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Review Journals : Molecular From Anonaceae

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Abstract. There are roughly 120 species of the Anona genus, which is found in tropical and subtropical areas. The diversity at the species level and similarity of the leaves, fruits, and stems that are produced as a result of plant cultivation and hybridization create new plants that are difficult to macroscopically identify. The diversity of the genus Anona can be accurately determined using molecular identification. For example, genetic analysis utilizing PCR and the RAPD and AFLP procedures is one way to gather molecular data. In this study, we inform the development of species-specific primers by differentiating the most agronomically interesting Annona species that, until recently, a new formal classification based on molecular phylogenetics has been developed in order to have a quick and precise method of distinguishing Annona between species agronomically.

Keyword: Annona, Annonaceae, Diversity, Molecular

Abstrak. *Genus Anona yang tersebar pada wilayah tropis dan subtropis memiliki kurang lebih 120 spesies. Budidaya tanaman serta hibridisasi menghasilkan tanaman baru yang memiliki keanekaragaman tingkat spesies dan mempunyai kemiripan daun, buah, dan batang menjadi penghalang untuk melakukan identifikasi secara makroskopis. Identifikasi molekuler digunakan untuk mengetahui informasi keanekaragaman genus Anona dengan data akurat. Ada beberapa metode yang dilakukan untuk memperoleh data molekulker seperti analisis genetik menggunakan PCR dengan metode RAPD dan AFLP. Untuk memiliki metode yang cepat dan akurat dalam membedakan Annona antar spesies secara agronomis, dalam penelitian ini kami menginformasikan pengembangan primer spesifik untuk spesies dengan membedakan spesies annona yang paling menarik secara agronomis yang sampai saat ini, klasifikasi formal baru berdasarkan filogenetik molekuler telah ditunda karena keterbatasan dalam representasi takson dan resolusi filogenetik. Rekonstruksi filogenetik di Annonaceae dimulai dengan analisis kladistik karakter makromorfologi dan palynologis*

Kata Kunci: *Annona, Annonaceae, Keanekaragaman, Molekuler*

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1. Introduction

In the world's tropical woods, the family Annonaceae includes a variety of trees, shrubs, and lianas [1]. These are some of the oldest plants among the angiosperms, having evolved in the tropics and subtropics and spreading over both hemispheres [2]. Beetles, thrips, bees, cockroaches, and the wind all pollinate the protogynous blooms. It has been widely dispersed across the Atlantic to other regions of the world since the mid-17th century, according to Annona spp. [1]. In addition to numerous species found in temperate and subtropical temperatures, the Annonaceae family is primarily found in the tropics. There are about 900 Neotropical species, 450 Afrotropical species, and additional species from the Indo-Malayan region. They are very significant for species diversity, particularly in tropical rainforest habitats. There are 120 species in the genus Annona, which is found across tropical and subtropical areas of the world [3]. Anemoya (Annona x atemoya Mabb.), guanabana or soursop (A. muricata L.), custard apple (A. reticulata L.), llama (A. macroprophyllata Donn. Sm.), pond apple (A. glabra L.), or soncoya (A. purpurea Moc. & Sessé ex Dunal) [4] [5].

The most crucial step in DNA isolation necessitates the destruction of the cell wall, cell membrane, and nuclear membrane in order to release all of the contents of the cell, including the DNA, into the buffer during the extraction process. This can be accomplished by washing the cells with a detergent like sodium dodecyl sulfate (SDS) or cetyltrimethylammonium bromide (CTAB) [6]. The AFLP approach and the RAPD method employing PCR were both used in genetic analysis. data evaluation For each fragment, amplified fragments were noted as absent (0) or present (1) in all subjects. The expression of two alleles at one locus, one dominant and one recessive, with the expression of the dominant allele being able to determine the existence of the band, results in these two potential states, which are referred to as molecular phenotypes [7] [8].

There are numerous species of the genus Annona all around the world. