The Pharma Innovation Journal 2023; 12(9): 1816-1819

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 1816-1819

www.thepharmajournal.com
Received: 20-06-2023
Accepted: 23-07-2023

Jnapika KH
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Sarvamangala Cholin
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Kulapati H
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

SG Gollagi
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Sanjeevaraddi GR
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Basavaraj Padashetti
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Corresponding Author:
Jnapika KH
College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

Genetic diversity and character association studies in *Annona* species under Northern dry zone of Karnataka

Jnapika KH, Sarvamangala Cholin, Kulapati H, SG Gollagi, Sanjeevaraddi GR and Basavaraj Padashetti

Abstract
The current study was conducted at the College of Horticulture in Bagalkot, Karnataka, from 2017 to 2019. Twelve cultivars of *Annona*, representing four distinct species, were assessed for morphological traits. On the basis of D2 values, twelve *Annona* genotypes were divided into four groups. The first cluster, which had cultivars No. 1, No. 2, Red Sitapahal, Island Gem, Red & Pink, Chance Seedling, Pink Mammoth, and Ramphal, contained the most cultivars (eight) of the four. The third cluster consisted of two cultivars, Arka Sahan and *Atemoya* x Balanagar, while clusters II and IV were solitary clusters made up of the cultivars *Atemoya* and Balanagar. The range of intra-cluster distance varied from a minimum of 0.00 in solitary clusters (II and IV) to a maximum of 314.43 in the first cluster, according to the assessment of inter- and intra-cluster distances using D2 for phenotypic variables. Indicating greater diversity between the cultivars belonging to clusters I and IV, the maximum inter-cluster distance was found between clusters I and IV (1567.97), while the minimum inter-cluster distance was found between clusters II and III (210.35). This higher diversity could be exploited for future hybridization programs in order to combine the desirable characters of these cultivars into a single variety.

Keywords: Genetic diversity, morphology, identification, *Annona* species

Introduction
According to Gupta *et al*. (2015) [5], *Annona* is a member of the family *Annona*ceae, which is one of the largest extant groups of primitive angiosperms in the clade Magnolid. The *Annona* genus contains 166 species, six of which are edible (*Annona squamosa* L., Sitaphal, Sharifa, Sugar apple, Sweet sop), *A. reticulata* (Bullock's Heart, custard apple), cherimoya (*A. cherimola*), *A. muricata* (Guanabana or soursop), *A. atemoya* (a natural hybrid of A. Except for *A. glabra*, which is said to be a tetraploid species with 2n = 4x = 28, the majority of *Annona* species are diploid in nature and have chromosome numbers 2n = 2x = 14 and 16. The *Annona* is a very abundant source of significant therapeutic substances (Pinto *et al*., 2005) [13]. The *Annona* fruit is a healthy fruit in and of itself, rich in beneficial fatty acids, vitamins, and minerals (Gyamfi *et al*., 2011; Leal, 1990) [6, 9]. It also has a high calorific content. Due to its richness, it could be a fruit crop that can help with the problems caused by malnutrition. However, due to a lack of high yielding variations, India's production of *Annona* cultivars is quite poor when compared to other nations. That is generally attributable to heavy fruit drop, which is primarily linked to moisture stress, inadequate nutrition, temperature changes, hormonal imbalance, greater wind speeds, insect pests and illnesses, etc. India is said to be *Annona squamosa*'s secondary place of origin. This allows for more *Annona* species diversity. Additionally, several new kinds have been created in order to increase genetic diversity and variability. The genetic diversity of *Annona* species has been researched by several authors (Jalikop and Kumar 2000; Sahoo *et al*. 2000; Onimawo 2002; Mathakar 2005) [7, 11, 10]. To create effective breeding techniques, a rigorous evaluation of nature, the degree of variety, and character association is required. Greater genetic potential and more room for selection are provided by greater variety. Selecting acceptable genotypes for fruits of higher quality can be greatly aided by the investigation of several morphological and biochemical features and how they interact. Making a successful breeding strategy requires knowledge of the type and degree of associations between various traits. In order to find the potential cultivars/species for economic features, the current study was conducted to thoroughly evaluate the 12 cultivars that correspond to four *Annona* species for morphological and biochemical traits.

