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POMOLOGICAL AND MOLECULAR CHARACTERIZATION AND ANTIOXIDANT PROFILING OF AONLA (*Phyllanthus emblica*) GROWN IN SUBTROPICAL ZONE OF INDIA

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ABSTRACT

Aonla (*Phyllanthus emblica*) is widely used in Ayurvedic and pharmaceutical industry due to the presence of medicinally important bioactive compounds. Seven commercial cultivars of *P. emblica* along with one wild genotype were characterized for morpho-molecular characters and antioxidant profiling. Morphological experiments were structured using a randomized block design, and molecular analysis was performed using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. The pomological traits revealed significant variation among the evaluated cultivars. The principal component analysis depicted 75.1% variation in fruit traits, and it clustered all the commercial cultivars together and bifurcated the wild variety separately. Further, the commercial cultivars were superior in all fruit traits and had high antioxidant potential as compared to the wild genotype. Molecular characterisation through molecular markers revealed the mean PIC value of 0.51 and 76.7% polymorphism. At cultivar level, these markers yielded polymorphic loci value ranging from 79.2 (cv. Kanchan) to 95.2% (cv. Krishna). Shannon's information index was between 0.28 (wild type) and 0.37 (cv. NA-7), indicating the genetic diversity. Cluster analysis through UPGMA method showed minimum similarity coefficient of 0.53 between wild type and cv. Kanchan. The pomological and molecular results authenticated the variability among the evaluated aonla cultivars and indicated the usefulness of pomological and molecular studies for precise characterization of *P. emblica* genotypes *viz-a-viz* genetic variability which could be exploited in future varietal improvement programmes.

Keywords: Antioxidant potential, genetic diversity, molecular characterisation, morphology, *Phyllanthus emblica*

INTRODUCTION

Phyllanthus emblica L., commonly known as Indian gooseberry or aonla, belongs to the family Euphorbeaceae and is considered as the mother of herbs. It is an important minor fruit crop used in pharma and commercial industries like food industry, nutraceuticals, cosmetics and is believed to attain the economy of about 50 million US dollars by ending 2025 (Muzaffar *et al*., 2022). The plant is widely distributed in subtropical and tropical areas of India, China, Thailand, Indonesia, Sri Lanka and Vietnam (Liu *et al*., 2008). It is one of the important indigenous fruits of Indian subcontinent (Gaur, 2003), with India ranking 1st in aonla cultivation and production (NHB, 2022). India has an area of 1.07 lakh ha under aonla cultivation, yielding 1.378 million t (NHB, 2022). Aonla cultivation is highly remunerative so its cultivation is becoming popular in Uttar Pradesh, Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Karnataka, Tamil Nadu and Himachal Pradesh with Uttar Pradesh as leading producer (384,000 t) of aonla. Kanchan, Krishna NA6, NA7 and NA10 are commercially cultivated varieties of aonla in India (NHB, 2022). Due to medicinal and therapeutic properties, aonla is considered as wonder fruit and is used to treat diseases like bronchitis, diabetes, fever, diarrhoea, anaemia, jaundice, cough, fever, dyspepsia, etc. (Luqman and Kumar, 2012). The fruits are used as anti-inflammatory, antipyretic, diuretic, hair tonic, laxative, and liver tonic (Balgia and Dsouza, 2011).

Aonla is rich in ascorbic acid, polyphenols, and minerals. The aonla fruit has strong anti-oxidant activity due to the presence of ascorbic acid, flavonoids, gallic acid, and other polyphenols (Kumari *et al*., 2024). Ascorbic acid has antioxidant, anti-inflammatory and antimutagenic properties and is an effective free radical scavenger. Antioxidants help in lowering the incidence of degenerative diseases like cancer and arthritis. Evidences show that phytochemicals in aonla potentially fight against SARS-CoV-2 virus by inhibiting its replication (Pandey *et al*., 2021).

