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LEAF QUALITY ASSESSMENT OF SUGAR APPLE (*ANNONA SQUAMOSA* L.) GENOTYPES THROUGH MORPHOLOGY AND PHYSIOCHEMICAL TRAITS

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Abstract: The sugar apple is a traditionally valued but underutilized fruit and medicinal crop in Bangladesh. This study assessed leaf quality attributes of sugar apple genotypes through morpho-biochemical characterization. This experiment was conducted using a randomized complete block design with four replications. Three genotypes, namely ACC AS1, ACC AS2, and ACC AS3, were utilized as planting materials. ACC AS2 exhibited superior leaf morphology, including length, breadth, area, and petiole length. ACC AS1 showed the highest internodal length, leaf moisture, and sodium content. ACC AS3 excelled in physiochemical traits, exhibiting higher SPAD values, leaf dry matter, ascorbic acid, protein content, and mineral concentrations of potassium, calcium, magnesium, iron, and zinc. Correlation and regression analyses revealed a strong positive relationship ($r > 0.8$, $p < 0.05$) between leaf length, leaf width, and leaf area. Additionally, leaf area was positively associated with SPAD values and leaf dry matter content. Regression analysis revealed strong positive relationships among leaf length, width, and area, with leaf area also positively associated with SPAD values and dry matter content. Ascorbic acid decreased significantly with increasing leaf pH, while sodium and potassium showed a negative relationship. Iron and zinc concentrations were positively associated, suggesting a coordinated accumulation of these minerals in sugar apple leaves. In principal component analysis (PCA), the first two components captured nearly all variance (99.99%), with PC1 and PC2 contributing 86.34% and 13.66%, respectively. PC1 captured key biochemical and nutritional traits, with ACC AS3 exhibiting higher values, reflecting superior physiochemical performance.

Keywords: Annona squamosa, leaf quality, minerals, physiochemical, correlation, regression and PCA

INTRODUCTION

The sugar apple (*Annona squamosa* L.), which belongs to the Annonaceae family, is considered an underutilized minor fruit and medicinal plant in Bangladesh. However, it is extensively cultivated in several Central and North American countries and some parts of Asia, especially in Thailand, Vietnam, the Philippines, Cambodia, Myanmar, India, and Nepal. (Jagtap and Bapat, 2018). The southern region of Bangladesh, encompassing the districts of Kushtia, Jhenaidah, Jessore, Meherpur, and Magura, is the main commercial producer of sugar apples.

Annona squamosa is commonly known in the Indian subcontinent by various vernacular names, such as sitaphal, sweet sop, chirimoya, misti ata, sharifa, and mewa, and is often referred to as a custard apple in South Asia, though that name more accurately denotes *Annona reticulata* L. (Hasan *et. al*., 2025). Sugar apple trees, starting from 3 m and extending up to 8 m, with broad, randomly scattered branches with brownish or light brownish bark and thin leaves (Kalidindi *et al*., 2015).

Approximately 80% of the world's population relies on herbal medicines for health care, and *Annona squamosa*, the second-largest genus of the Annonaceae family, is both a fruit plant and a medicinal plant with proven pharmacological effects (Safira *et al*., 2025). Sugar apple plant parts contain high levels of proteins, carbohydrates, vitamins, minerals, phenolics, antioxidants, antimicrobials, and insecticidal properties, indicating their potential for use in human and animal nutrition as well as ecofriendly integrated pest management in crop cultivation practices. Sugar apple leaves contain high levels of essential minerals (P, K, Fe, Ca, Mg, Na, Cu, Se, and Zn) and vitamins (A, C, E, B1, B2, B3, and B9) that are necessary for human health. These nutrients support bone and muscle function, blood and nerve activity, immunity, and overall metabolic health (Akram *et al*., 2020).

Correlation and regression analyses aid in identifying correlations and predicting trends across leaf attributes, whereas PCA biplots visually summarize multivariate variation, emphasizing patterns and clustering among sugar apple genotypes. These methods are important for selecting superior genotypes and assessing the nutritional and biochemical content of leaves (Rezaei *et al*., 2018).

It is crucial to evaluate the morphological and physicochemical characteristics of genotypes when the origin is unknown in order to identify superior genotypes. This study focused on the appraisal of the morphology and physiochemical traits of leaves from sugar apple genotypes. These evaluations illustrate the potential of nutrition and the variation in leaf morphology among genotypes. This study helps to

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facilitate the selection, classification, and conservation of valuable germplasm for future enhancement initiatives.