Material and Methods

The current study was carried out in 2017–19 at the University of Horticultural Sciences' fruit orchard in Bagalkot, Karnataka. It was situated at an altitude of 610 m at 16°12′N and 75°45′E. With an average annual rainfall of only 518 mm, the climate is hot and dry all year long and is classified as semi-arid tropical. In the current investigation, twelve unique genotypes originating from *Annona* squamosa, *A. atemoya, A. cherimoya, and A. reticulta* were employed. There were noted significant morphological and biochemical characteristics. Insect infestations (mealy bug, fruit fly, and myrid bug), illnesses (dry rot), and physiological problems (percent of mummified fruits) were also examined for in the cultivars. When a fruit reaches physiological maturity, it is harvested. Fruits were taken to the lab after collection and maintained there at -4 °C until additional observation recording. Utilizing the Cherimoya descriptor created by Bioversity Internationale and CHERLA in 2008, cultivars were thoroughly characterized for qualitative parameters. Quantitative characteristics for tree, leaf, and fruit traits were estimated using standard SI units. According to the prescribed protocols, tree and leaf parameters were recorded. Fruit characteristics such as shape, color, stem end cavity, shape, and color of the seeds, as well as physical and morphological characteristics, were noted. Vernier callipers were used to measure the fruit's length and width.

On an electric weighing balance, the fruit's weight was recorded. The amount of Total Soluble Solids (TSS) was calculated using a portable refractometer. The fruit juice's acidity was measured by titrating it against 0.1N NaOH and expressing the results as a percentage of citric acid, while the sugar content was examined using techniques developed by the Association of Official Analytical Chemists (AOAC) in 1984. For the current analysis, a Randomized Complete Block Design (RCBD) with three replications and 12 cultivars was used. Variance analysis (ANOVA) was applied to the quantitative traits found in the phenotypic data. The software Windowstat (version 8.2) was used to do the analysis of variance. Using the statistical program SPSS (version 16.0), descriptive statistical analysis, such as mean, range, and standard error, was performed on the mean data of all twelve cultivars for 21 quantitative attributes. The D2 technique proposed by Mahalanobis (1936) was used to evaluate the genetic divergence. The Rao (1960) [14] described ward minimum approach was used to cluster all n (n-1) /2 D2 values.

Results and Discussion

12 *Annona* cultivars were examined for several phenotypic variables in the current study, including 16 morphological and 5 biochemical parameters. All of these traits were then subjected to a diversity analysis. Twelve cultivars were assessed for fruit quality, quantity, and physico-chemical characteristics, as well as morphological and biochemical variables. The most effective of these were selected, propagated clonally, and preserved using both in-situ and ex-situ techniques. Among all cultivars, Island Gem, Arka Sahan, and Balanagar had the ideal fruit characteristics of a high pulp percentage, a low seed percentage, and a small quantity of seeds. Asymmetrical fruits were produced by the cultivars Island Gem, No. 1 Pink Mammoth, and Arka Sahan, which decreased their market value. The fruit morphology of cultivars Balanagar and *Atemoya* x Balanagar was pleasing. Ramphal has the longest shelf life of the examined cultivars (5 days). Among the fruit morphology. clusters, cluster I contained the most cultivars (eight), including No. 1, No. 2, Red Sitapahal, Island Gem, Red & Pink, Chance Seedling, Pink Mammoth, and Ramphal. The third cluster comprised of two cultivars—No. 1, No. 2, Arka Sahan, and *Atemoya* x Balanagar—while clusters II and IV were composed only of the cultivars *Atemoya* and Balanagar. Two hybrids, Atemoya x Balanagar and Arka Sahan, belong to one cluster (Cluster III), whereas their parents, Atemoya and Balanagar, belong to two different clusters, II and IV, respectively. This difference highlights how the hybrids differ from their parents. The majority of the cultivars in Cluster I belonged to the widely cultivated A. squamosa species (No. 1, No. 2, Red Sitaphal, Red and Pink, Chance Seedlings, Red Sitaphal), and they were grouped with cultivars from the *A. reticulata* (Ramphal) and Pink Mammoth (*A. atemoya*) species, demonstrating how difficult it is to distinguish between species using morphological markers alone. The range of intra-cluster distance varied from a minimum of 0.00 in solitary clusters (II and IV) to a maximum of 314.43 in the first cluster, according to the assessment of inter and intra-cluster distances using D2 for phenotypic variables. The highest inter-cluster distance was found between clusters I and IV (1567.97), while the lowest inter-cluster distance was found between clusters II and III (210.35), indicating that future hybridization programs could take advantage of the higher diversity between the cultivars in clusters I and IV to combine the desirable traits of these cultivars into a single variety (Table 2). Cluster IV outperformed other clusters in terms of stem girth (10.17 cm), fruit diameter (25.38 mm), fruit length (6.22 cm), number of seeds (37.67), fruit volume (137.33 cm3), rind weight (68.77g), percentage of mummified fruits (11.48), TSS (14.320B), acidity (26.43%), and reducing sugar (22.97%), while cluster I performed best in terms of fruit weight (22.97%), followed by cluster IV.

