generally exhibited low values of seed-related (e.g., SWt, SP, and NSP) and leaf bioactive parameters. The genotypes, such as CHESM-30 (NSP and PIP), CHESM-29 (SP), CHESM-33 (SL and SWt), CHESM-6 (ArL, ArW, ArWt, PWt, ArP, and PW), and CHESM-24 (Acid and PlWt) had positive value(s) of certain traits, suggesting that these traits distinguished these genotypes from others and may be utilized in genetic studies and genetic improvement programs. Based on the hierarchical clustering heatmap, three white aril types (CHESM-4, CHESM-6, and CHESM-20) and two red aril types (CHESM-27, CHESM-31, and CHESM-33) were adjudged to be promising in terms of desirable traits (Fig. 4).

Genotype	TSS	Acid	TA	AA	TS	ArPt
CHESM-1	20.27 ± 1.08c-g	0.98 ± 0.04ab	20.65 ± 1.79 h	92.70 ± 6.06gh	15.44 ± 0.75a-e	5.28 ± 1.97a-c
CHESM-2	20.36 ± 0.96c-g	0.65 ± 0.04d-g	31.63 ± 3.41b-f	92.0 ± 8.19gh	14.89 ± 0.75b-e	5.79 ± 0.99a-c
CHESM-3	21.49 ± 0.71b-g	0.62 ± 0.05e-g	35.07 ± 4.0a-c	129.95 ± 5.38a-c	15.75 ± 0.52a-e	3.57 ± 1.08bc
CHESM-4	22.47 ± 2.90a-f	0.69 ± 0.03d-f	32.66 ± 5.15b-e	138.49 ± 3.97a	17.48 ± 2.26ab	6.25 ± 1.23ab
CHESM-5	23.49 ± 0.70a-e	0.77 ± 0.03 cd	30.44 ± 1.96b-g	127.37 ± 4.19a-c	16.94 ± 0.46a-c	4.31 ± 0.97a-c
CHESM-6	19.53 ± 0.25e-g	0.54 ± 0.05 g	36.33 ± 2.73a-c	110.62 ± 7.26d-f	13.81 ± 0.28c-e	4.18 ± 0.84a-c
CHESM-7	18.20 ± 0.36 fg	0.55 ± 0.04 fg	32.96 ± 1.71b-d	84.18 ± 4.95 h	12.52 ± 0.59e	3.79 ± 0.92a-c
CHESM-10	17.33 ± 0.57 g	0.79 ± 0.04 cd	21.96 ± 0.44gh	87.75 ± 3.75 h	12.45 ± 0.38e	5.34 ± 0.73a-c
CHESM-12	22.50 ± 0.70a-f	0.61 ± 0.05e-g	37.01 ± 3.26ab	85.37 ± 4.39 h	16.92 ± 0.53a-c	4.14 ± 1.28a-c
CHESM-17	23.10 ± 0.53a-e	0.85 ± 0.05bc	27.39 ± 2.48c-h	133.93 ± 5.13ab	16.62 ± 0.42a-d	4.89 ± 0.98a-c
CHESM-20	24.20 ± 0.79a-d	1.02 ± 0.04a	23.76 ± 1.43e-h	125.10 ± 3.48a-d	17.62 ± 0.57ab	5.24 ± 0.23a-c
CHESM-22	18.13 ± 1.46 fg	0.65 ± 0.03d-g	27.92 ± 2.34b-h	82.54 ± 2.85 h	12.88 ± 1.03e	4.78 ± 1.80a-c
CHESM-24	24.70 ± 0.99a-c	1.07 ± 0.09a	23.21 ± 2.52f-h	133.47 ± 4.85ab	17.56 ± 0.70ab	3.15 ± 0.44c
CHESM-26	21.63 ± 0.85b-g	0.84 ± 0.05bc	25.75 ± 2.57d-h	93.17 ± 3.47gh	15.77 ± 0.66a-e	4.95 ± 1.08a-c
CHESM-27	23.71 ± 2.67a-e	0.72 ± 0.07c-e	33.05 ± 5.47b-d	119.75 ± 4.58b-e	17.16 ± 1.82a-c	6.32 ± 0.81a
CHESM-28	26.46 ± 1.96a	0.77 ± 0.04 cd	34.46 ± 3.76a-d	115.75 ± 4.55c-f	18.81 ± 1.39a	4.67 ± 0.49a-c
CHESM-29	25.50 ± 0.50ab	0.60 ± 0.03e-g	42.79 ± 1.77a	132.073 ± 3.04ab	16.99 ± 1.10a-c	5.64 ± 0.25a-c
CHESM-30	19.45 ± 2.87e-g	0.86 ± 0.06bc	22.54 ± 1.88f-h	82.83 ± 3.79 h	14.03 ± 2.04c-e	5.53 ± 0.12a-c
CHESM-31	20.02 ± 2.35d-g	0.95 ± 0.07ab	21.24 ± 3.27 h	92.06 ± 2.05gh	14.27 ± 1.98b-e	5.26 ± 0.43a-c
CHESM-32	17.45 ± 1.07 g	0.61 ± 0.03e-g	28.45 ± 1.06b-h	102.79 ± 3.91 fg	12.60 ± 0.79e	4.75 ± 0.39a-c
CHESM-33	18.56 ± 1.92 fg	0.66 ± 0.07d-g	28.38 ± 3.05b-h	89.49 ± 3.75gh	13.29 ± 1.45de	3.19 ± 0.43c
CHESM-34	20.50 ± 0.70c-g	0.61 ± 0.05e-g	33.68 ± 1.47b-d	108.38 ± 8.06ef	14.89 ± 0.46b-e	5.96 ± 0.34ab
Mean	21.32	0.75	29.61	107.26	15.40	4.86
CV	12.51	21.02	20.05	18.51	12.63	18.92
F	9.90	31.33	12.70	49.797	9.09	2.873
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002

Table 5. Analysis of variance (ANOVA) and mean comparisons for aril protein and chemical properties related traits in *Pithecellobium dulce* genotypes. The differences between the means indicated by different letters in the same column are significant at the $p < 0.001$ level. TSS- total soluble solids (°Brix), Acid- acidity (%), TA- total soluble solids: acidity, AA- ascorbic acid (mg 100 g^{-1}), TS- total sugars (%), and ArPt- aril protein (%).

Discussion

Although Manila tamarind could be a promising alternative crop for enhanced food production from marginal lands, there have been no systematic attempts to properly assess its genetic resources for the development of improved cultivars. Because Manila tamarind continues to be grown in certain niche tribal and marginalized areas as a subsistence crop[16], adequate emphasis on its genetic improvement is a prerequisite for promoting its commercial cultivation. An improved understanding of the fruit's physicochemical, mineral, and biochemical characteristics is crucial for developing Manila tamarind cultivars that are appropriate for various end uses[38]. There is a growing worldwide interest in non-conventional plant foods to meet the daily needs for vital nutrients and health-promoting bioactive chemicals. The global food industry is also increasingly exploring locally adapted and underutilized natural products that contain a blend of vital nutrients and bioactive compounds[74]. To our knowledge, there is a lack of comprehensive understanding regarding the genetic diversity for fruit and leaf nutritional and medicinal properties of Manila tamarind; our study seeks to address this knowledge gap[5].

In this study, we characterized 15 white and 7 red aril *P. dulce* accessions using pod physical attributes (13), aril chemical composition (6), minerals (3), bioactive compounds (3), and leaf bioactive compounds (3) to assess the extent of genotypic variability and to identify the promising genotypes. Our findings demonstrated highly significant ($p < 0.001$) genotypic differences for almost all the traits examined, suggesting the scope for further selection. Hitherto, our understanding of the fruit quality attributes in Manila tamarind comes largely from the investigations on a few non-descript landraces from countries, such as the Philippines[18], Thailand[24], Mexico[50], Pakistan[23], and India[6,16,27]. Interestingly, one of the major shortcomings in the majority of these studies is that they examined nutritional and bioactive compound profiles exclusively in fruit[50,74], or bark/leaf samples[22,27]. They are thus not comprehensive because only a small number of genotypes were screened, overlooking several desirable characteristics, which makes it challenging to draw reasonable conclusions. Our study addresses these gaps, at least partly, by investigating horticultural, nutritional, and antioxidant traits in fruits of 22 *P. dulce* accessions, and establishing their relationships with leaf bioactive compounds. The majority of the economically significant attributes in our study showed a high degree of variation, indicating ample scope for the selection of elite genotypes[16].

Higher fruit weight and aril yield are two crucial factors in selecting the superior cultivars of arillate fruits[39,59,62]. The weight of the fruit and arils may have varied across the Manila tamarind accessions due to the varying translocation of photosynthates from the leaves to developing pods and seeds[74]which is also influenced