MATERIALS AND METHODS

Experimental site and location

The experiment was set up from February to May 2023 at the Germplasm Centre of Bangladesh Agricultural University (BAU-GPC) in Mymensingh, Bangladesh. The experimental site occupied a location in the Old Brahmaputra Floodplain, at 24°44′ north latitude and 90°23′ east longitude, in the north-central region of the nation. The region resided between 15 and 20 meters above sea level and is characterized by a low-lying topography that has been influenced by ancient alluvial deposits. The soils in Mymensingh are primarily silty loam, which contains an ideal mix of silt, clay, and sand that is suitable for a wide variety of agricultural crops. The soil reaction was slightly acidic to almost neutral, with pH values fluctuating between 6.0 and 6.8. Organic matter content was moderate, averaging between 0.8 and 1.0 percent, while available nitrogen contents could vary from low to moderate. The detailed data about the soil properties were collected from the laboratory of Soil Science of Bangladesh Agricultural University (BAU), Mymensingh, and described in Table 1.

Table 1.

Morphology, physiochemical, microbial activity, and microbiome of the soil of the experimental area

Category	Constituents/Properties	Characteristics/ Value
Morphological	Location	BAU-GPC, Mymensingh
	Soil tract	Old Brahmaputra Alluvium
	Land type	Medium high land
	General soil type	Non-calcareous dark grey floodplain
	Soil series	Sonatola
	Agro-ecological zone (AEZ)	AEZ-9
	Topography	Fairly level
Physiochemical	Sand (%)	35.8
	Silt (%)	58.98
	Clay (%)	6.75
	Textural class	Silty loam
	Particle Density (g/cc)	2.673
	Bulk Density (g/cc)	1.358
	Porosity (%)	46.73
	Soil pH	6.8
	Organic Matter (%)	1.29
	Organic carbon (%)	0.83
	Total nitrogen (%)	0.18
	Available potassium (me/100g soil)	0.02
Microbial activity	Microbial biomass carbon (μg C g^{-1} soil)	574
	Microbial biomass nitrogen (μg N g^{-1} soil)	48
	Microbial quotient (MBC/SOC)	2.73
Soil Microbiome	Dominant bacteria	*Bacillus* spp., *Pseudomonas* spp., *Rhizobium*, *Streptomyces* spp.
	Dominant phyla	Proteobacteria, Actinobacteria, Firmicutes
	Dominant fungal community	*Aspergillus* spp., *Fusarium*, *Penicillium* spp., *Rhizopus* spp.

Weather and climate

Mymensingh has a humid subtropical monsoon climate with distinct rainy and dry seasons. The monsoon season runs from May to October, bringing in an average of 210 to 220 centimeters of rain per year, with July and August seeing the highest amounts. The dry winter of November to February is distinguished by colder temperatures and less rainfall, whereas the pre-monsoon summer of March to April is hot and dry, with maximum temperatures often topping 36°C. Average low temperatures in the winter can fall to 12-17°C, while relative humidity stays high, ranging from 60 to 96%, especially during the monsoon season. Flooding and temporary waterlogging are widespread in this region as a result of heavy monsoon rains, river overflow, and overflow from neighboring countries highlands, all of which have an impact on farming practices and soil qualities. Mymensingh's soil and climate are ideal for growing a variety of crops, including rice, carrots, tomatoes, mangoes, bananas, and legumes. The weather conditions during the experimental period were collected from the weather station of BAU and presented in Figure 1.

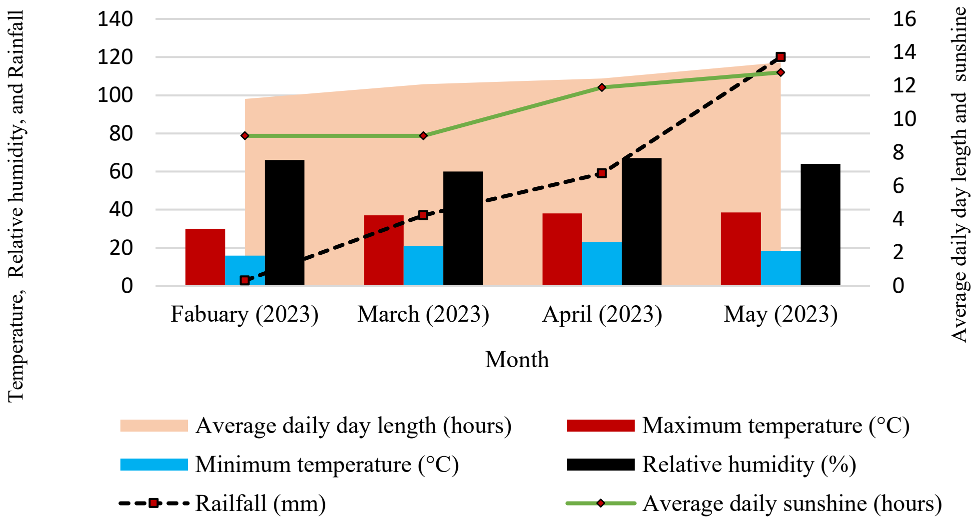

Fig. 1. Maximum and minimum temperature, rainfall, average day length, and average daily sunshine of the experimental area during the conduction of the experiment.

