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RESEARCH ARTICLE

Variability in Morpho-physicochemical Traits and Selection of Superior Genotypes of Aonla (*Phyllanthus emblica* L.) from Northeast India

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Abstract

Aonla (*Phyllanthus emblica* L.), commonly known as Indian gooseberry, is of great importance due to its medicinal and nutritional value. The current investigation was carried out to study the morphological, physical, and biochemical characteristics of 84 aonla genotypes growing in the wild from Mizoram, Meghalaya, and Tripura states of Northeastern India. Variation was observed in the qualitative parameters (11 parameters) and significant to highly significant differences were observed in the quantitative parameters (22 parameters) among the studied genotypes. The fruit weight varied from 2.6 to 9.78 g, TSS from 7.67 to 18 °Brix, vitamin C from 206.3 to 1392.5 mg/100 g, titrable acidity from 1.7 to 4.9%, total sugar from 3.8 to 12.3%, phenol from 1022.80 to 4210.00 mg/100 g and protein from 2.1 to 6.2%. Six genotypes were found promising and they can be recommended for direct cultivation as well as for breeding programs. These selected genotypes can serve as valuable genetic resources for further research and development efforts.

Keywords: Ascorbic acid, Genetic resources, Indian gooseberry, Phenol, Morphology.

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Introduction

Wild aonla (*Phyllanthus emblica* L. family Phyllanthaceae), generally known as Indian gooseberry, is indigenous to India, Pakistan, China, Sri Lanka, Malaysia, and Southeast Asia (Parmar and Kaushal, 1982). Aonla is a rich source of vitamin C, amino acids, and minerals, which make the fruits very nutritious. Aonla is used for various ailments by Ayurveda, Siddha, Unani systems of India, Sri Lankan, Tibetan, and Chinese Systems of medicine. It is used as a healing option and immunity booster for respiratory disorders, heart disorders, scurvy, diarrhea and dysentery, aging, and hair tonic (Krishnaveni and Mirunalini, 2010). Its therapeutic potential is attributed to its antioxidant properties, high vitamin C content, and other beneficial compounds that contribute to its medicinal value across different cultures and traditional healing practices.

Aonla, found naturally in India, is widespread across various regions, including the Himalayas, Bihar, West Bengal, Odisha, Chota Nagpur, Deccan, Karnataka, and the Western Ghats (Rawat and Uniyal, 2003). Typically, wild aonla is found in mixed forests with rugged terrain, sometimes challenging to access. The northeastern part of India, especially in Lower Assam, Meghalaya, Mizoram, and Tripura, harbors a diverse genetic pool of aonla (Sankaran and Dinesh, 2020)

Aonla holds immense importance globally for its various health benefits. However, there is a need to understand and characterize the diverse genetic pool of aonla present in the Northeastern regions of India, particularly local aonla genotypes growing in

Figure 1: Distribution map of the 84 selected genotypes of aonla

Meghalaya, Mizoram, and Tripura. The study aims to assess the morpho-physical-biochemical properties of wild aonla genotypes from these regions to identify superior varieties suitable for cultivation and breeding programs. Additionally, the research seeks to contribute valuable insights from the conservation of genetic resources and inform future genetic improvement endeavors.

Material and Methods

Wild aonla trees were surveyed and a total of 84 aonla genotypes were selected for the study. A minimum of 100 m distance between the two genotypes was taken into consideration while selecting them. However, in cases where there was noticeable variation in morphological traits, the distance criterion was compromised to some extent to ensure a diverse sample. The 84 genotypes were sampled from the states of Mizoram, Meghalaya, and Tripura, comprising eight districts (32 genotypes) from Mizoram, five districts (20 genotypes) from Tripura and seven districts (32 genotypes) from Meghalaya. The sites ranged from 42 to 1447 m. amsl in elevation and latitude from 22°30'15'' to 25°54'59.56'' N, longitude 90°1'25.14'' to 93°21'54.38'' (Figure 1). The geographical details of the selected genotypes have been given in Supplementary file 1. The survey was done in July and August of 2019 and the fruits were collected in three phases: early harvest in October and November, mid-harvest in December 2019 and January 2020, and late harvest till March of 2020. The healthy fruits from each genotype were collected and transported immediately to the lab of the Forestry Department, Mizoram University, and kept in a deep freezer (-80°C) for further physicochemical characterization.