Genotype	ArTP	ArFlav	ArTAA	ArK	ArP.1	ArCa	LTP	Lflav	LTAA
CHESM-1	7.41 ± 0.96c-g	0.57 ± 0.02e-g	20.83 ± 1.25bc	280.35 ± 4.42 cd	25.32 ± 2.88hi	19.44 ± 3.57e-g	12.11 ± 1.51a-c	0.96 ± 0.13bc	12.41 ± 1.54c-f
CHESM-2	3.21 ± 0.45i-k	0.19 ± 0.05kl	6.78 ± 0.18 g-i	220.44 ± 4.69 h	53.55 ± 3.56a-c	14.06 ± 2.95 g	7.25 ± 0.35c	0.66 ± 0.22d-g	16.86 ± 3.36c-f
CHESM-3	2.62 ± 0.14jk	0.41 ± 0.06 h-j	5.46 ± 1.10hi	273.53 ± 4.89de	23.85 ± 2.94hi	17.93 ± 3.14 fg	9.18 ± 1.99bc	0.41 ± 0.12hi	21.73 ± 2.90a-d
CHESM-4	3.25 ± 0.39i-k	0.45 ± 0.11 g-i	8.84 ± 1.48f-i	233.56 ± 2.886e-h	21.63 ± 3.41i	34.04 ± 5.07a-c	9.28 ± 2.61bc	0.47 ± 0.11 g-i	22.82 ± 4.04a-c
CHESM-5	3.48 ± 0.49 h-k	0.56 ± 0.03e-g	17.56 ± 0.67 cd	264.49 ± 4.31d-g	41.45 ± 5.62c-e	14.56 ± 2.07 g	12.38 ± 1.81a-c	0.94 ± 0.11bc	12.39 ± 4.29c-f
CHESM-6	2.28 ± 0.59k	0.18 ± 0.04kl	4.11 ± 0.22i	267.887 ± 7.52d-f	25.24 ± 3.06hi	39.12 ± 4.82ab	7.69 ± 1.35c	0.59 ± 0.13f-h	17.57 ± 4.33c-f
CHESM-7	2.34 ± 0.29k	0.12 ± 0.05 L	4.47 ± 0.15i	225.29 ± 3.96gh	30.54 ± 3.84e-i	22.39 ± 1.99d-g	7.54 ± 0.76c	0.80 ± 0.24c-f	19.29 ± 4.99b-f
CHESM-10	2.90 ± 0.22jk	0.51 ± 0.07f-h	10.49 ± 1.47f-h	220.81 ± 2.04 h	43.90 ± 4.65 cd	33.10 ± 2.94a-c	7.42 ± 1.85c	0.74 ± 0.21c-f	15.96 ± 3.36c-f
CHESM-12	8.19 ± 0.99b-d	0.56 ± 0.05e-g	19.01 ± 1.57c	288.33 ± 7.75 cd	35.72 ± 4.92d-h	26.50 ± 4.95c-f	12.41 ± 2.97a-c	0.94 ± 0.25bc	21.15 ± 5.34a-e
CHESM-17	5.50 ± 0.35f-h	0.37 ± 0.04 h-j	12.37 ± 1.27ef	283.62 ± 3.95 cd	40.11 ± 4.84d-g	29.95 ± 3.94a-e	11.98 ± 4.05a-c	0.84 ± 0.23c-e	17.54 ± 3.88c-f
CHESM-20	4.51 ± 0.40 g-j	0.36 ± 0.03ij	9.66 ± 0.67f-h	298.83 ± 6.90b-d	42.74 ± 2.93c-e	32.07 ± 3.01a-d	12.51 ± 2.89a-c	0.92 ± 0.09bc	9.98 ± 1.37ef
CHESM-22	2.13 ± 0.34k	0.35 ± 0.04ij	13.42 ± 0.58d-f	227.54 ± 7.38f-h	39.01 ± 4.74d-g	29.21 ± 2.95a-e	8.74 ± 2.48bc	0.79 ± 0.17c-f	13.05 ± 2.59c-f
CHESM-24	5.44 ± 0.50f-h	0.69 ± 0.04c-e	19.68 ± 0.52c	301.03 ± 4.87b-d	27.14 ± 3.01 g-i	32.54 ± 2.94a-d	9.45 ± 3.36bc	0.83 ± 0.15c-f	17.71 ± 2.65c-f
CHESM-26	7.83 ± 0.33c-e	0.74 ± 0.06b-d	21.17 ± 1.33bc	271.44 ± 4.94de	40.57 ± 3.90c-f	33.79 ± 3.78a-c	10.58 ± 2.84a-c	0.87 ± 0.09 cd	22.94 ± 6.68a-c