Experimental setup

Three sugar apple (*Annona squamosa* L.) genotypes, designated as ACC AS1, ACC AS2, and ACC AS3, were utilized as planting materials and experimental treatments in this study. These genotypes, originally collected from unspecified locations in the southern region of Bangladesh, are currently conserved at the Germplasm Centre of Bangladesh Agricultural University, Mymensingh. The fruits of ACC AS1 and ACC AS2 exhibit a green outer rind and white-colored flesh, whereas ACC AS3 bears a distinct pinkish-red rind and creamy-colored flesh. All genotypes were approximately seven years of age at the time of experimentation. The trial was laid out in a randomized complete block design (RCBD) comprising four replications to ensure experimental precision and minimize environmental variation. Physiochemical properties except ascorbic acid of sugar apple leaves were estimated in the Laboratory of Agricultural Chemistry of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Morphological data collection

Leaf shape, leaf margin, leaf apex and leaf base was visually observed in the research field and noted and the leaf color of three genotypes was recorded with the help of a color chart. The chlorophyll content was measured using a Minolta® SPAD 502 chlorophyll meter. Randomly Five leaves from each twig were selected, Leaf length (LL) was measured from tip to base (without petiole) using a measurement scale (meter scale). leaf breadth (LB) was measured considering the widest section of the leaves, and petiole length (PL) was calculated taking the leaf stalk into consideration. The mean value for these three parameters was calculated in centimeters. The internodal distance was estimated by measuring the distance between two consecutive leaves by using a meter scale and calculating the mean value. The following Equation (1) was used to measure the leaf area of three genotypes:

$$\text{Leaf area (cm}^2) = \text{Leaf length (cm)} \times \text{leaf breadth (cm)} \times \text{Correction coefficient} \quad \ldots\ldots\ldots (1)$$

Estimation of physiochemical properties

The chlorophyll content (SPAD) of fresh *Annona squamosa* leaves was measured without damaging the leaves with a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan).

The pH of fresh *Annona squamosa* leaves was measured after homogenization in distilled water. A mortar and pestle were used to macerate approximately 5 g of fresh leaf tissue with 25 mL of water that had been double-distilled. The homogenate was passed through filters using a muslin filter, and the filtrate was taken away. The pH of the leaf extract was determined at room temperature using a digital pH meter calibrated with standard buffer solutions at pH 4.0 and 7.0 (Jackson, 1973; AOAC, 2016).

Ten grams of fresh leaves were weighed and subsequently placed in a forced-air oven at 70°C for 48-72 hours to achieve a consistent weight. The following Equation (2) was used to determine the moisture content (MC) of *Annona squamosa* leaves:

$$\%\ \text{MC} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Fresh weight (g)}} \times 100 \quad \ldots\ldots\ldots (2)$$

The following Equation (3) was used to determine the dry matter (DM) contents of *Annona squamosa* leaves:

$$\%\ \text{DM} = \frac{\text{Dry weight (g)}}{\text{Fresh weight (g)}} \times 100 \ \ldots\ldots\ldots (3)$$

The ascorbic acid (AA) content of fresh *Annona squamosa* leaves was determined following the 2,6-dichlorophenol indophenol (DCPIP) titration method (AOAC, 2016). Ten grams of fresh leaves were taken, extracted with 4% oxalic acid, filtered, and titrated against DCPIP. The following Equation (4) was used to determine the ascorbic acid content of fresh *Annona squamosa* leaves:

$$\text{Ascorbic acid (mg 100 g}^{-1}\text{ FW)} = \frac{\text{Titre}\times\text{Dye factor}\times\text{volume made up (ml)}}{\text{Volume of extract of leaves}\times\text{weight of sample}} \times 100 \ \ldots\ldots (4)$$

The protein percentage of oven-dried leaf powder was ascertained through the Kjeldahl method (AOAC, 1990), which involves digestion with H_2SO_4 in the presence of a catalyst, distillation, and titration against HCl.

The sample for wet digestion (AOAC, 1990) with the mixture of HNO_3 and $HClO_4$ was prepared by utilizing one gram of oven-dried leaf powder. Spectrophotometry was employed to estimate the concentrations of minerals, including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). Details of the mineral estimation procedure are provided in the following Table 2.