Currently, limited research has been conducted for characterization of *P. emblica* germplasm This gap account the urgent need for orderly and comprehensive assesssment. Additionally, it offers in-depth research material to analyse the genetic diversity of this species. However, systematic cahacterization of this crop on both morphological and molecular level is still lacking..The most convenient way to identify any plant species is morphological characterization. But these methods often unsuccessful to accurately identify some species due to morphological relatedness and phenotypic flexibility to adjust in varied environmental conditions. The morphological determinants with in *Phyllanthus emblica* are highly variable under different agro-climatic conditions due to cross pollination and wider adoptability.

Molecular markers are a reliable mean to identify plants with specific characteristics as these are not affected by the environmental factors. The possibility of genetic evaluation during seedling stage make them swift and aid in varietal identification. Molecular marker techniques have been applied in a wide range of crops to assess their genetic diversity, and evaluate genetic relationships between accessions or cultivars and estimate their relatedness. DNA-based molecular markers is more reliable as the genetic information is unique for each species and is independent of age, and physiological and environmental factors (Joshi *et al*., 2004). DNA markers can potentially be used for characterizing and managing the plant germplasm. Markers have widely been used in genetic diversity studies because of their co-dominance, high polymorphism rate, good reproducibility, and rapid excavation at low cost (Nasim *et al.,* 2020). In recent years random amplified polymorphic DNA (RAPD), a useful DNA marker system, has been used to assess the genetic relationships between different sets of germplasm accessions/cultivars of *P. emblica* with estimation of relatedness (Chaurasia *et al.,* 2009; Rane *et al*., 2012; Singh *et al*., 2014).

Inter-simple sequence repeats (ISSR) markers is a technique in which DNA sequences amplified at a distance between two identical repeat regions in opposite direction (Luc *et al.*, 2020). ISSR primers sequence consist of di- or tri- nucleotide simple sequence repeat amplifying the regions. As compared to RAPD, ISSR markers are more reliable, reproducible and economical. ISSR has successfully used to estimate the extent of genetic diversity at inters- and intra-specific level in a wide range of medicinal plant species (Sarwat *et al*., 2016), but very scarce reports are available on the molecular characterization of aonla (Wang *et al.,* 2025). The present study was aimed to generate useful data based on morpho-molecular characterization of seven aonla (*Phyllanthus emblica*) varieties, assess genetic diversity based on pomological traits and principal component analysis and cluster analysis and molecularly characterize them through DNA markers and dendrogram construction. This two-step analysis may provide information about the biodiversity of valuable genetic resources which could prove helpful in future diversification breeding programmes.

MATERIALS AND METHODS

Sample collection

The fruits and leaves of aonla (*Phyllanthus emblica*) genotypes *viz*., Krishna, Kanchan, Banarasi, Chakaiya and Hathijhool were collected from Regional Horticulture Research & Training Station (RHR&TS), Jachh, Kangra, Himachal Pradesh (India) while the fruits and leaves of genotypes NA-7, NA-10 and a wild type were obtained from the College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh (India). The study was conducted during the year 2020 to 2021.

Pomological characterization

The experiment was set up in a randomized block design and each treatment replicated thrice. The fruits of different aonla cultivars were collected to analyse their colour, fresh weight, diameter (horizontal and vertical), pulp weight, stone weight, pulp/stone weight ratio, and dry fruit weight. The observations were recorded for fruit parameters as per aonla descriptor (Pathak, 2003). Quantitative characters were measured by using a precise electronic balance and digital Vernier's calliper. The colour characters were observed through visual assessment as mentioned in Royal Horticulture Society (RHS) colour illustrating charts (Royal Horticultural Society, 2001).

Antioxidant profiling of aonla

Fresh mature fruits of aonla genotypes were separately crushed in a pestle mortar and 10 mg crushed sample of each was dissolved in 10 mL methanol. Methanolic fruit extracts were used to estimate percent scavenging activity and total antioxidant activity. Free radical scavenging activity of methanolic fruit extracts of aonla genotypes were estimated by using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay (Williams *et al*., 1995). The absorbance of samples was measured at 517 nm by using UV-vis spectrophotometer (Thermo Scientific Evolution 201). The ability to scavenge DPPH radical was calculated by using formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total antioxidant capacity (TAC) of aonla fruits was analysed as per method of Preito (1999). The absorbance of samples was measured at 695 nm spectrophotometrically. Ascorbic acid was used as standard and the results expressed as µg ascorbic acid equivalents (AAE) mg^{-1} fresh fruit extract. The antioxidant profiling experiments were conducted in triplicate in a completely randomized block design (Gomez and Gomez, 1984).