CHESM-27	10.22 ± 0.88ab	0.65 ± 0.04d-f	27.09 ± 1.66a	365.79 ± 6.84a	57.36 ± 7.72ab	39.74 ± 3.34a	16.95 ± 1.65a	1.21 ± 0.06a	30.54 ± 2.05a
CHESM-28	10.25 ± 1.79ab	0.62 ± 0.03d-f	24.98 ± 0.97ab	317.11 ± 6.32bc	25.71 ± 3.96hi	38.09 ± 5.83ab	15.84 ± 1.92ab	0.43 ± 0.11 g-i	9.92 ± 1.71f
CHESM-29	11.18 ± 1.08a	0.84 ± 0.04ab	26.76 ± 5.29a	289.24 ± 7.42 cd	46.34 ± 4.62b-d	35.03 ± 4.94a-c	14.41 ± 2.48a-c	1.13 ± 0.07ab	23.23 ± 8.43a-c
CHESM-30	6.42 ± 0.30d-g	0.59 ± 0.05ef	17.11 ± 1.64c-e	295.58 ± 4.97b-d	23.07 ± 3.03hi	28.72 ± 5.04b-e	16.95 ± 0.66a	1.16 ± 0.13ab	29.33 ± 3.62ab
CHESM-31	5.84 ± 0.39e-g	0.29 ± 0.03jk	10.55 ± 0.42 fg	303.18 ± 3.05b-d	24.66 ± 3.97hi	29.12 ± 4.46a-e	9.04 ± 1.65bc	0.39 ± 0.08hi	8.61 ± 0.89f
CHESM-32	5.15 ± 0.75 g-i	0.88 ± 0.03ab	17.85 ± 1.91 cd	333.09 ± 4.28ab	62.34 ± 3.75a	37.53 ± 2.83ab	9.68 ± 3.87a-c	1.12 ± 0.14ab	10.18 ± 2.34ef
CHESM-33	9.12 ± 0.55a-c	0.93 ± 0.04a	16.28 ± 1.23c-e	334.33 ± 3.87ab	58.52 ± 5.59ab	30.21 ± 3.72a-d	8.79 ± 1.14bc	0.34 ± 0.08i	9.08 ± 1.166f
CHESM-34	5.11 ± 0.20 g-i	0.83 ± 0.04a-c	16.66 ± 0.87c-e	298.22 ± 6.32b-d	28.24 ± 3.73f-i	33.03 ± 2.91a-c	8.27 ± 1.89c	0.60 ± 0.07e-h	10.82 ± 1.85d-f
Mean	5.65	0.53	15.05	281.53	37.14	29.55	10.84	0.78	16.96
CV	50.22	43.64	46.33	13.86	34.18	25.63	28.14	33.94	37.85
F	55.794	72.449	201.14	155.16	26.435	12.017	5.262	9.773	8.555
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 6. Analysis of variance (ANOVA) and mean comparisons for aril minerals, aril, and leaf bioactive compounds in *Pithecellobium dulce* genotypes. The differences between the means indicated by different letters in the same column are significant at the $p < 0.001$ level. ArTP- aril total phenols (GAE mg g^{-1}), ArFlav- aril flavonoids (CE mg g^{-1}), ArTAA- aril total antioxidant activity (AAE mg g^{-1}); ArK- aril potassium (mg 100 g^{-1}), ArP.1- aril phosphorus (mg 100 g^{-1}), ArCa- aril calcium (mg 100 g^{-1}), LTP- leaf total phenols (GAE mg g^{-1}), LFlav- leaf flavonoids (CE mg g^{-1}), and LTAA- leaf total antioxidant activity (AAE mg g^{-1}).