Table 2.

Mineral estimation methods with references from three sugar apple (*Annona squamosa*) leaf samples

Mineral	Procedure	References
Na	The digested sample was analyzed by a flame photometer at a 589 wavelength.	Chapman and Pratt, 1961; AOAC, 1990
K	The digested sample was analyzed by a flame photometer at a 766 wavelength.	Chapman and Pratt, 1961; AOAC, 1990
Ca	The digested sample was analyzed by atomic absorption photometer at a 422.7 wavelength.	AOAC, 1990; Vaessen and van de Kamp, 1990
Mg	The digested sample was analyzed by atomic absorption photometer at a 285.2 wavelength.	AOAC, 1990; Vaessen and van de Kamp, 1990
Fe	The digested sample was analyzed by atomic absorption photometer at a 248.3 wavelength.	Stookey, 1970; Allen et al., 1974; AOAC, 1990
Zn	The digested sample was analyzed by atomic absorption photometer at a 213.9 wavelength.	Lindsay and Norvell, 1978; Reichen and Lakin, 1949; AOAC, 1990

Statistical analysis

The collected data on various parameters was statistically analyzed using a statistical computer program, namely Statistix 10. The significance of differences between treatments was estimated by least significant difference (LSD) at the 1% and 5% level of probability (Gomez and Gomez, 1984).

RESULTS

The qualitative leaf morphological attributes of sugar apple (*Annona squamosa*) exhibited a significant extent of genotypic variability (Table 3). The genotypes that were examined exhibited distinct variations in leaf apex and base form and coloration. The color spectrum of the leaves ranged from green to deep green, and their shape varied from elliptic-oblong to oblong (Table 3). Variation was observed in the leaf apex, which ranged from acute to acuminate, and in the leaf base, which extended from obtuse to acute, despite the uniformity in leaf margin across all genotypes (Table 3).

Table 3.

Leaf qualitative characteristics of the sugar apple (*Annona squamosa*) genotypes

Genotype	Leaf shape	Leaf color	Leaf margin	Leaf apex	Leaf base
ACC AS1	Elliptic oblong	Green	Entire	Acute	Obtuse
ACC AS2	Elliptic	Green	Entire	Acute	Obtuse
ACC AS3	Elliptic oblong	Dark green	Entire	Acuminate	Acute

The three genotypes of sugar apple (*Annona squamosa*) exhibited substantial differences in leaf length, breadth, thickness, and area (Table 4). Genotype ACC AS2 had the longest leaves, measuring 10.33 cm, with genotype ACC AS3 following closely behind at 10.33 cm. The maximum leaf breadth was observed in ACC AS2 (4.283 cm), with ACC AS1 (4.236 cm²) following in this order (Table 4). The highest leaf thickness was recorded in ACC AS3 (0.420 mm), with ACC AS2 (0.330 mm) following in the same order. Similarly, the leaf area of ACC AS2 was the largest at 45.09 cm², while ACC AS3 had a leaf area of 36.90 cm² (Table 4). The genotypes also exhibited substantial variation in petiole length and internodal distance. The maximum petiole length was recorded in ACC AS2 (1.475 cm), while the greatest internodal distance was observed in ACC AS1 (3.475 cm) (Table 4).

Table 4.

Morphological traits of three sugar apple (*Annona squamosa*) genotypes

Genotype	LL (cm)	LB (cm)	LT (mm)	LA (cm2)	PL (cm)	IL (cm)
ACC AS1	9.550^{b}	4.236^{a}	0.260^{c}	30.34^{c}	1.200^{b}	3.475^{a}
ACC AS2	10.53^{a}	4.283^{a}	0.330^{b}	45.09^{a}	1.475^{a}	3.100^{b}
ACC AS3	10.33^{a}	3.572^{b}	0.420^{a}	36.90^{b}	0.875^{c}	2.650^{c}
$LSD_{0.05}$	0.256	0.483	0.04	2.91	0.170	0.311
$LSD_{0.01}$	0.388	0.732	0.061	4.41	0.259	0.472
CV (%)	1.46	6.92	7.00	4.50	8.33	5.86
Here, means having same letter within a column do not differ significantly at 5% level of probability, LL= Leaf length, LB= Leaf breadth, LT= Leaf thickness, LA= Leaf area, PL= Petiole length, and IL= Internodal length						

There were significant differences in SPAD value, moisture content, dry matter content, leaf pH, ascorbic acid concentration, and protein content across the three different sugar apple (*A. squamosa* L.) genotypes (Table 5). The SPAD measurement indicated that genotype ACC AS3 had the maximum chlorophyll content (49.36). Moisture and dry matter content varied significantly across genotypes, with ACC AS1 possessing the highest moisture content (78.12%) and ACC AS3 containing the highest dry matter content (27.85%) (Table 5). The leaf pH remained slightly acidic across all genotypes, ranging from 6.53 to 6.93. The maximum ascorbic acid concentration in fresh leaf weight (FW) was reported in ACC AS3 (18.85 mg 100^{-1} FW), while the lowest was in ACC AS1 (18.38 mg 100^{-1} FW). The leaf protein content of the genotypes ranged from 3.42% to 4.60% (Table 5).