DNA extraction, PCR amplification and product detection for genetic diversity studies

Total genomic DNA was extracted from the frozen leaves of aonla using cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) with slight modifications. Polyvinyl pyrrolidone (2 g) was added to the extraction buffer to control the exudation of phenols from leaves. Isolated genomic DNA was subjected to RNase treatment (0.5 µL) in each vial for successive purification. These vials were incubated at 37˚C for 45 min in hot water bath (Genaxy). DNA concentration was determined by UV-vis spectrometer and DNA quality was checked through electrophoresis using 0.8% agarose gel. The isolated DNA was stored at 4ºC until use.

Different genotypes of aonla were genotyped using total 33 RAPD primers and 15 ISSR primers to estimate genetic variability among different aonla cultivars. The concentrations of different components PCR was standardized for 25 µL reaction mixture. The optimized PCR mixture consisted of 50 ng template DNA, IU Taq DNA polymerase, 1X Taq buffer (10 X), 0.1 mM each dNTP mix, 200 nM primer and 1.5 mM $MgCl_2$. The amplification was carried out in a thermocycler (Biorad) the program set up to initial denaturation at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C, 1 min at annealing (as per Tm of primer), and 1 min at 72°C; with a final 7 min extension at 72°C. The PCR amplified product of 10 µL was analysed on 1.2% agarose gel. The gel was viewed under gel documentation system (Imagene) to take a photograph of gel.

All amplified band was accounted across all the eight cultivars of aonla for genetic analysis with respect to all informative primers. The presence of a band was designated as '1' and absence as '0'. Numerical taxonomic and multivariate analysis system (NTSYS-pc) version 2.2 software programme was used to predict genetic diversity between genotypes through RAPD and ISSR data. Matrix was constructed which computes a variety of similarity using Jaccard's coefficient (Jaccard, 1908) based on SIMQUAL function. Dendrogram was constructed by unweighted pair group method analysis (UPGMA) using arithmetic averages (Sokal and Sneath, 1963) based on sequential agglomerative hierarchical nesting (SAHN) function of NTSYS-pc. A polymorphic information index (PIC) for band profile of both markers was calculated as $PIC = 1 - p^2 - q^2$, where, p is band frequency and q is no band frequency (Ghislain *et al.*, 1999). Population genetic analysis was done by using Popgen32 to calculate Nei's (1978) genetic diversity (h), Shannon's information index (I), observed number of alleles (na), percent polymorphic loci (PPL) and effective number of alleles (ne).

Statistical analysis

The data generated was analysed using one-way analysis of variance (ANOVA). The differences at $p < 0.05$ were considered significant. The statistical analysis was carried out using MS-Excel and OPSTAT. The data was expressed as mean ± standard error. Eigen values were used to determine the contribution of principle components. Further, the cultivars were grouped into different clusters using an unweighted pair group method analysis (UPGMA).

RESULTS AND DISCUSSION

Pomological characters of aonla

Fruit quality is a multifaceted character dependent on multiple factors and in this study the physical parameters of fruit cultivars exhibited a significant variation (Table 1). Fruit weight in different aonla genotypes varied from 9.8 ± 0.4 to 36.34 ± 1.4 g with maximum fruit weight in cv. Banarasi and lowest in wild genotype. The fruit weight of cv. Banarasi was at par with that of cv. Kanchan and Hathijhool. The diverse genetic backgrounds of parents could be the reason for variation in fruit weight of genotypes. The variations may be caused by differences in the genetic makeup of cultivars, since they were propagated through seeds (Mishra *et al.*, 2018). The maximum pulp weight was found in cv. Banarasi (32.58 ± 1.16 g) which was at par with cv. Hathijhool and Krishna. However, fruits of wild genotype showed lowest pulp weight (8.43 ± 0.42 g). Similar trend for pulp weight has been reported by Bakshi *et al*. (2015).