Phenotypic diversity of the germplasm was recorded based on quantitative and qualitative traits related to trees, leaves and fruits. Standard SOPs given by Mahajan *et al.* (2002) and guidelines for conducting DUS testing on Indian Gooseberry given by PPV & FRA (2014) have been followed. Matured leaves from the middle of tertiary branches were selected for the observations on the leaf. Each genotype was replicated three times to ensure the reliability and accuracy of the data. Within each replication, a sample of 20 fruits was collected for analysis. The physical parameters of the fruits were observed in terms of the fruits' shape, surface, stalk and stone shape. Vernier caliper measured fruits' length and diameter, and the weight was taken in on an electric weighing balance. The fruit stalks of diameter above 1-mm were considered thick and diameters below 1-mm were considered as thin stalks. The color of the fruits was

recorded with a visual evaluation of the color chart of the Royal Horticultural Society (RHS), London. The pulp of each fruit was weighed separately and the pulp percentages were worked out. Fruit pulp was dried in an oven at 70°C until a constant weight was achieved and moisture content was expressed in percentage. A hand refractometer was used to determine the total soluble solid (°Brix) and values were corrected to 20°C. Total phenolic content was analyzed as suggested by Ranganna (1986). Acidity was determined by titrating the fruit juice against 0.1N NaOH. Total sugar and reducing sugar were determined by the titration method (Lane and Eynon, 1923) and it was expressed in percentage. Ascorbic acid (mg/100 mL) was analysed as described by Ranganna (2004). In contrast, protein was estimated as per methods described by Lowry *et al.* (1951). The "Weighted-Ranged" method was modified to determine the promising aonla genotypes (Guleryuz *et al.*, 1998). Ascorbic acid content, phenolic content, pulp-stone ratio, TSS, total sugar, fruit weight, protein and yield were taken into consideration while giving the ranking.

Statistical Analysis

The data were subjected to ANOVA analysis by following a completely randomized design. Significance and non-significance differences between different treatments were determined by calculating the respective *F* value and comparing with the appropriate value of *F* at 5% probability level.

Results and Discussion

Morphological Characters of Aonla Trees and Fruits

In this study, a wide range of diversity was observed in the trees, leaves, and fruit traits of 84 aonla genotypes growing in NE India. Detailed morphological characters of the aonla genotypes are presented in Supplementary File 2, and the number and percentage of the genotypes' morphological characters are shown in Figure 2. The tree height of the genotypes studied ranged between 4.5 and 14.4 m. The highest tree height was recorded in CRR1 from Garo Hills, whereas the shortest was recorded in KYR5 from West Jaintia Hills. Among the genotypes studied, the tree group between 9 to 12 m accounted for 45.24% (38 genotypes), followed by the 6 to 9 m group, which accounted for 38.10%, the 12 to 15 m group at 11.90%, and the 3 to 6 m group at 4.76%. The highest tree girth was observed in TSP3 (196 cm), and the minimum girth was observed in WNG2 (20 cm). Girth between 25 to 30 cm. accounted for 65.71%, followed closely by 50 to 70 cm (34.52%), and girths below 25 cm accounted for only 4.76%. Late maturity (54) accounted for 64.29%, followed by mid (25) and early maturity (5), accounting for 29.76 and 5.95%, respectively, of the total genotypes studied. Of all the genotypes, 34 (40.48%) showed a spreading tree shape, followed by semi-spreading (33), which accounted for 39.29%, while erect and drooping contributed 13.10% (11) and 7.14% (6) of tree shape among genotypes (Supplementary Figure 1). 55.95% exhibited sparse foliage, and 44.05% exhibited dense foliage. Oblong (61 trees, 72.62%) leaf shape was recorded maximum, followed by oval (19.05%) and elliptical (8.33%). The average pinnate leaf size ranged from 0.9 (WNG2) to 2 mm (CRR1). The majority of the leaf apexes revealed an acute apex, accounting for 59.52% (50), while obtuse apexes accounted for 40.48% (34) (Supplementary Figure 2). Glabrous leaf surface was recorded to be dominant, which showed 95.24% (80), and moderately glabrous leaf surface showed 4.76% (4) (Supplementary Figure 3). Similarly, variations in morphological characters of aonla genotypes were also recorded by Singh *et al.* (2016) from northeastern regions of India and Singh *et al.* (2021) from semi-arid conditions.