In 2008, Dikshit *et al* [2]. investigated the diversity of Maharashtra's custard apple germplasm pools. During an exploration program, they gathered 21 custard apple germplasm accessions from six regions in Maharashtra. The average fruit weight ranged from 90.8 to 375 g, as did the total soluble sugar content (19 to 26%), the number of seeds per fruit (21.2 to 73), the weight of pulp per fruit (44.4 to 188 g), and the fruit to pulp ratio (37 to 54.2). TSS was found to be lowest in cluster II (4.17%) and highest in cluster IV (14.320B) among the biochemical parameters. Total sugars and reducing sugars fared best in Cluster III (23.53% and 22.27%, respectively), while they were lowest in Cluster II (17.40% and (11.80%), respectively.

Cluster III (9.47%) had the highest non-reducing sugar concentration, followed by cluster I (5.59%), while cluster IV (3.03%) had the lowest concentration. Similar to this, Rao and Subramanyam (2011) [15] assessed custard apple germplasm for a region with little rainfall when it rains. *Atemoya* x Balanagar (highest fruit weight), Y. Palli-12 (highest pulp weight), NLD-8 (maximum T.S.S.), Balanagar SR (more number of fruits per tree), and Ramphal (highest yield per tree) are the varieties out of 35 germplasm evaluated that can be suggested for scarce rainfall zones under rainfed conditions.

To the best of our knowledge, study conclusions were drawn after a thorough review of the data utilizing a variety of statistical techniques, including genetic variability estimates,

correlation analysis, and diversity analysis. Even though there were only 12 *Annona* cultivars chosen for phenotypic characterization, they contained local germplasm that is commercially grown in northern dry zones of Karnataka. As a result, the information gleaned from this study would be useful for both crop improvement in the Northern Dry Zone of Karnataka as well as for fruit breeders worldwide.

In light of this, the current study on the phenotypic characterization of *Annona* cultivars for fruit parameters would be very beneficial for a variety of stakeholders, including students, researchers who are interested in learning about the *Annona* diversity, and even farmers who can choose the varieties based on the data provided by fruit breeders to decide about the varieties to be planted when establishing the orchard. Mahalanobi's D2 analysis is regarded as one of the most effective methods for the study of genetic diversity, particularly when using the diversity of physical traits as a criterion for choosing different parents for mating. Regardless of the number of qualities included in the study, it provides precise information on the number of characters contributing to the diversity by taking the genetic variation of the relevant traits in the population. According to the diversity of the cultivars, the cultivars are divided into various clusters, and information on the number of cultivars in each cluster, intra- and intercluster distances, and the mean values for each trait under study for the corresponding cluster can be obtained. These data are useful for selecting diverse cultivars from the diverse cluster in accordance with the breeding goals. *Annona squamosa*'s genetic diversity was examined by Bharad *et al*. (2009) [1] using morphological and biochemical markers. The cultivars that were taken from various areas displayed a great deal of variability, according to the results obtained using eight molecular markers and biochemical tests. The 11 sugar apple cultivars were divided into four separate clusters based on the morphological data collected on various characteristics. The 11 cultivars that were tested were polymorphic, according to the findings. Four *Annona* species that were discovered in Nigeria and were considered valuable but underutilized species were researched by Folorunso and Olorode in 2006. An intra-generic connection and genetic diversity among *Annona* species were indicated by morphological characteristics. *Annona*ceae species with elliptic leaves, such *A. senegalensis*, A. *suqamosa*, and *A. reticulata*, were strongly associated with clustering.

There are many different locally adapted landraces of *Annona* as a result of the plant's evolution through natural and artificial selection in various elevational zones and under various cropping techniques with honey bees acting as the carrier of cross-pollination. These landraces reflect a wide variety of crop diversification patterns and have evolved over time to adapt into regional cropping patterns and various end uses. The collection and identification of suitable clones or cultivars with the desired characters to multiple traits, including productivity, quality, tolerance to abiotic stresses, and resistance to significant pests and diseases, are the fundamental requirements in the crop improvement program of this perennial crop species.