Table 1: Pomological characters of eight genotypes of aonla (*Phyllanthus emblica*)

Cultivars	Fruit weight (g)	Seed weight (g)	Pulp weight (g)	Dry fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Pulp: seed ratio
Chakaiya	22.8 ± 1.2	1.4 ± 0.1	20.7 ± 1.1	3.5 ± 0.3	29.9 ± 0.8	35.0 ± 0.5	14.2 ± 0.3
Krishna	31.1 ± 0.9	1.5 ± 0.1	28.6 ± 0.9	3.7 ± 0.3	34.8 ± 0.2	38.6 ± 0.4	18.2 ± 0.9
Hathijhul	35.2 ± 1.4	2.5 ± 0.1	31.5 ± 1.3	8.9 ± 0.8	36.7 ± 0.3	40.8 ± 0.6	12.3 ± 0.7
Kanchan	34.4 ± 0.9	1.8 ± 0.1	31.3 ± 0.7	7.1 ± 0.2	37.0 ± 0.6	38.6 ± 0.6	16.7 ± 0.6
Banarsi	36.3 ± 1.4	2.4 ± 0.1	32.5 ± 1.1	12.5 ± 0.4	34.3 ± 0.9	41.4 ± 0.5	13.1 ± 0.4
NA- 10	27.9 ± 0.4	1.6 ±0.2	25.3 ± 0.4	5.9 ± 1.0	31.1 ± 0.3	37.9 ± 0.5	15.3 ± 0.8
NA -7	26.5 ± 0.4	1.6 ± 0.1	24.0 ± 0.3	8.6 ± 0.6	32.8 ± 0.4	36.2 ± 0.3	14.6 ± 0.5
Wild	9.8 ± 0.4	1.1 ± 0.1	8.43 ± 0.4	5.7 ± 0.6	21.5 ± 0.6	26.2 ± 0.2	7.3 ± 0.2
$CD_{0.05}$	3.07	0.33	2.82	2.16	1.93	1.53	1.96

Data represents the mean ± standard error of three replicates

The seed weight of eight cultivars of aonla ranged from 1.14 ± 0.03 to 2.57 ± 0.13 g with maximum seed weight in cv. Hathijhool followed by cv. Banarasi. Among the evaluated cultivars, maximum dry fruit weight was observed in cv. Banarasi and lowest in cv. Chakaiya. The dry fruit weight of cv. Hathijhool was at par with cv. Kanchan and NA 10 at 5% level of significance. The variation in pulp weight and stone/seed weight in aonla might be due to the variations in fruit to stone ratio, genetic makeup, accumulation and transport of photosynthates transport within fruit tissues (Singh *et al.,* 2020). The fruits with higher pulp content in relation to peel and stone is preferred by the consumers and so would benefit the breeders to choose the best parents in their hybridization programmes.

The fruits of evaluated cultivars varied from 21.56 ± 0.6 to 37.0 ± 0.7 mm with maximum fruit length in cv. Kanchan which was at par with Hathijhool and lowest fruit length in cv. Wild. The fruit diameter of aonla genotypes varied from 26.2 ± 0.2 to 41.46 ± 0.6 mm with maximum diameter recorded in cv. Banarsi was at par with cv. Hathijhool (40.0 ± 0.6 mm) and minimum fruit diameter in wild aonla. Kumar and Khatkar (2015), Bakshi *et al*. (2015) and Gocher and Gochar (2020) also reported maximum fruit length and diameter in cv. Banarasi as compared to the wild types. Differences in genetic features of individual variety, soil properties and climatic conditions appear to be the main cause for the observed variation in fruit length and diameter (Rozar *et al.,* 2024).

A considerable variation (7.3 ± 0.2 to 18.2 ± 0.9) was observed in pulp to seed ratio in the evaluated cultivars with highest value noted in cv. Krishna and lowest in wild type. Pulp to stone ratio is a vital factor in identifying a superior genotype by breeders. Our results align with Hazarika and Laltluangkimi (2019) and Chandra *et al*. (2020). Kumar and Khatar (2016) observed highest pulp stone ratio in fruits of cv. Kanchan (19.54) and lowest in desi fruits. The overall comparison for morphological characters across different studies showed remarkable variation which appear to be influenced by soil and environmental factors. The results demonstrated great diversification in fruit characters in commercial aonla.