Table 5.

Physiochemical traits of three sugar apple (*Annona squamosa*) genotypes

Genotype	SPAD	MC (%)	DM (%)	pH	AA (mg 100 g^{-1} FW)	Protein (%)
ACC AS1	38.75^{c}	78.12^{c}	21.88^{c}	6.93^{a}	18.38^{a}	3.42^{c}
ACC AS2	41.63^{b}	76.47^{b}	23.53^{b}	6.75^{a}	12.50^{b}	4.06^{b}
ACC AS3	49.36^{a}	72.15^{a}	27.85^{a}	6.53^{b}	18.85^{a}	4.60^{a}
LSD0.05	1.256	0.918	0.611	0.138	1.2	0.489
LSD0.01	1.904	1.332	0.926	0.209	1.78	0.742
CV (%)	1.68	1.05	1.45	1.19	2.25	7.03
Here, means having same letter within a column do not differ significantly at 5% level of probability, SPAD= Soil plant analysis development (chlorophyll), MC= Moisture content, DM= Dry matter, and AA= Ascorbic acid						

Marked genotypic variation was identified in the mineral content of sugar apple (*Annona squamosa*), including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) (Table 6). On a dry weight (DW) basis, the highest Na concentration was reported in ACC AS1 (276.50 mg 100^{-1} DW), closely followed by ACC AS2 (249.75 mg 100^{-1} DW). Subsequently, the most notable K content had been reported in ACC AS3 (1687.5 mg 100^{-1} DW), with ACC AS2 (1287.3 mg 100^{-1} DW) ranking next (Table 6). Conversely, ACC AS3 revealed greater Ca (715.25 mg 100^{-1} DW) and Mg (120.00 mg 100^{-1} DW) accumulation in contrast to the other genotypes. The maximal amounts of Fe (30.25 mg 100^{-1} DW) and Zn (7.74 mg 100^{-1} DW) concentrations were also reported in ACC AS3 (Table 6). Overall, genotype ACC AS3 consistently demonstrated enhanced mineral accumulation efficiency, suggesting a more effective nutrient uptake and translocation mechanism relative to ACC AS1 and ACC AS2 (Table 6).

Table 6.

Physiochemical traits of three sugar apple (*Annona squamosa*) genotypes

Genotype	Na (mg 100^{-1} DW)	K (mg 100^{-1} DW)	Ca (mg 100^{-1} DW)	Mg (mg 100^{-1} DW)	Fe (mg 100^{-1} DW)	Zn (mg 100^{-1} DW)
ACC AS1	276.50^{a}	1234.0^{c}	607.75^{c}	105.75^{c}	20.120^{c}	3.2500^{c}
ACC AS2	249.75^{b}	1287.3^{b}	631.25^{b}	114.75^{b}	25.750b	6.4425^{b}
ACC AS3	187.00^{c}	1687.5^{a}	715.25^{a}	120.00^{a}	30.250a	7.7375^{a}
LSD0.05	4.128	8.017	4.43	3.198	1.343	0.719
LSD0.01	6.255	12.14	6.712	4.845	2.034	1.09
CV (%)	1.00	0.33	0.39	1.63	3.06	7.16
Here, means having same letter within a column do not differ significantly at 5% level of probability, K= Potassium, Na= Sodium, Ca= Calcium, Mg= Magnesium, Fe= Iron, Zn= Zinc, FW= fresh weight, and DW= Dry weight						

Correlation heatmap

The correlation heat map (Fig. 2) demonstrated substantial relationships between morphological and physiochemical features, providing information about their interrelationships. There is a substantial positive association ($r > 0.8$, $p < 0.05$) between leaf length (LL), leaf width (LB), and leaf area (LA). Furthermore, LA correlated positively with SPAD and DM, and additionally significant positive associations ($p < 0.05$) were detected among the physiochemical parameters, including ascorbic acid (AA), protein, and mineral nutrients (Ca, Mg, Fe, and Zn) (Fig. 2). In addition, K demonstrated substantial positive correlations with Ca and Mg, while Na demonstrated negative correlations with the majority of minerals, suggesting ionic competition in assimilation. pH, protein content, and AA were positively correlated with essential minerals (Ca, Mg, Zn, and Fe) among biochemical variables, while Na was negatively associated (Fig. 2). There was a substantial positive relationship between protein content, AA levels, and leaf SPAD values. DM also correlated positively with SPAD, Ca, and K, and in contrast, Na and pH showed negative associations with most biochemical parameters (Fig. 2).