by the genetic makeup of the accessions[31,41,71,72]and different growing environments[2,68]. In our case, considerable variability was recorded for PWt (10.22–32.92 g), PW (12.24–19.65 mm), and PlWt (1.21–5.12 g). Although such variability has not previously been documented in *P. dulce*, similar results have been reported in tamarind[26,34,55]. In contrast to the findings of Narayan[46] for a lower pod width (9.33–13.60 mm), our results suggest a greater variability in this attribute. In comparison with white aril genotypes (1.66–2.62 g), we observed that red aril genotypes showed comparatively more diversity in seed weight (1.19–3.40 g), suggesting that red aril types may provide greater possibilities for the selection for lower seed weight. Pio-Leon et al.[50] also reported lower seed weight in 'white' and higher seed weight in 'red' aril *P. dulce* genotypes. Our results for NSP are slightly higher than those reported in Goyal et al.[16], and Narayan[46]. Overall, the results of this study for seed physical properties revealed rich genetic diversity, and agree with the previous findings in *P. dulce*[16,50]and tamarind[26,34,55]. We recorded higher ArWt in white aril genotypes while it was lower in red aril genotypes. The aril physical properties in our study are more variable than values previously reported in *P. dulce* by Pio-Leon et al.[50].

One of the major contributions of the present study was that we examined genotypic variations in biochemical attributes, such as TSS, acidity, TSS: acidity ratio, total sugars, ascorbic acid, and protein contents; these factors significantly improve the nutritional value and eating quality of Manila tamarind arils. Such variations have also been reported in other fruit crops[34,38,77]. The highly variable values of TSS (17.33–26.46 °Brix), acidity (0.54–1.07%), TA (21.24–36.33), AA (82.54–138.49 mg 100 g^{-1}), TS (12.45–18.81%), and ArPt (3.15–6.32%) recorded by us on the whole meet fresh consumption and aril processing standards[9,24,49,53,74]. Our results agree with the findings of Pio-Leon et al.[50], we also found that white aril genotypes had greater levels of ascorbic acid; nevertheless, in contrast to them, we found that white aril types had higher TSS whereas red aril types had higher ArPt and TS. Such differences are reasonable on account of a comparatively large number of *P. dulce* accessions assessed by us. Because their high sugar content provides a quick energy boost, the consumption of Manila tamarind arils is considered an alternate food source for the marginalized people in the semi-arid tropics. Similarly, in addition to antioxidant properties, higher ascorbic acid levels can also alleviate vitamin C deficiencies[9,70].