Principal component analysis (PCA)

The PCA Eigen value and it contribution to total variation in pomological trait in different cultivars is given in Table 2. PC1, PC2 and PC3 accounted for 75.13, 21.2 and 2.59% variation with Eigen value of 5.26, 1.48 and 0.18, respectively. PC1 was found positively and strongly related with fruit weight, seed weight, pulp weight, fruit length, fruit diameter and pulp seed ratio. PC2 value was highest for dry fruit weight (0.793). PCA has widely been explored in fruit quality assessment, and it provides in depth knowledge of trait inter-relationships which can be exploited in selection of superior cultivars (Gantiat *et al*., 2021).

Table 2: Principal components analysis (PCA), Eigen value and contributions to the total variation (%) in different aonla cultivars

Variables	PC1	PC2	PC3
Fruit weight	0.997	-0.041	-0.018
Seed weight	0.845	0.490	-0.213
Pulp weight	0.996	-0.074	-0.008
Dry fruit weight	0.526	0.793	0.306
Fruit length	0.961	-0.191	-0.027
Fruit diameter	0.984	-0.062	-0.053
Pulp seed ratio	0.625	-0.754	0.197
Eigen value	5.260	1.486	0.182
Variability (%)	75.137	21.225	2.596
Cumulative %	75.137	96.362	98.959

UPGMA dendrogram of the same data is shown in Fig. 1. In this dendrogram wild aonla cultivars is separated from all commercial cultivars with maximum mean Euclidean distance. According to fruit characters NA-10 and NA-7 shared 100% similarity.

Determination of antioxidants in aonla

The major cause of disease is the production of harmful substances which induce oxidative stress due to free radical formation. The compound which protect body against such stress are known as

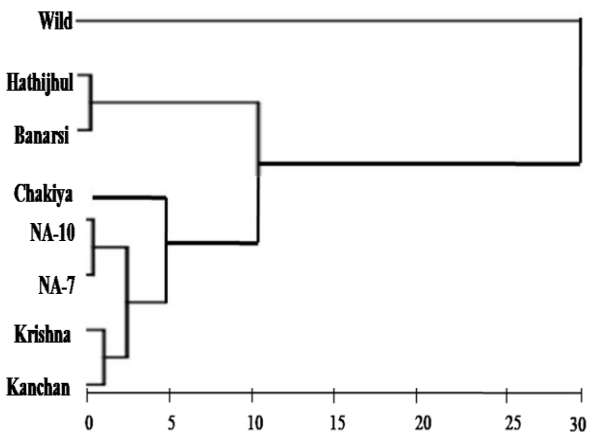

Fig. 1: UPGMA dendrogam of aonla cultivars based on mean Euclidean distance of fruit traits

Table 3: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging activity and total antioxidant capacity in various aonla cultivars

Cultivars	DPPH (% inhibition)	Total antioxidant capacity (µg AAE mg^{-1} fresh fruit extract)
Chakaiya	51.1 ± 0.90	264.8 ± 1.9
Krishna	30.2 ± 0.79	257.1 ± 1.6
Hathijhul	30.4 ± 1.12	237.4 ± 1.5
Kanchan	38.1 ± 0.79	247.9 ± 1.8
Banarsi	33.8 ± 0.68	210.5 ± 1.5
NA-10	29.2 ± 1.03	247.5 ± 1.6
NA-7	35.6 ± 0.79	232.4 ± 2.0
Wild	27.3 ± 0.90	208.8 ± 1.2
$CD_{0.05}$	1.76	5.17

