Indian Journal of Ecology (2025) 52(6): 1466-1471
DOI: https://doi.org/10.55362/IJE/2025/4676

Manuscript Number: 4676
NAAS Rating: 5.38

Genetic Variability and Character Association Studies in Anola (*Phyllanthus emblica* L.), Germplasm from South Western Punjab

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Abstract: Aonla (*Phyllanthus emblica* L.), holds significant medicinal and nutritional benefits. The present investigation was carried out at Punjab Agricultural University, Regional Research Station, during 2019-20 to assess the genetic variability of 30 accessions of *Phyllanthus emblica* L. The results showed a wide range of diversity among the germplasm. Mean sum of squares owing to genotypes (GCV) was highly significant for all of the characters under study. The phenotypic coefficient of variation (PCV) for all traits was greater than the genotypic coefficient of variation, indicating the presence of environmental factors that can have impact on the the phenotypic expression of some traits. The high estimates of PCV and GCV were for fruit weight and plant girth and the lowest estimates of PCV and GCV were observed for pH and genetic advance as percent of mean was observed for fruit yield, stone weight, and plant girth. The significant positive correlation between fruit yield was with fruit breadth, fruit diameter, and fruit weight Smilarly, the fruit weight exhibited a significant correlation with fruit breadth and diameter. The path correlation analysis indicated that girth has the greatest positive direct effect on yield per plant, followed by fruit length, stone weight, and pH. The PCA showed a considerable amount of the total variability, accounting for 80.15% of the cumulative variance contribution rate across the first 5 PCs identified. The PC was highly contributed by positive loading with fruit characters viz. fruit weight, fruit breadth, fruit diameter, fruit yield, plant girth, fruit length, plant height, TSS, pH indicating that these characters should be considered as selection criteria for increasing fruit yields in breeding programs.

Keywords*:* Anola, *Phyllanthus emblica* L., South Western Punjab, Genetic variability

Aonla (*Phyllanthus emblica* L), is a hardy and extremely nutrient-dense fruit crop of immense medicinal and economic value. It is the richest source of vitamin C, antioxidants, and phyto chemicals .Tropical Southeast Asia is its native habitat, especially central and southern India, which is thought to be the origin of aonla. In terms of area (99000 ha) and production (1216000 MT), India is leading country (Anonymous 2021-22). The fruit has multiple medicinal uses and can be turned into a variety of goods with added value. *Phyllanthus* species offer a variety of therapeutic qualities, including antiviral, antibacterial, antipyretic, anti-inflammatory, anti-hepatotoxic, antioxidant, and analgesic activity. It includes numerous bioactive substances like as flavonoids, phenolics, tannins, alkaloids, kaempferol, gallic acid, and quercetin. The plant is modest to medium in height, with a spreading crown. It is the perfect crop for arid and semi-arid areas like South-Western Punjab, where soil salinity and water scarcity frequently restrict the development of traditional fruit crops. This is due to its versatility under a variety of agro-climatic conditions. Since there are no standard commercial methods of vegetative propagation, farmers have been propagating the plant from seeds, and as a result, there is a large variability among the existing populations in morphology, plant shape, fruit color, fruit size, and chemical constituents of the fruits. However, no systematic research has been undertaken to yet on the genetic diversity of wild populations in order to identify elite varieties with horticultural essential fruit attributes. Exploration of available germplasm and identification of acceptable genotypes are critical components of every breeding program. High heterozygosity and frequent cross-pollination resulted in the current heterogeneity in seedling populations, from which promising genotypes were chosen (Dinesh and Vasugi 2010). Genetic variability studies using leaf and fruit metrical features may reveal genetically diverse wild genotypes with crop improvement attributes (Nogueira et al., 2012).The evaluation of genetic characteristics such as genotypic coefficient of variation, phenotypic coefficient of variation, heritability, and genetic advancement is necessary for effective selection and improvement in the base population. Path analysis using the phenotypic correlation coefficient reveals additional information about the contribution of several variables to yield. The current study was aimed to elucidate the phenotypic diversity among the natural populations and to select the elite types that may have very important horticultural characteristics.