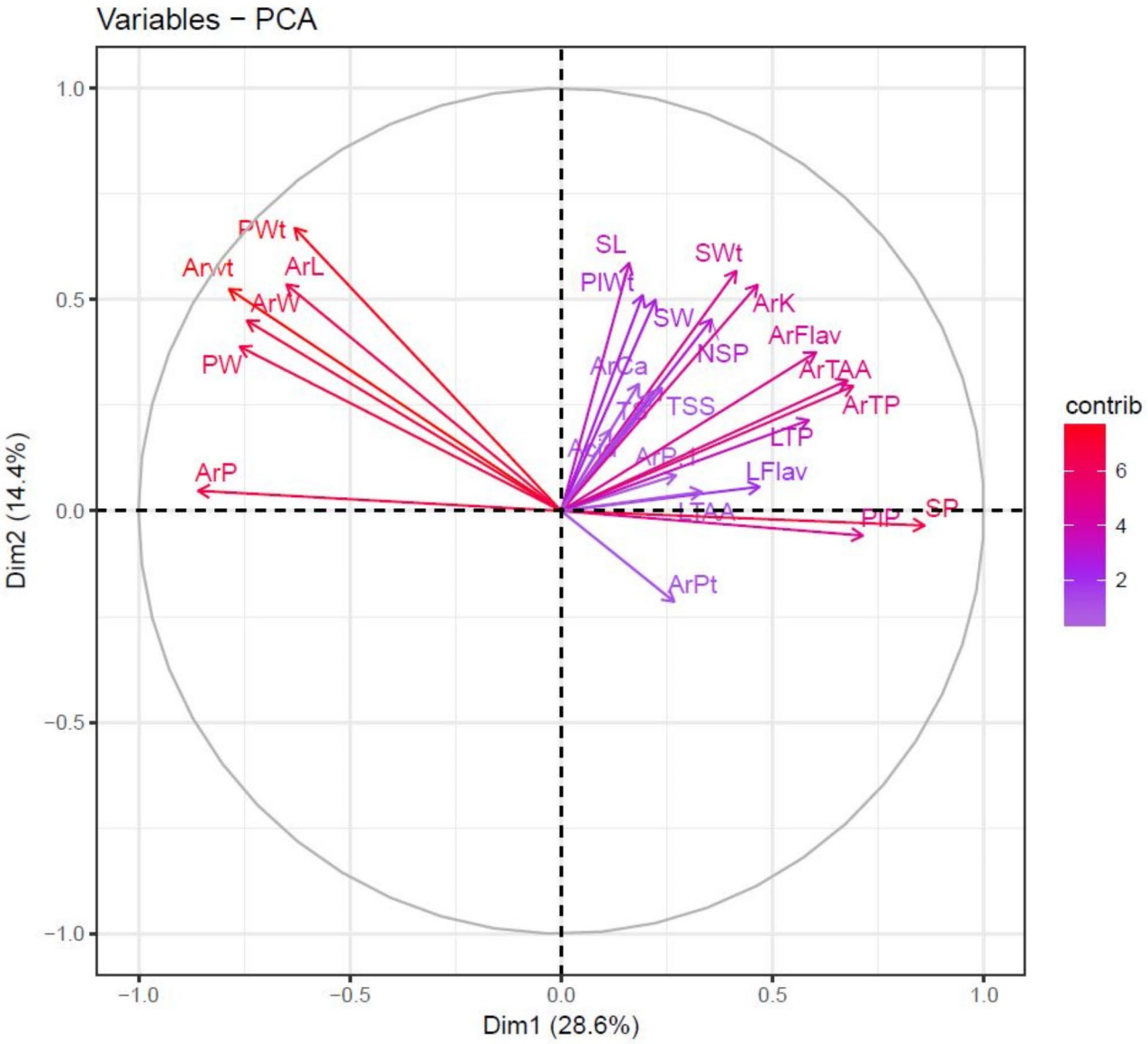

Fig. 2. Correlation of variables in a 2-dimension plot of *Pithecellobium dulce* genotypes. Abbreviations: PWt, pod weight (g); PW, pod width (mm); PlWt, peel weight (g); PlP, peel percentage (%); SWt, seed weight (g); SP, seed percentage (%); SL, seed length (mm); SW, seed width (mm); NSP, number of seed/pod; ArWt, aril weight (g); ArL, aril length (mm); ArW, aril width (mm); ArP, aril percentage (%); TSS, total soluble solids (°Brix); Acid, acidity (%); AA, ascorbic acid (mg/100 g); TS, total sugars (%); ArPt, aril protein (%); ArTP, aril total phenols (GAE mg g^{-1}); ArFlav, aril flavonoids (CE mg g^{-1}); TAA, aril total antioxidant activity (AAE mg g^{-1}); ArK, aril potassium (mg g^{-1}); ArP.1, aril phosphorus (mg g^{-1}); ArCa, aril calcium (mg g^{-1}); LTP, leaf total phenols (GAE mg g^{-1}); LFlav leaf flavonoids (CE mg g^{-1}); LTAA, and leaf total antioxidant activity (AAE mg g^{-1}).

P. dulce accessions varied appreciably with each other in aril mineral content and bioactive compounds. The variability in ArK (220.44–334.33 mg 100 g^{-1} FW), ArP (21.63–62.34 mg 100 g^{-1} FW), and ArCa (14.06–39.12 mg 100 g^{-1} FW) observed in this study are supported by the findings of Pio-Leon et al.[50] in Mexican *P. dulce* and Yadav et al.[76] in Indian *P. dulce*. It is also reported that red aril types had higher ArCa and ArK contents than white aril types[50,53]. The range for ArTP content (2.13–11.18 GAE mg g^{-1}FW) in our study conforms the findings of Kubola et al.[24] in Thai *P. dulce*. However, Recuenco et al.[54] recorded low ArTP content in Philippines *P. dulce* accessions whereas Rao et al.[53] recorded a fairly high ArTP in white types; supporting our findings. Our results for total phenols are comparable to the values reported for Mexican *P. dulce* accession[50,74], but higher than those reported for Thai Manila tamarind[57]. The range for ArFlav content (0.12–0.93 CE mg g^{-1}FW) in our study is consistent with the earlier findings[10,50,74]. We recorded a high variability for ArTAA (4.11–27.09 AAE mg g^{-1}), which has previously been substantiated in *P. dulce* from other countries, including Thailand[24], India[6,51], and the Philippines[54]. Leaf nutraceutical parameters, including LTP (7.25–16.95 GAE mg g^{-1}FW), LFlav (0.34–1.21