Data represents mean ± standard error of 3 replicates

antioxidants. In present study, the antioxidants produced in different varieties were estimated as DPPH free radical scavenging activity and total antioxidant capacity. All the cultivars of aonla fruit showed appreciable free radical-scavenging activity and cultivars varied significantly at $p > 0.05$ (Table 3). The methanolic extract of cv. Chakaiya recorded highest antioxidant activity (51.17 ± 0.90% DPPH scavenging) and lowest antioxidant activity (27.344 ± 0.902% DPPH scavenging) was observed in wild genotype. The fresh fruits of cv. Chakaiya also had highest total antioxidants (264.827 ± 1.974 µg AAE mg^{-1}) and lowest total antioxidants in wild aonla (208.8 ± 1.2 µg ascorbic acid mg^{-1}). Yadav *et al.* (2007), Haripriya *et al*. (2012) and Sumalatha (2013) also reported antioxidant activity in anola, while Kumar and Khatkar (2016) found significant variations in antioxidant activities in the methanolic extracts of various aonla cultivars. Saleh *et al.* (2020) emphasised that aonla plays a crucial role in reducing lipid peroxidation, oxidative stress and reactive oxygen species (ROS); and simultaneously enhance the levels of different types of antioxidants, thereby hinder the process of disease development in plants.

Genetic diversity in aonla cultivars

Molecular characterization by using molecular markers: Thirty three RAPD (operon series) and fifteen ISSR primers were screened, out of which only fifteen primers RAPD and nine ISSR showed amplification of different loci of isolated genomic DNA from different varieties. Fifty one and twenty nine bands were obtained in PCR amplification of genomic DNA of aonla using fifteen RAPD and nine ISSR primers, respectively (Table 4). A total of 88 alleles were developed, of which 56 (70%) were found polymorphic and 6 (7.5%) were unique bands, with an average of 3.33 bands per primer, while remaining 18 (22.5%) were monomorphic among the studied aonla cultivars. The amplicon size of the product varied from 150-1500 bp and the PIC value ranged from 0.25 to 0.83. The average PIC value was 0.51 which signified that the test markers were informative.

Genetic diversity studies at varietal level: The results of genetic diversity using RAPD and SSR markers are presented in Table 5. At population level, the PPL of eight genotypes was reported to range from 79.2% Kanchan to 95.4% (cv. NA-7) and 95.2 (cv. Kanchan), H was between 0.10 (wild type) and 0.23 (cv. NA-7); and I was between 0.28 (wild type) and0.37 (cv. NA-7), indicating that the genetic diversity existed among different genotypes. However, relatedness was shared by two cultivars *viz*., Krishna and NA-7.

Table 4: The primer used and PCR banding profile details for analysis of genetic diversity among different *Phyllanthus emblica* genotypes

S. No.	Primer name	Total amplified bands	Poly-morphic locus	Mono-morphic locus	Unique bands	Percent poly-morphism	Per cent mono-morphism	PIC value	Size range (bp)
1	OPA-1	5	4	1	0	80.0	20.0	0.76	400-1200
2	OPA-3	1	1	0	0	100.0	0.0	0.50	800
3	OPA-4	5	4	1	0	80.0	20.0	0.76	200-750
4	OPA-11	5	3	2	0	60.0	0.0	0.62	200-600
5	OPA-13	3	2	1	0	66.6	33.3	0.44	250-1100
6	OPA-14	3	2	1	0	66.6	33.3	0.42	450-900
7	OPA-15	1	1	0	0	100.0	0.0	0.50	300
8	OPB-1	7	3	3	1	42.8	42.8	0.44	300-1300
9	OPB-2	7	3	4	0	42.8	57.1	0.42	150-1100
10	OPD-9	2	1	1	0	50.0	50.0	0.36	300-600
11	OPD-10	3	2	1	0	66.6	33.3	0.42	400-800
12	OPM-8	2	2	0	0	100.0	0.0	0.47	500-600
13	OPM-9	4	4	0	0	100.0	0.0	0.26	200-900
14	OPM-11	1	1	0	0	100.0	0.0	0.31	400
15	OPM-15	2	1	0	1	50.0	0.0	0.25	600-1300
16	ISSR 2	2	2	0	0	100.0	0.0	0.27	650-1100
17	ISSR 3	4	3	0	1	75.0	0.0	0.67	300-900
18	ISSR 17	3	2	1	0	66.6	33.3	0.51	250-800
19	ISSR 5	5	3	2	0	60.0	40.0	0.48	600-1500
20	ISSR 7	3	2	0	1	66.6	0.0	0.42	600-800
21	ISSR 22	3	3	0	0	100.0	0.0	0.83	300-900
22	ISSR 24	4	3	0	1	100.0	0.0	0.71	400-1200
23	UBC 836	1	1	0	0	100.0	0.0	0.66	250
24	ISSR 18	4	3	0	1	66.6	0.0	0.71	150-700
Total		80.00	56.00	18.00	6.00	1,840.2	363.1	12.25	
Average		3.33	-	-	-	76.7	15.1	0.51	