Detailed fruit morphological characters of aonla genotypes are shown in Supplementary File 3, and their numbers and percentages are shown in Figure 3. Three types of fruit shapes were recorded, where flattened round accounted for 47.62%, round shape accounted for 42.86%, and triangular shape accounted for 9.52%. Of the total genotypes studied, 65 (77.38%) had a smooth fruit surface, and 17 (20.24%) had a rough surface. Among the genotypes, 54.76% had a thin stalk, and 45.24% had a thick stalk. Five types of stone shapes (Figure 4) were observed in the present study: round shape (32.14%), oval shape (28.57%), oval round shape (19.05%), triangular shape (17.86%), and flattened round (2.38%) as shown in Figure 3. All fruit colors fell under yellow-green with different shades, except for

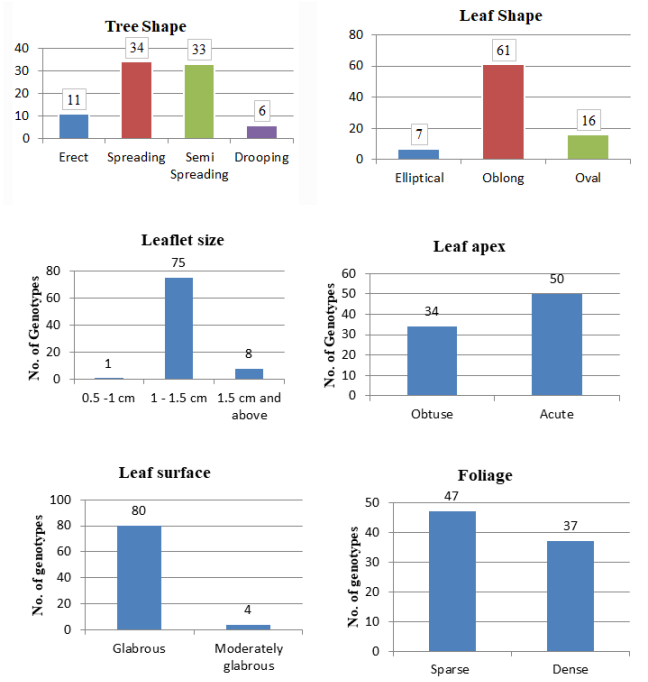

Figure 2: Frequency of aonla genotypes falling under different morphological categories

the MZU-1 sample, which showed an orange-red color. The highest percentage of fruit color was found to be yellow-green 153C and 145C, accounting for 17.86% each, followed by yellow-green 151A, accounting for 14.29%, while the lowest was found to be orange-red 35B (Supplementary Figure 4), accounting for only 1.19% of the total genotypes studied. These findings are in close proximity to the results reported by Rai et *al.* (1993) and Singh *et al.* (2016).

Physical Characters of Aonla Fruits

Aonla genotypes were found to be statistically different ($p < 0.05$) in the context of all studied fruit quality parameters. The result in Table 1 (Supplementary files 4, 5, and 6) revealed that the maximum average fruit length was recorded in JAINTIA1 (24.72) followed by TRA1 (24.6 mm) JAINTIA3 (23.54 mm), and the minimum was recorded in NGP13 (12.77 mm). Among the germplasms, the maximum diameter was recorded in JAINTIA1 (28.04 mm) successively by WNG3 (26.86 mm), subsequently by JAINTIA4 (25.77 mm), whereas the shortest diameter was observed in LTL1 (14.67 mm), The highest L-D ratio among the fruits was observed in WNG2 (1.2 mm) and the lowest ratio was observed in KLS3 with a ratio of 0.78. The highest L-D ratio observed in WNG2 was due to its typical shape where the pulp was found attached in the fruit's stalk. Our findings of fruit length and diameter are in agreement with other findings of Singh *et al.* (2016), who also reported variation in fruit length of aonla in the range of 12.6 to 25.3 mm and 12.7 to 21.00 mm, they also reported variation in fruit diameter ranged between 12.7 to 25.7 mm and 12.7 to 24.4 mm.