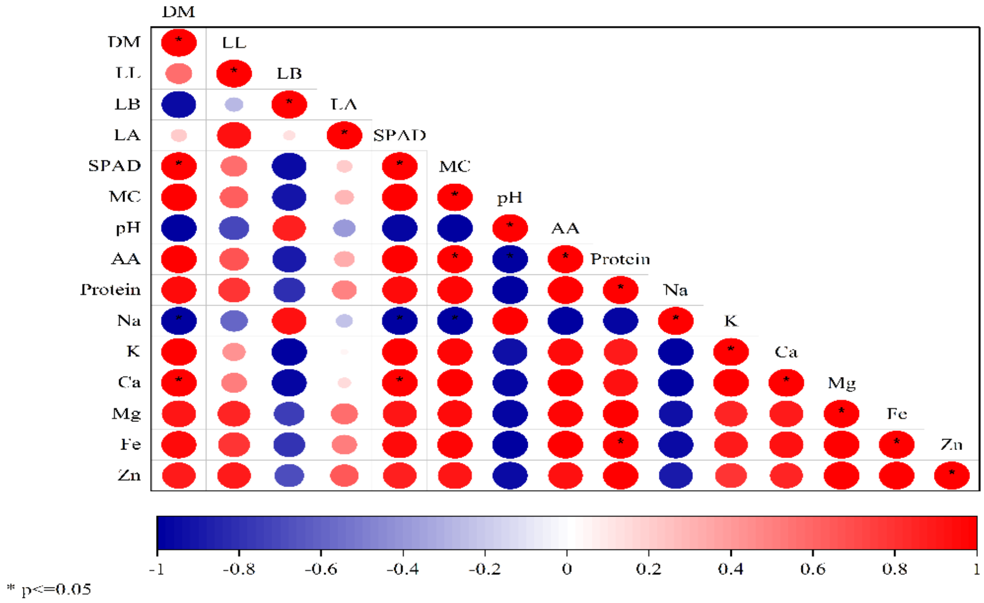

Fig. 2. The correlation heatmap illustrates the relationships among morpho-biochemical traits in the study. The intensity and direction of Pearson's correlation coefficients are represented by the size and color of the circles. Positive correlations are shown in red, while negative correlations are marked in blue. Larger and more intensely colored circles indicate correlations that are more robust. Significance levels are indicated by asterisks (*p≤0.05).