Table 5: Population genetic analysis using popgene software

Genotypes	na*	ne*	h*	I*	PPL
Kanchan	1.8	1.31	0.21	0.34	79.2
Banarsi	1.92	1.20	0.20	0.36	91.7
Hathijhul	1.94	1.26	0.19	0.33	93.8
Chaikya	1.83	1.24	0.18	0.30	83.3
Krishna	1.96	1.26	0.19	0.34	95.2
NA-7	1.98	1.24	0.23	0.37	95.4
NA-10	1.87	1.32	0.22	0.36	87.5
Wild	1.83	1.1	0.10	0.28	80.0

na = observed number of alleles; ne = effective number of alleles, h = genetic diversity, I = Shannon's information index (I), PPL = percent polymorphic loci

Cluster analysis

The RAPD and ISSR fragments obtained after the amplification of genomic DNA from eight cultivars of aonla were scored in binary form *i.e.,* 1 (presence of band) and 0 (absence of band). The similarity matrix obtained in present study was used to construct dendrogram by UPGMA. The similarity value obtained by using combined data of both marker systems ranged from 0.53 to 0.87. The maximum similarity coefficient (0.87) was found in cv. NA-7 and Krishna (Fig. 2). Minimum similarity coefficient of 0.53 was observed in cv. Kanchan and wild. Chaurasia *et al*. (2009) estimated genetic variability among cultivated aonla cultivars through RAPD and observed close similarity between cv. Chakaiya and Kanchan with similarity coefficient of 0.81. Rout *et al*. (2010) studied relationship among twelve aonla species by using molecular markers and reported that similarity values ranged from 0.35 to 0.76 in RAPD markers with very high polymorphism (96.13%) while ISSR similarity values ranged from 0.31 to 0.76. Rana *et al*. (2012) also reported 56-60% polymorphism among 20 genotypes of aonla using RAPD markers. Singh *et al*. (2014) reported 56.18% polymorphism among 8 cultivars

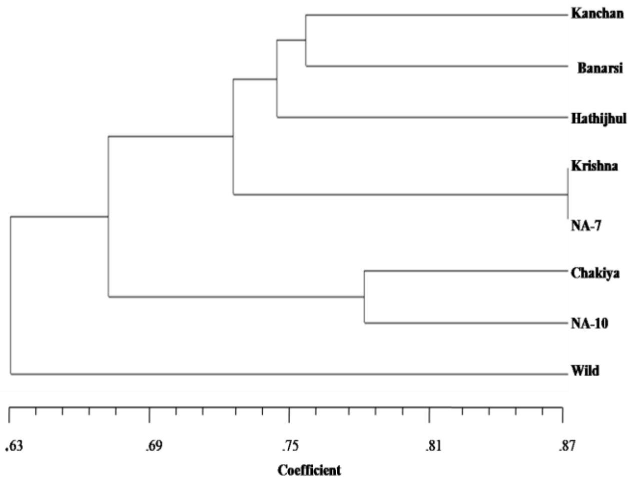

Fig. 2: Phylogenetic tree of aonla genotypes constructed using NTSYS-pc version 2.0 software

of aonla using 10 RAPD primers. The findings are consistent with Poeaim *et al*, (2025) which emphasized the efficacy of RAPD markers in assessing the genetic diversity in plants, particularly in identifying polymorphic loci and constructing genetic relationships.

On the basis of present study, it may be concluded that the commercial cultivars possess higher level of desired characters as well as the compounds of medicinal importance. The pomological and molecular studies diversified wild genotype from all the other commercial cultivars cultivars and exhibited highest diversity with cv. Kanchan. Future studies have to be focused on screening the genetic diversity using genic SSR to improve the resolution of genetic relationships. The integration of molecular data with the desirable traits such as higher vitamin C content, larger fruit size, greater yield, disease resistance and stress tolerance, would improve the targeted aonla breeding.

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