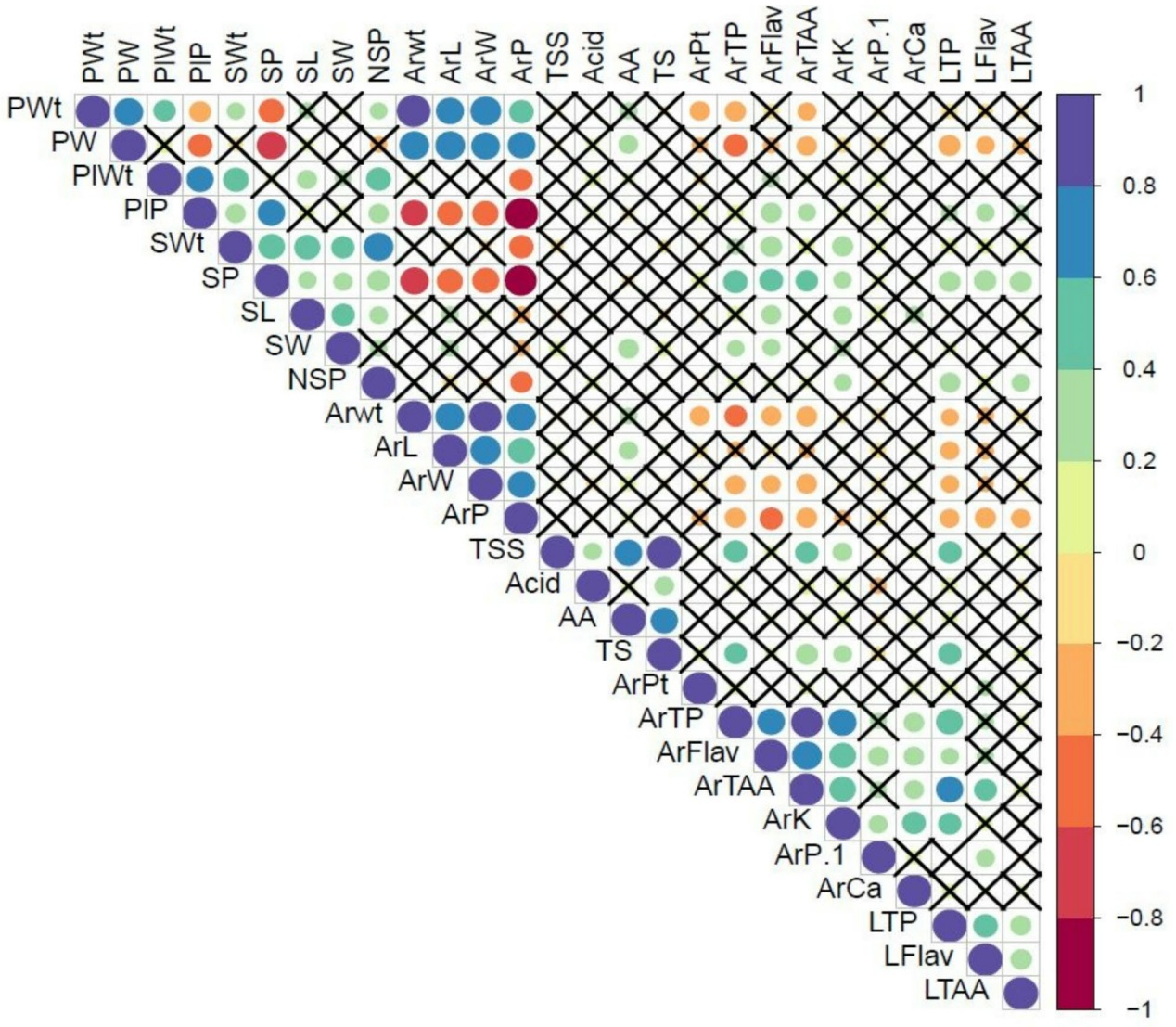

Fig. 3. Correlation plot showing Pearson's bivariate correlations between the measured traits in *Pithecellobium dulce* genotypes. Ellipse size and color reflect the strength and direction (positive or negative) of the correlation. Individual cells marked with a cross (X) denote non-significant correlations. For abbreviations, see Fig. 2.

CE mg g^{-1}FW), and LTAA (9.08–30.54 AAE mg g^{-1}) also varied remarkably among Manila tamarind genotypes in the present study. In general, leaves were found to have more bioactive compounds than arils. Likewise, red aril types were observed to contain more bioactive compounds in both leaves and arils when compared with white aril accessions. It is known that plants belonging to the Fabaceae family are frequently rich in secondary metabolites, such as tannins, flavonoids, and other phenolic compounds[11]. Several factors, including genetic, agronomic, and environmental aspects, can alter the nutraceutical levels in the red and white aril varieties[38,74].

Highly significant correlations between PWt on one hand and ArW, PW, ArL, and PlWt on the other indicate that increases in pod weight covary with the increases in other pod components[26]. The inverse relationships of PWt with PlP and SP suggest that selection for a higher pod weight can result in significant reductions in non-edible components of the pod[21,34]. We observed significant negative correlations of SP with aril physical attributes, such as ArP and ArWt. Kanupriya et al.[21]reported that the seed percent had significant negative effects on pulp mass and pulp percentage in tamarind and that this could be useful in selecting the genotypes with a higher pulp weight. The ArWt had strong positive correlations with ArP, ArL, and ArW, and negative correlations with ArTP. A negative relationship between pulp weight and tannin content has previously been demonstrated in tamarind[34] and may be useful in selecting less acrid and more palatable *P. dulce* genotypes. TSS showed strong positive correlations with TS, AA, ArTP, LTP, and ArTAA, substantiated by previous reports in red-pulped guava[38]and tamarind[34]. In our study, ArTP showed strong positive correlations with ArTAA, ArFlav, ArK, and LTP which is supported by the findings of Nagmoti et al.[44]. They demonstrated the better