The fruit weight was observed maximum in genotype JAINTIA1 (10.71 g) subsequently, by UMR3 (9.99 g), WNG3 (9.78 g), while the minimum weight was observed in genotype LTL1 with 2.45 g only. These findings are consistent with previous research by Chiranjeevi *et al.,* (2018), who also documented variations in fruit physical parameters among different aonla varieties. Similarly, Chandra *et al.* (1998) observed variations in fruit physical parameters among aonla genotypes collected from the Garo Hills of Meghalaya, further supporting the observed diversity in fruit weight within the species. The variation in these fruits may be mainly due to its genetic constitution and the location where they were grown. From all the genotypes, the fruits from MAMIT-4 have the highest dry matter content (23.22%)

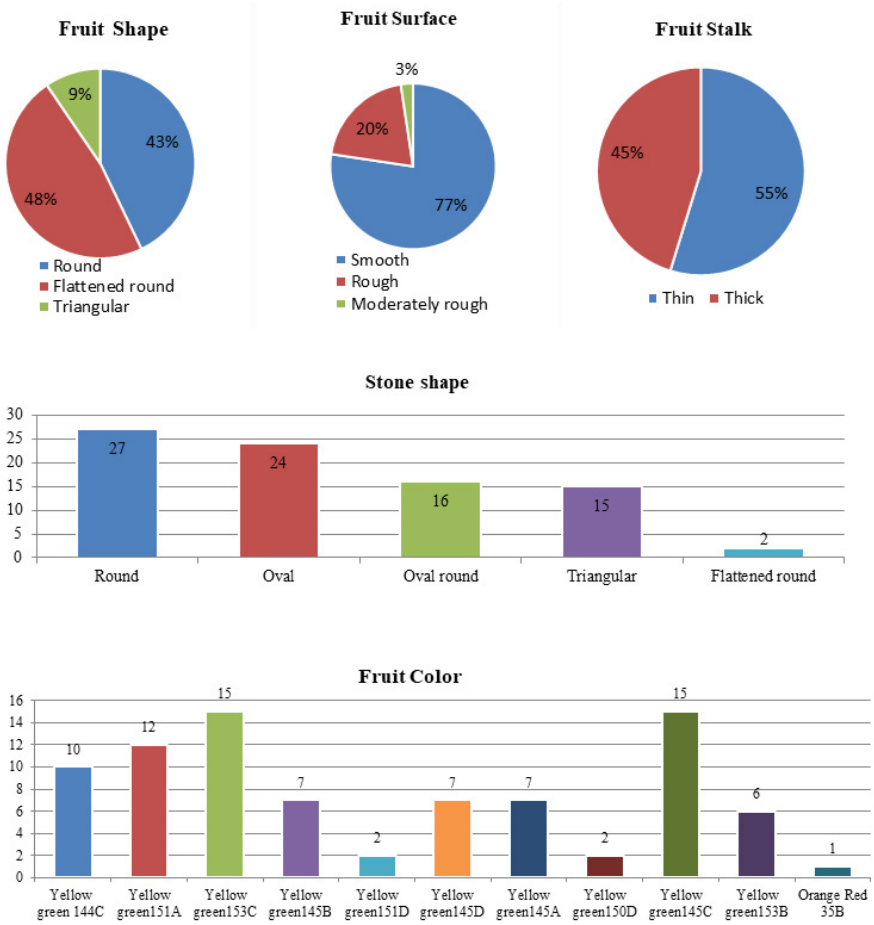

Figure 3: Fruit characteristics of *P. emblica* genotypes (n = 84)

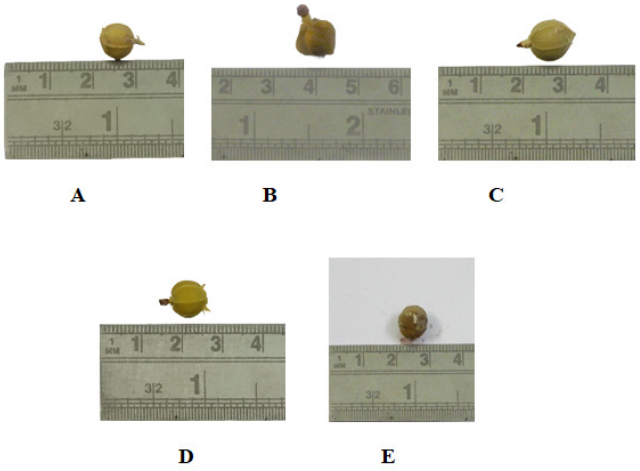

Figure 4: Stone shapes of aonla fruits: Round shape (A), Triangle shape (B), Oval shape (C) and oval round (D) and Flattened round (E)

followed by DACOPGKE (23.18%), whereas the lowest dry matter content was observed in CHITOKTE (14.09%). Kumar *et al.* (2013), Thakur *et al.* (2018) and Chandra *et al.* (2020) also reported similar values of aonla fruits.