MATERIAL AND METHODS

The experiment was carried out in 2019-20 on ten-year-old trees of 30 desi Aonla seedlings grown and maintained at the experimental farm PAU, Regional Research Station, (30º11'23.0"N 74º57'18.6”E) Bathinda, Punjab, using a randomized block design with three replications (one plant per replication). The plantation was done according to the square system and the plants were kept under uniform cultural operations in accordance with the PAU's package of practices for Aonla production (Anonymous 2020). The climate is arid with lowest and maximum temperatures ranging from 16 to 32ºC and an average annual rainfall of 480 mm. The soil was sandy loam with an 8.31 pH, 0.32% organic carbon, 0.24 dS/m electrical conductivity, 216 kg/ha available N, 22.7 kg/ha available P, and 368.0 kg/ha available potassium. The data was recorded on various vegeative, fruits, quality and yield parameter (Table 1, 2).

Total soluble solids in fruits were conducted at ambient temperature using a handheld refractometer with a 0-32 range (ERMA). The determination of titratable acidity was performed according to the method suggested in A.O.A.C (2010).The measurement of juice pH was taken by pH metre by placing the rod into the juice beaker and the value was taken as displayed by the pH metre.

Statistical analyses: The data from 30 desi Aonla seedlings involving 14 traits were analyzed by R software version 4.3.1. The correlation analyses used parametric Pearson correlations to analyze the quantitative and qualitative traits. Heritability in broad sense and estimates of appropriate genetic variance components were substituted for the parameters to predict expected genetic gain. Genotypic coefficient of correlation (rg) and phenotypic coefficient of correlation (rp) were computed as per Robinson et al. (1949). Path coefficient was measure of direct and indirect effects of each character on fruit yield was estimated using a partial regression coefficient (Dewey and Lu 1959). The PCA biplot was generated using the R packages ggplot 2, factoextra, grid extra, corrplot, and facto mine R, along with path coefficient analysis (Dewey and Lu 1959).

RESULTS AND DISCUSSION

Analysis of variance for characters studied during the experiment was significant among treatments (Table 1 and 2). The mean values of the characters, ranges, genotypic mean sums of squares, standard error (SE) of means, and coefficients of variation also showed sufficient amounts of variation for morphological and biochemical components of aonla genotypes. The genotypic and phenotypic coefficients of variation are reliable parameters for measuring the degree of variability demonstrated by different traits. Higher PCV than GCV was in all the parameters indicating the role of environments on the expression of characters but differences were very minor. The slight variation between the genotypic and phenotypic coefficients of variation indicates that expressions of traits are predominately governed by genetic factors. The selections based on these traits are likely to be effective with equal chances of success. The high PCV and GCV were found for the fruit weight plant girth, stone weight, stone breadth, yield and height offers a good scope of improvement with the selection of these traits. The findings corroborate with findings of Singh et al. (2012) in aonla.

High heritability (>97%) for all the traits studied in this research suggested that genetic factors rather than the environment mainly controlled the expression of traits. The yield, girth and stone weight were the most heritable traits (97%), whereas, the plant height (90.87%) were the least

Table 1. Analysis of variance of different characteristics in Aonla

Source of variation	d.f.	Mean sum of squares						
		Height (m)	Girth (cm)	Fruit Dia	Fruit breadth	Fruit length	Stone Dia	Stone breadth
Replication	2	77.38	1.39	1358.51	306.46	253.79	279.51	26.27
Treatment	29	192.48*	11.36*	1538.98*	362.76*	319.21*	235.21*	140.14*
Error	58	12.47	0.22	42.34	10.22	7.22	8.05	3.06

* Significant at 1.0 percent level

Table 2. Analysis of variance of different characteristics in Aonla

Source of variation	d.f.	Mean sum of squares						
		Stone length	Stone (Wt)	Fruit (Wt)	Acidity	pH	TSS	Yield
Replication	2	30.82	13.22	5916.29	6.51	7.85	376.75	9631.74
Treatment	29	146.56*	75.56*	54775.94*	8.61*	6.48*	615.38*	26696.59*
Error	58	3.18	1.48	1122.89	0.23	0.16	16.59	514.33