Six species of this broad family of *Annona*ceae are edible, therefore identification of the cultivars at the species level would aid plant breeders in sifting through the segregating population after hybridization for the desired qualities from the appropriate species. The manifestation of the economic element, i.e., fruit-related features, however, takes longer time because to its prolonged juvenile phase, and the necessity to maintain each segregant in the orchard makes it laborious and a waste of resources. As a result, in the current study, an effort has been made to characterize the cultivars of *Annona* from 4 different species, including *A. squamosa*, *A. Atemoya*, *A. reticulata*, and *Atemoya* cultivars (hybrid between *A. squamosa* and *A. cherimoya*), involving a total of 12 cultivars, both at the phenotypic and genotypic levels. For the purpose of organizing upcoming missions to explore germplasm and, later, for the effective application of such germplasm in crop improvement programs, it is crucial to have an understanding of the genetic diversity patterns of a crop species in any particular region or nation.

Table 1: Based on quantitative (morphological and biochemical) features, the cluster composition of various *Annona* cultivars was determined by D2 analysis.

Cluster No.	No. of cultivars	Name of cultivars
Cluster I	8	No. 1, No. 2, Red Sitapahal, Island Gem, Red& Pink, Chance Seedling, Pink Mammoth,Ramphal
Cluster II	1	Atemoya
Cluster III	2	Arka Sahan, *Atemoya* x Balanagar
Cluster IV	1	Balanagar

Table 2: D^2 analysis of quantitative (morphological and biochemical) variables for various *Annona* cultivars: inter- and intra-cluster distance

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	314.43	530.07	640.02	1567.97
Cluster II	530.07	0.00	210.35	504.22
Cluster III	640.02	210.35	158.01	665.56
Cluster IV	1567.97	504.22	665.56	0.00

Table 3: Cluster Means: Tocher Method

Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
1	Plant height (m)	3.71	3.64	3.75	3.26
2	Stem girth (cm)	10.82	9.71	10.12	10.17
3	East West Canopy (m)	2.81	2.58	2.65	2.16
4	Fresh Fruit weight (g)	188.50	156.60	107.48	143.75
5	Fruit length (m)	0.66	0.51	0.44	0.62
6	Fruit diameter (m)	0.07	0.084	0.003	0.023
7	Ripe fruit weight (g)	162.12	138.00	114.98	71.87
8	Pulp weight (g)	106.68	78.30	57.66	60.08
9	Number of seeds	21.21	27.00	20.00	37.67
10	Seed weight (g)	10.62	8.67	6.19	11.48
11	Percentage of mummified fruits	7.04	4.17	14.21	14.32
12	TSS (°Brix)	24.88	23.03	24.97	26.43
13	Acidity (%)	0.19	0.22	0.35	0.12
14	Total sugars (%)	19.94	17.40	23.53	22.97
15	Reducing sugars (%)	12.73	11.80	14.07	22.27
16	Non- reducing sugars (%)	5.59	5.60	9.47	3.03
17	Seed percentage	5.86	5.54	5.62	8.24
18	Fruit volume (cm^3)	89.44	69.00	102.50	137.33
19	Shelf life (days)	2.71	3.00	2.50	3.00
20	Rind weight (g)	68.66	66.72	53.64	68.77
21	Number of fruits / plant	7.63	18.00	16.17	20.67

Conclusion

Twelve cultivars in all were assessed for biochemical and morphological characteristics, including fruit quality,

quantity, and physico-chemical parameters. The most effective of these were clonally multiplied and preserved both in-situ and ex-situ. Among all cultivars, the top two fruits with the ideal fruit characteristics: high pulp percentage and low seed percentage with a minimum amount of seeds were Island Gem and Balanagar. Asymmetrical fruits were produced by the cultivars Island Gem, No. 1 Pink Mammoth, and Arka Sahan, which decreased their market value. The fruit shape of cultivar Balanagar, No. 2, *Atemoya* x Balanagar was pleasing. Ramphal had the longest shelf life out of all the cultivars that were examined. To the best of our ability, research results were drawn after a thorough review of the data collected using a variety of statistical methods of diversity analysis. Even though there were only 12 *Annona* cultivars chosen for phenotypic characterization, they contained local germplasm that is commercially grown in northern dry zones of Karnataka; as a result, the information gleaned from this study would be useful not only for fruit breeders worldwide but also for crop improvement in those regions.