Regarding pulp percentage, the maximum was observed in WNG3 with 92.91% followed by TSP5 (92.8%), while the lowest was recorded in CM7 with only 71.84%. Similarly, the maximum pulp-stone ratio was recorded in WNG3 (13.10) followed by TSP5 (12.89), minimum pulp-stone was recorded in CM7 (2.48). Pulp-to-stone ratio is a vital factor in identifying a superior genotype by breeders. These results align with previous studies conducted by Kumar *et al.* (2013), Hazarika and Laltluangkimi (2019) and Chandra *et al.* (2020).

In the present study the stone weight ranges from 0.28 to 1.54 g. The maximum value was recorded in SM1 (1.54 g) and minimum in TSP5 (0.28 g). Our result is in agreement with the findings of Chandra *et al.* (1998); Singh and Singh (2016) and Hazarika and Laltluangkimi (2019) from North-east India.

Significantly higher fruit yield of aonla genotype was recorded from TSP1 with 163.54 kg/tree followed by TSP2 with 152.35 kg/tree. While, the lowest yield was recorded from WNG3 with only 31.25 kg/tree. One possible explanation for the increase in production is that there are more fruits per determinate shoot. Our findings showed a higher yield as compared to aonla cultivar Balwan (85.33 kg/tree) recorded from Punjab by Aulakh *et al.* (2013). Variation in fruit yield of aonla was also reported by Pandey *et al.* (2016).

Biochemical Parameters of Aonla Fruits

Table 2 (Supplementary files 7, 8 and 9) reveals that the total soluble solid of the genotype ranged from 7.5° (KYR6) to 18 °Brix (SCH1). Among the studied germplasm, WNG2 was observed to have the highest titriable acidity (4.90) and the minimum was observed in UMR31 (1.65). TSS and acidity ratio ranged between 1.65 and 8.91, the maximum was

Table 1: Mean and range of physical attributes of fruits of aonla in different North-East states

State	*Number of trees*	*Fruit length (mm)*	*Fruit diameter (mm)*	*Ld ratio*	*Pulp (%)*	*Stone weight (G)*	*Pulp weight (G)*	*Pulp-stone ratio*	*Fruit weight (g)*	*Dry matter content (%)*	*Yield (kg/tree)*
meghalaya	32	19.12 (12.77–24.72)	20.96 (15.57–28.04)	0.91 (0.79–1.20)	87.90 (75.87–92.91)	0.71 (0.32–1.4)	5.5 (1.74–11.14)	8.08 (3.15–13.10)	5.969 (2.61–10.71)	18.78 (14.09–23.18)	57.23 (31.25–111.07)
Mizoram	32	17.23 (12.78–23.47)	19.24 (14.67–24.85)	0.9 (0.78–0.99)	85.64 (71.84–92.80)	0.66 (0.28–1.54)	4.21 (1.66–7.95)	6.94 (2.48–12.89)	4.63 (2.45–9.10)	19.12 (15.34–23.22)	75.86 (39.61–163.54)
Tripura	20	18.15 (15.30–20.80)	20.27 (16.97–23.13)	0.91 (0.81–1.01)	88.7 (81.38–91.77)	0.65 (0.44–0.96)	5.21 (3.16–6.86)	8.41 (5.16–11.22)	5.38 (3.58–7.61)	18.93 (16.26–23.07)	58.31 (32.66–100.39)
-											
$CD_{0.05}$		0.84	0.90	0.03	1.62	0.083	0.497	1.10	0.60	1.11	
-											
CV		5.24	5.09	4.30	1.15	7.628	6.191	8.82	12.80	3.62	

*CD and CV of each parameter are calculated for 84 genotypes combined

Table 2: Mean and range values of biochemical parameters of aonla fruits in different northeastern states

State	*TSS (°Brix)*	*Titriable acidity (%)*	*TSS : acidity*	*Ascorbic acid (mg/100g)*	*Total sugar (%)*	*Reducing sugar (%)*	*Non-Reducing sugar (%)*	*Phenol (mg/100 g)*	*Protein (%)*
Meghalaya	11.47 (7.50–17.50)	2.70 (1.65–4.90)	4.6 (2.11–7.66)	663.51 (180.00–1255.00)	8.15 (5.46–12.24)	5.13 (3.51–8.17)	3.28 (0.96–6.35)	1285.77 (687.73–3201.41)	3.92 (2.18–6.24)
Mizoram	12.36 (8.07–18.00)	2.78 (1.66–3.93)	4.69 (2.22–8.91)	686.42 (195.00–1393.00)	8.19 (4.96–11.33)	5.2 (3.43–6.53)	3.25 (0.80–6.28)	1331.6 (620.55–2598.16)	3.86 (2.31–6.13)
Tripura	11.56 (8.17–17.17)	2.93 (1.70–4.39)	4.17 (2.11–6.95)	753.93 (300.70–1176.00)	8.52 (3.90–13.05)	5.48 (2.47–9.10)	3.29 (1.10–5.59)	1605.38 (863.66–2979.61)	4.12 (2.48–5.58)
$CD_{0.05}$	0.89	0.28	0.57	39.63	0.79	0.39	0.81	100.13	0.51
CV	4.66	6.17	7.74	3.54	5.92	4.65	15.26	4.50	8.02