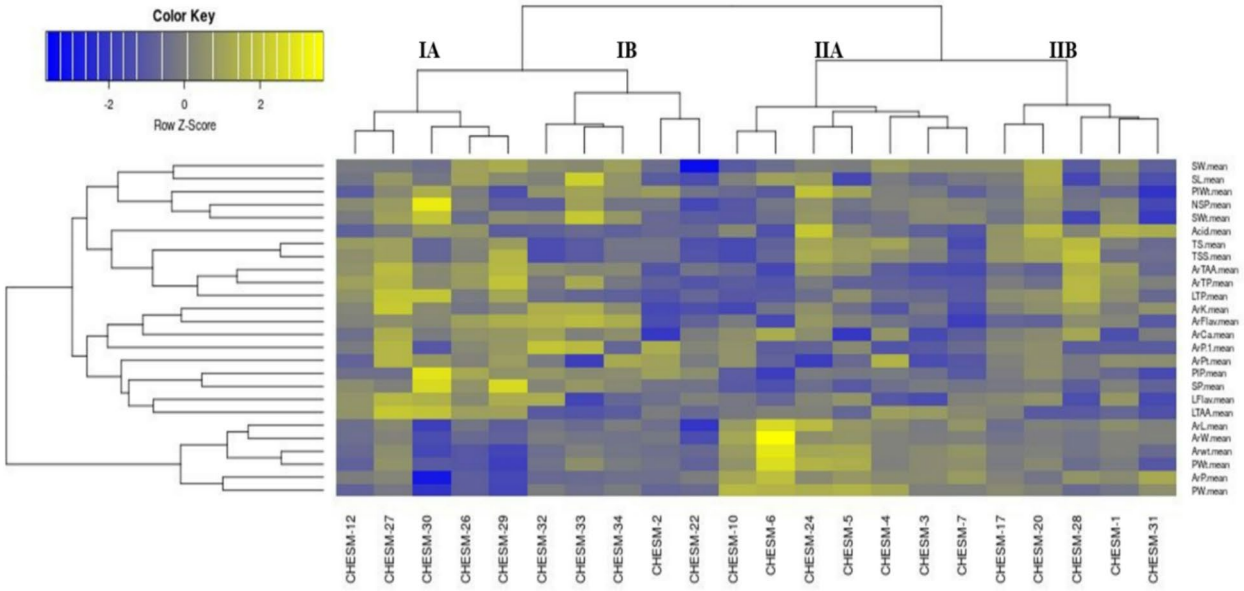

Fig. 4. Hierarchical clustering heat map showing the clustering of genotypes and traits of *Pithecellobium dulce* genotypes based on Ward's linkage method. Data were scaled before clustering, and the values were normalized for each trait by Z-Fisher transformation. Yellow and blue represent high and low trait values, respectively. For abbreviations, see Fig. 2.

antioxidant and free radical scavenging potential of *P. dulce* seeds extract powder via an in-vitro assay compared with standard antioxidants, and attributed high antioxidant activity in *P. dulce* to its high phenolic content. ArTAA exhibited strong positive correlations with LTP, ArK, and ArFlav. Such positive correlations between various phenolic and antioxidant compounds have previously been demonstrated[22,57]. These results indicate that the aril and leaf extracts of this plant are an important source of natural antioxidants[22,73]. Principal Component Analysis (PCA) and cluster analysis highlighted significant variations among *P. dulce* genotypes. The first three Principal Components accounted for over half of the cumulative variance in data suggesting that PCA efficiently reduced the dimensionality and summarized the major patterns in data. These results are largely agreed with the previous reports on tamarind[21,26]and guava[38]. Hierarchical cluster analysis revealed both the similarities and differences among the Manila tamarind genotypes in terms of various physical, biochemical, and bioactive compound attributes[26,45]. The identification of specific traits deriving clustering can inform breeding strategies aimed at improving crop performance and sustainability[15].

Conclusions

Our findings demonstrated a high degree of diversity among 22 *P. dulce* accessions for several traits of interest. Our results assume significance because these 22 accessions represent an unexplored gene pool of *P. dulce*. The genetic divergence for pod physical and chemical quality traits along with leaf and aril bioactive components is expected to assist in selecting the suitable genotypes for various end uses. Significant variations were observed across all accessions for pod quality and phytochemical attributes. Among the white aril genotypes, CHESM-6 exhibited superior pod physical characteristics, including a higher pod weight (32.92 g), aril weight (28.45 g), and aril percentage (86.31%). However, among red aril genotypes, CHESM-27 recorded the higher pod weight (22.21 g), aril weight (15.72 g), aril protein (6.32%), and total antioxidant activity (27.09 AAE mg g^{-1}); CHESM-28 recorded the maximum TSS (26.46 °Brix) and total sugars (18.81%), while CHESM-31 showed the higher aril percentage (81.04%). Bioactive compounds varied significantly, with strong correlations between key traits, suggesting these genotypes as promising candidates for both nutritional and bioactive value. Some promising accessions identified (CHESM-4, CHESM-6, CHESM-24, CHESM-27, CHESM-31, CHESM-33) these genotypes stand out as excellent candidates for future breeding programs targeting both yield and quality improvements. A fairly high variability in ascorbic acid, minerals, and antioxidant compounds in *P. dulce* accessions also indicates their suitability for fresh consumption, and for use in processing industries.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions
DSM conceived the idea, conducted the experiments, and recorded the data. MKB and VVA helped in analyzing fruit biochemical, bioactive compounds, and mineral contents. AMS performed the statistical analyses. DSM, AMS, and PR wrote the manuscript. AMS, VY, DKS, JR, YT, and AK edited the manuscript before submission. All authors read and approved the final draft.

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Declarations

Competing interests
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Correspondence and requests for materials should be addressed to D.S.M. or A.K.