* Significant at 1.0 percent level

heritable (Table 3). Traits such as fruit weight yield and fruit diameter exhibited high genetic advance, indicating that their heritability is mainly due to additive gene effects and direct selection based on these traits in genetically diverse genotypes could be effective for desired improvement. The phenotypic correlation coefficients were lower than the genotypic correlation coefficients for traits studied, indicating a strong genetic link. Plant height, plant girth, fruit breadth, fruit diameter, fruit length and fruit weight were showed positive relationship with yield/plant. Hence, selecting genotypes with higher values of these traits may improve crop yield.

Singh et al (2012) reported similar results for these traits in aonla. Genotypic and phenotypic coefficient of variance was used to assess the nature and level of relationships between traits for strategic improvement programs through selection or hybridization. The observations are in accordance with the findings of Patel et al. (2015).These characters also exhibited high GCV, therefore, selection based on phenotypic performance for these traits would be effective in improving these characters directly in the population.

Correlation analysis: Significant positive associations were found between fruit diameter, and yield, fruit width and yield, pH and yield, and girth and yield (Fig. 1). Stone length and breadth were shown to be negatively correlated with fruit weight. There was a positive association between fruit weight and both fruit diameter and width. Hazarika et al. (2025) also observed strong positive relationship between fruit weight and TSS, fruit length, and fruit diameter. Ganopoulos et al. (2018), Khadvi et al. (2019), and Srivastava et al. (2019) have published similar results, showing a strong positive association between fruit weight and fruit length and diameter.

Path coefficient analysis: Path correlation analysis revealed girth exhibits the highest positive direct effect on yield per plant, followed by fruit length, stone weight and pH (Table 4). The negative direct effects were observed on yield per plant for, stone length, fruit breadth and stone diameter .This indicates that there is a real correlation between these traits and yield, and selection for this characteristic directly will increase strawberry yield. Maximum indirect positive effect was found for fruit yield with fruit weight) via stone length followed by fruit breadth via stone length, fruit diameter via fruit length. The highest negative indirect effects were for stone breadth via stone length followed by fruit weight via fruit breadth, acidity via stone length

Principal component Analysis of various parameters: To ascertain the relationship between genotypes and the associations between various attributes within the subsets,

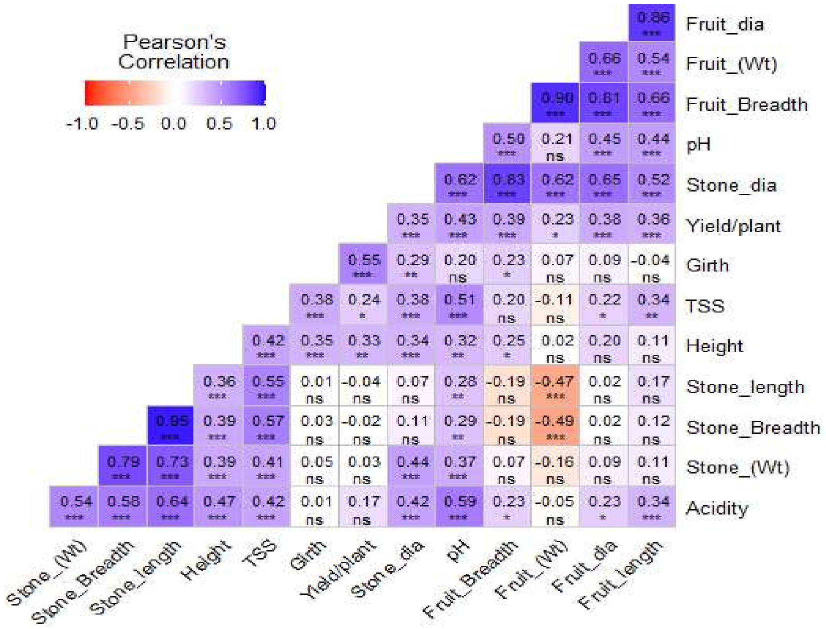

Fig. 1. Linear correlation among the various attributes such as vegetative, fruit, quality and yield attributes calculated using Pearson correlation. The $p \geq 0.05$: non-significant, $^{*}p<0.05$ (significant at 5 % level of significance); $^{**}p<0.01$ (significant at 1 % level of significance); $^{***}p<0.001$ (significant at 0.1 % level of significance)