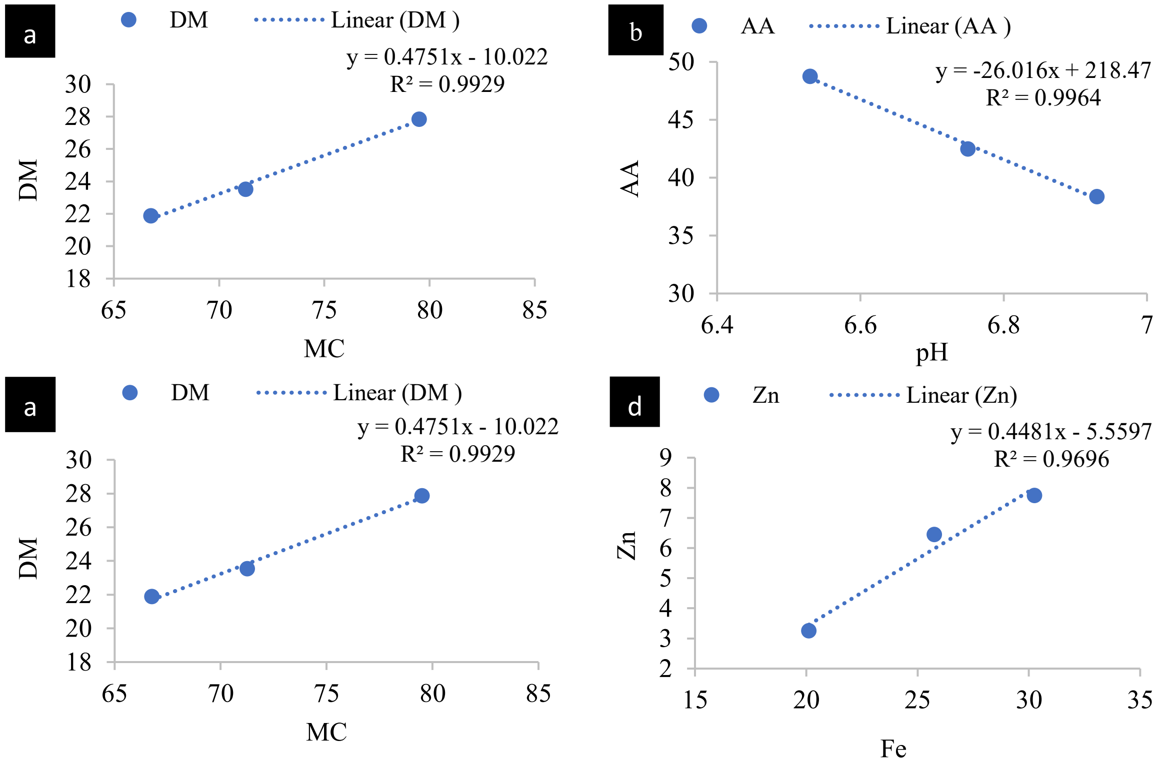

Fig. 3. Linear regression between morpho-physiochemical traits: **(a)** DM (%) and MC (%), **(b)** AA (mg 100 g^{-1} FW) and pH, **(c)** K (mg 100 g^{-1} DW) and Na (mg 100 g^{-1} DW), **(d)** Zn (mg 100 g^{-1} DW) and Fe (mg 100 g^{-1} DW).

Regression analysis

A simple linear regression analysis was utilized to investigate the relationship between MC and DM in *Annona squamosa* leaves (Fig. 3a). The fitted regression equation was y = 0.4751x - 10.022. The model exhibited a high coefficient of determination (R^2 = 0.9929), accounting for 99.29% of the change in DM through MC (Fig. 3a). The positive regression coefficient indicates a strong, direct linear relationship between MC and DM. Ascorbic acid (AA) content had a strong negative linear association with leaf pH (y = -26.016x+218.47, R^2 = 0.9964), showing that higher pH values led to a significant drop in AA concentration (Fig. 3b). Na and K concentrations had a substantial negative correlation (y = -5.3008x+2663.2, R^2 = 0.9652), indicating an antagonistic interaction between these elements (Fig. 3c). The regression equation y = 0.4481x - 5.5597 with a coefficient of determination R^2 = 0.9696 revealed a significant positive linear association between Fe and Zn concentration in sugar apple leaves (Fig. 3d). This suggests that as Fe concentrations increased, it also raised Zn levels, indicating a synergistic interaction between these two minerals. High coefficients of determination in all circumstances indicate that the fitted regression models are quite reliable.

Principal component analysis (PCA)

The first two principal components (PC1 and PC2) accounted 99.99% of the total variance, accounting for 86.34% and 13.66%, respectively (Fig 4). The PCA biplot clearly separated the genotypes into three clusters, showing significant genetic and physiological differences. The traits associated with PC1 were primarily biochemical and nutritional parameters such as protein, AA, MC, DM, SPAD value, Ca, K, Mg, Fe, and Zn, which were positively correlated and heavily loaded on the right side of the biplot (Fig. 4). These characteristics were strongly associated with ACC AS3, indicating higher physiochemical performance. ACC AS2 was positively positioned along PC2, impacted mostly by LA and LL, indicating its dominance in leaf morphological expression (Fig. 4).