Acknowledgement
The authors are extremely grateful to the Director of Research at the University of Horticultural Sciences in Bagalkot for giving funding to support their research at the college of horticulture there in Karnataka, India. Conceptualization of the research (by Sarvamangala Cholin and Kulapati Hipparagi); design of the tests (by Sarvamangala Cholin and Kulapati Hipparagi); execution of field/lab experiments and data gathering (Jnapika, K.H. and Sarvamangala Cholin), analysis of data, and interpretation; Kulapati Hipparagi and K.H. Jnapika prepared the manuscript.

Declaration
The authors declare that there is no conflict of interest.

References
1. Bharad SG, Kulwal PL, Bagal SA. Genetic diversity study in custard apple (*Annona squamosa* L.) by morphological, biochemical and RAPD Markers, Acta Hort. 2009;839:615-624.
2. Dikshit N, Bharad SG, Badge MP. Diversity in custard apple germplasm collections from Maharashtra, Indian J Pl. Genetic Res. 2008;21(1):95-96.
3. Escribano P, Viruel MA, Hormaza JI. Molecular analysis of genetic diversity and geographic origin within an *ex-situ* germplasm collection of cherimoya by using SSRs. J American Soc. Hortil. Sci. 2007, 132(3).
4. Folorunso AE, Olorode O. Biosytematic studies in *Annona*ceae, vegetative and floral morphological studies of some species of *Annona* in Nigeria. Res. J Bot. 2006;1(3):118-124.
5. Gupta Y, Pathak AK, Singh K, Mantri SS, Singh SP, Tuli R. De novo assembly and characterization of transcriptomes of early-stage fruit from two cultivars of *Annona squamosa* L. with contrast in seed number.BMC Genomics. 2015;16(1):86.
6. Gyamfi K, Sarfo D, Nyarko B, Akaho E, Serfor-Armah Y, Ampomah-Amoako E. Assessment of elemental content in the fruit of graviola plant, *Annona muricata*, from some selected communities in Ghana by instrumental neutron activation analysis. Elixir Int J. 2011;41:5671-5675.
7. Jalikop SH, Kumar SP. New fruit varieties for arid regions - pomegranate 'Ruby' and custard apple 'Arka Sahan'. Indian Hort. 2000;45(2):19-20.
8. Larranaga N, Hormaza JI. DNA barcoding of perennial fruit tree species of agronomic interest in the genus *Annona* (*Annona*ceae). Frontiers in Pl. Sci. 2015;6:589.
9. Leal F. Fruits of tropical and subtropical origin composition, properties and uses. (eds.) S. Nagy, Shaw, P. E. and Wardowiski W. F. Florida Sci. Source, Inc., Lake Alfred, Fl; c1990. p. 149-58.
10. Mahalanobis PC. On the generalized distance in statistics. Proceedings of National Institute of Science. India. 1936;2:49-55.
11. Mathakar TD. Assessment of custard apple (*Annona squamosa* L.) hybrids M. Sc. (Agri.) thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra), India; c2005.
12. OnimawoI A. Proximate composition and selected physico-chemical properties of the seed, pulp and oil of sour sop (*Annona muricata* L.). Pl. Foods Human Nutrit. 2002;57(2):165-171.
13. Pal DK, Kumar PS. Changes in the physico-chemical and biochemical composition of custard apple (*Annona squamosa* L.) fruits during growth, development and ripening. J Hortil Sc. 1995;70:569-572.
14. Pinto ACQ, Cordeiro MCR, Andrade SRM, Ferreira FR, Filgueiras HAC, Alves RE. *Annona* species. International centre for under utilized crops, University of Southampton, Southampton; c2005. p. 263.
15. Rao CR. Multivariate analysis: An indispensable tool in statistical and in applied research. Sankhya. 1960;22:317-38.
16. Rao Dhanunjay K, Subramanyam K. Growth and yield performance of custard apple germplasm under scarce rainfall zone. Indian J Agric. Sci. 2011;45(2):156-160.
17. Sahoo SC, Panda JM, Mohanty D. Studies on floral biology of custard apple (*Annona squamosa* L.) under Bhubaneshwar, Orissa. J Hort. 2000;28(1):43-45.