Table 3. Genetic variability among different characters in Aonla

Name of character	GM	Minimum	Maximum	CV	PCV	GCV	H² (%)	GA	GAM
Height (m)	6.88	4.10	9.50	6.74	22.32	21.28	90.87	2.87	41.78
Girth (m)	0.93	0.30	2.00	6.68	39.41	38.84	97.12	0.73	78.84
Fruit diameter (mm)	28.60	22.00	35.00	2.98	14.90	14.61	96.10	8.44	29.50
Fruit breadth (mm)	13.63	10.40	17.30	3.08	15.21	14.87	95.64	4.08	29.96
Fruit length (mm)	12.46	9.30	15.20	2.83	15.53	15.28	96.75	3.86	30.95
Stone diameter (mm)	13.10	0.25	1.37	2.84	12.78	12.47	95.14	3.28	25.05
Stone breadth (mm)	4.02	2.30	6.40	5.70	31.89	31.41	97.07	2.57	63.76
Stone length (mm)	4.31	2.10	6.60	5.43	30.41	29.94	96.92	2.62	60.72
Stone (Wt)	2.84	1.00	5.15	5.63	33.17	32.69	97.12	1.88	66.36
Fruit (Wt)	59.80	7.17	14.20	7.35	42.40	41.75	96.98	50.65	84.70
Acidity	1.98	1.00	2.43	3.19	16.11	15.79	96.04	0.63	31.88
pH	2.19	1.80	2.80	2.38	12.50	12.30	96.83	0.55	24.93
TSS (°Brix)	15.11	10.40	21.40	3.53	17.84	17.48	96.06	5.33	35.30
Yield (kg/plant)	76.90	35.00	100.00	3.87	23.00	22.67	97.17	35.40	46.03

Table 4. Path correlation among differeent traits with yield of the plants

Attributes	Height	Girth	Fruit_D	Fruit_Br	Fruit_L	Stone_D	Stone_Br	Stone_L	Ston_Wt	Fruit_Wt	Acidity	pH	TSS
Height	0.124	0.136	0.028	0.072	-0.169	0.020	0.014	-0.126	0.085	-0.037	-0.007	-0.021	-0.032
Girth	0.027	0.621	0.022	-0.010	-0.165	-0.023	-0.006	0.100	-0.031	-0.008	0.010	-0.014	-0.052
Fruit_D	-0.030	-0.118	-0.116	-0.299	0.343	-0.086	-0.022	0.269	-0.086	0.137	0.014	-0.020	0.073
Fruit_Br	-0.020	0.013	-0.075	-0.458	0.177	-0.191	-0.039	0.471	-0.097	0.206	0.014	-0.001	0.076
Fruit_L	-0.046	-0.224	-0.086	-0.177	0.458	-0.019	-0.012	0.102	-0.072	0.098	0.006	-0.018	0.025
Stone_D	-0.009	0.050	-0.035	-0.304	0.030	-0.287	-0.017	0.251	0.089	0.128	0.004	0.036	0.027
Stone_Br	0.029	-0.068	0.043	0.307	-0.095	0.083	0.058	-0.645	0.266	-0.147	-0.017	-0.001	-0.097
Stone_L	0.023	-0.091	0.045	0.315	-0.068	0.105	0.055	-0.686	0.240	-0.144	-0.020	-0.007	-0.089
Stone_Wt	0.030	-0.054	0.028	0.126	-0.093	-0.072	0.044	-0.466	0.354	-0.067	-0.015	0.027	-0.050
Fruit_Wt	-0.022	-0.023	-0.075	-0.448	0.214	-0.176	-0.040	0.471	-0.113	0.210	0.013	-0.007	0.085
Acidity	0.025	-0.173	0.043	0.172	-0.070	0.029	0.027	-0.372	0.145	-0.074	-0.036	0.042	-0.007
pH	-0.014	-0.045	0.012	0.001	-0.043	-0.052	0.000	0.024	0.050	-0.008	-0.008	0.195	-0.025
TSS	0.018	0.151	0.039	0.162	-0.053	0.036	0.026	-0.285	0.083	-0.083	-0.001	0.023	-0.215