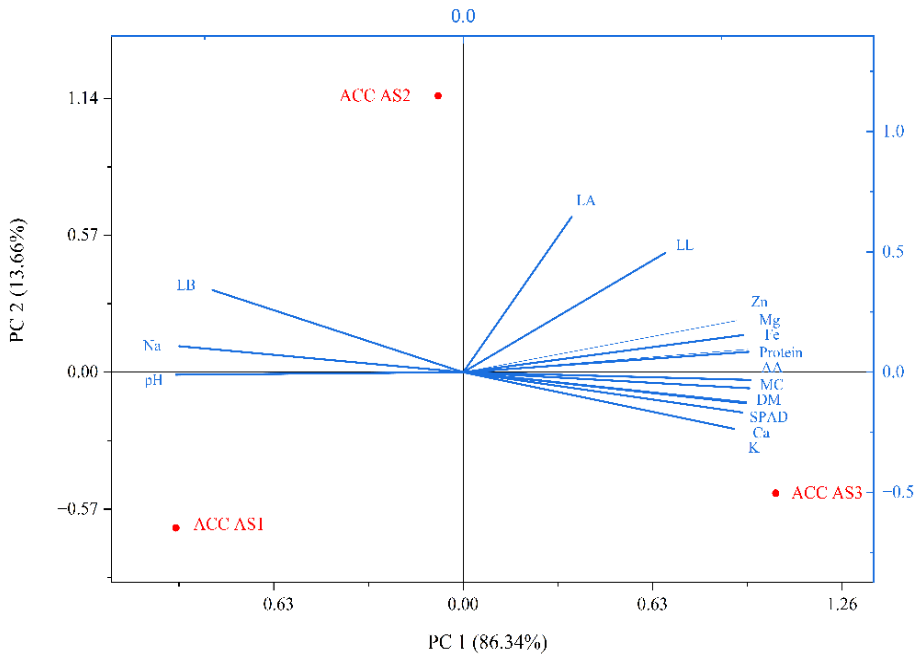

Fig. 4. Principal component analysis biplot depicting the distribution of genotypes and their association with morpho-physiochemical traits. Red vectors represent genotypes, while blue vectors indicate key contributing traits.

DISCUSSION

According to the findings of this study, the sugar apple genotypes tested exhibit distinct genotypic differences in foliar and stem morphological attributes, as well as unique physiological and biochemical differences, indicating potential variability in metabolic activity and adaptive ability. Ha *et al*. (2024) observed *A. squamosa* leaves and reported that they diversify, are only approximately 9 to 13 cm long and 3 to 5 cm broad, are a deeper green, and have an acute apex. The high SPAD values observed in the genotype are attributed to its darker green leaf coloration, which indicates a higher chlorophyll concentration (Pinzón-Sandoval *et al*., 2022). Shukry *et al*. (2019) reported that sugar apple leaves have the highest concentration of K and Fe content, ranging from 20.78 to 27.64 mg 100 g^{-1} DW, and AA, from 11.98 to 16.78 mg 100 g^{-1} FW. K is an essential macronutrient that promotes morphology, plant growth, and physiological functions, whereas excessive Na disrupts ionic balance and inhibits growth and development (Adams and Shin, 2014). In this study, the correlation heat map reveals that increases in one morphological trait are reliably connected to increases in others. This illustrates that larger leaves have proportional dimensions in both length and breadth, which adds to greater surface area and possible photosynthetic efficiency. Furthermore, LA associated favorably with SPAD and DM, showing that leaf expansion supports higher chlorophyll content and biomass accumulation. Low Mg levels in the three sugar apple genotypes led to reduced SPAD values, as supported by Wadas and Dziugieł (2020), who stated that SPAD values tend to depend on N and Mg levels. DM also associated favorably with SPAD, Ca, and K, demonstrating that leaves with greater chlorophyll (Tränkner *et al*., 2018) and mineral content acquire more dry mass. In contrast, Na and pH revealed negative relationships with most biochemical measures, indicating a deleterious function in affecting the nutritional and physiological health of the leaves. The K content was found to be closely correlated with the AA content and the concentration of mineral nutrients in leaves, which was employed to facilitate the estimation of vegetative yield and the quality of the leaves and fruit (Huang *et al*., 2021). From the regression analysis of this study, it was demonstrated that when the MC (%) increases, simultaneously the DM (%) increases. Concurrently, both MC (%) and DM (%) increased, indicating that water retention was improved and that biomass accumulation was increased. This may indicate that physiological activity was more active. Patel & Kousar (2011) found a considerable correlation between AA and pH (R^2 = 0.97), indicating that pH is a significant predictor of AA levels in leaves. A linear regression study found a substantial negative slope between leaf Fe and Zn (Kolberg *et al*., 2022), while Kronzucker *et al*. (2013) identified a K^+-Na^+ antagonist association in fruit leaves. The PCA biplot in this investigation revealed that ACC AS3 was the most promising genotype due to its relationship with the majority of the physiochemical features and PC1, which explained 86.34% of the overall variance.

CONCLUSION

Based on the findings of this study, the genotype ACC AS2 exhibited superior performance in terms of morphological characteristics, whereas ACC AS3 demonstrated dominance in physio-chemical traits. Therefore, it is recommended that multi-locational trials and advanced genetic studies, including marker-assisted selection and quantitative trait loci (QTL) mapping, be conducted to accurately identify and confirm the superior genotypes of Annona squamosa for future breeding and improvement programs.

AUTHORS CONTRIBUTIONS

Md. Mahfuzul Hasan: Formal analysis, investigation, resources, writing - original draft, and software; Md. Mokter Hossain: Conceptualization, methodology, supervision, project administration, and fund acquisition; Mst. Nusrat Jahan Pakhom: Data Curation, validation, and writing - review & editing; and Md. Rahat Sazzad Tusar: Data curation, visualization, and writing - review & editing.

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CONFLICT OF INTERESTS

All the authors declared that they have no conflict of interest.

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