*CD and CV of each parameter are calculated for 84 genotypes combined

recorded from LTL1 and the minimum was recorded from both NKL1 and LCR1. These findings of aonla genotypes closely align with Singh and Singh (2016), Singh *et al.* (2016), and Hazarika and Laltluangkimi (2019). Accession MMT1 (1392.5 mg 100 g^{-1}) recorded the highest ascorbic acid and for the same least value was calculated in genotype KYR6 (180 mg 100 g^{-1}). More or less similar variation in Ascorbic acid was observed from the Garo Hills of Meghalaya, which ranged between 322.5 to 982.50 mg/100 g (Chandra *et al.,* 1998) from northeast India ranging between 375 to 1428.50 mg/100 mL (Singh and Singh, 2016; Singh *et al.,* 2016) and from Garhwal Himalaya, ranging between 191.13 mg 100 - 495.21 mg 100 g^{-1} (Naithani *et al.*, 2020).

The total sugar of the genotype ranged from 3.89 to 13.05%, in which the maximum was observed in LCR1 and the minimum in SNM2. The average Total sugar was determined between 7.50 to 13.68% from 39 aonla genotypes (Singh *et al.,* 2016), between 3.2 to 6.0% (Kumar *et al.,* 2013). In the present study, it was also revealed that genotype LCR1 had a significantly higher level of reducing sugar (9.1%) and TURA1 had the highest non-reducing sugar (6.35%). However, the minimum reducing sugar was determined in SNM2 (2.47%) and the lowest non-reducing in LTL1 (0.8%). Some other researchers also recorded variations in reducing sugar and non-reducing sugars (Kumar *et al.,* 2013; Hazarika and Laltluangkimi, 2019).

Among all genotypes, polyphenol content as gallic acid equivalent was recorded as the maximum value in UMR-2 (320.41 mg/100 g) and MMT4 recorded the minimum value (620.55 mg/100 g) of phenol. Singh *et al.,* (2016) reported that there was a significant variation of phenolic content in Aonla genotypes selected from North East India, with the value range between 944.85 to 4969.50 mg. Kumar and Rao, (2011) also determined the phenolic content (Gallic acid equivalent) between 3505.61 to 6155.71 mg/100 mL of different aonla cultivars. Parveen and Khatkar (2015) reported that desi aonla had a low protein of about 2.12%. In this study average protein content in the selected genotype of fruit ranged between 2.18 to 6.24%, the highest in CHITOKTE and the lowest in GBD5 genotype. The variation in protein content among different genotypes was also reported by other researchers (Kafkas *et al.,* 2020).

Conclusion

This study provides a comprehensive overview of the morphological diversity present in aonla genotypes in Northeast India. KOLASIB, WNG-3, TPN-7, TSP-1, TSP-3 and TURA-4 (Supplementary Figure 5) are observed as promising genotypes on the basis of the "Weighted-Ranged" method. The findings of the study indicate a high degree of diversity in the morphological characters of aonla genotypes. These findings provide important information for the selection and breeding of aonla genotypes with desirable morphological traits. However, further studies are necessary to assess

the genetic diversity of these genotypes and understand the underlying molecular mechanisms that control these morphological characters. Such studies could facilitate the identification of key genes involved in morphological traits and enable the development of molecular markers for selecting desirable genotypes. Additionally, further research could investigate the relationship between morphological traits and aonla fruit quality attributes such as nutritional and medicinal properties. Overall, the study provides an important baseline for future research on aonla genotypes, which could facilitate the development of improved aonla cultivars with desirable morphological and quality traits. The study provides a valuable contribution to the understanding of aonla diversity in northern India and can aid in the development of a sustainable aonla production system.

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Supplementary Files

https://ispgr.in/public/site/supplementary-files/23-21-supplementary-files.pdf