Fruit_D: Fruit diameter; Fruit_Br: Fruit diameter; Fruit_L: Fruit diameter: Stone_D: Stone diameter: Stone_Br: Stone Breadth: Stone_L: Stone length; Stone_Wt: Stone weight; Fruit_Wt: Fruit WeighT

Table 5. Eigen values, variance and cumulative variance (%) with principle components

Variables	PC1	PC2	PC3	PC4	PC5
Height	-0.15	0.21	-0.28	0.21	-0.46
Girth	0.01	0.53	-0.23	0.29	0.13
Fruit diameter	0.32	-0.28	0.08	0.31	0.10
Fruit breadth	0.39	-0.10	-0.27	0.09	-0.07
Fruit length	0.22	-0.38	0.26	0.29	0.29
Stone diameter	0.21	-0.15	-0.61	0.03	0.02
Stone breadth	-0.39	-0.20	-0.06	0.26	0.07
Stone length	-0.38	-0.23	0.01	0.21	0.05
Stone weight	-0.26	-0.26	-0.44	0.20	0.02
Fruit weight	0.40	-0.13	-0.21	0.07	-0.09
Acidity	-0.25	-0.25	-0.14	-0.34	-0.16
pH	-0.03	-0.05	-0.30	-0.54	0.57
TSS	-0.22	0.10	-0.09	0.34	0.47
Yield plant	0.11	0.41	-0.02	0.07	0.28
Eigen values	5.15	2.12	1.65	1.21	1.09
Variance (%)	36.79	15.14	11.78	8.65	7.80
Cumulative variance (%)	36.79	51.93	63.71	72.35	80.15

principal component analysis or PCA was done utilized (Ruiz and Egea 2008). PCA results indicated that since the eigen values (λi) were greater than 1, the first five PCs may be used to examine the relationship between the qualities assessed in our study. The combined variance proportion of the first two PCs was 51.93%. (Table 5).The first PC accounted for 36.79% of the total variance, while the second PC explained 15.14% (Figs. 2, and 3). After analyzing the qualities'

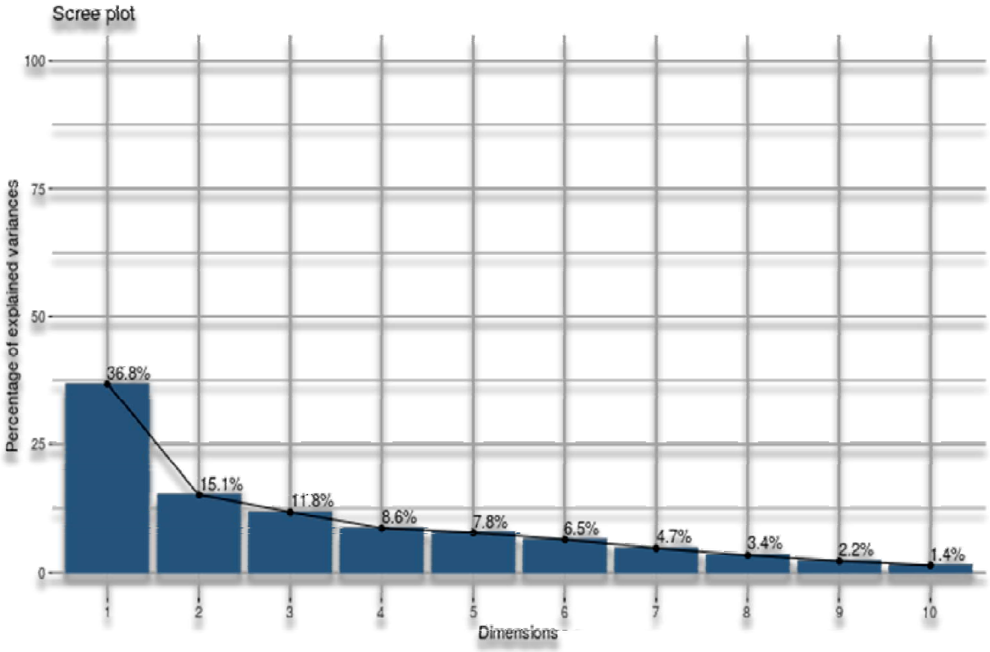

Fig. 2. Scree plot depicting the variability explained by each Principal component of the various vegetative, fruit, quality and yield attributes of amla

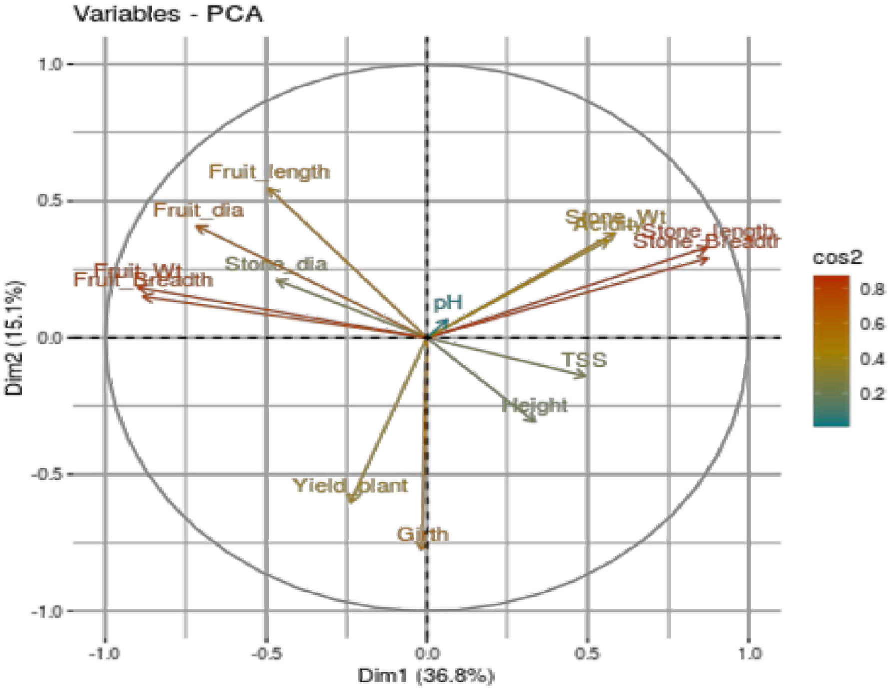

Fig. 3. Principal component analysis with the contribution percentage of various vegetative, fruit, quality and yield attributes to variability

contributions, that PC1 and PC2 benefited more from the levels of fruit weight and yield, respectively. Hazarika et al. (2025), also observed variation among aonla accessions, as demonstrated by scatter plots and scree plots along PCA biplots, which explained 86.66% of the total variation. Fruit length, diameter, weight, yield, TSS, acidity, and other attributes that are represented in the PCs are integrated to emphasize the significance of these crucial quantitative factors.

CONCLUSION

The present investigation on Aonla accessions exhibited significant variability in the studied in the vegetative , fruiting and biochemical characters offering a valuable resource for including them in breeding programmes and mainstreaming them. The existence of wide ranges of variability for most of the characters among the aonla germplasm provides opportunities for genetic gain through selection or hybridization. The fruit weight and girth showed the high estimates of PCV and GCV, and genetic advance as percent of mean was observed highest for fruit yield stone weight and plant girth. Fruit yield showed strong positive and significant correlations with fruit breadth, fruit diameter, and fruit weight. Thus, selection may be possible for these characters for improving yield.

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Received 07 July, 2025; Accepted 25 November, 2025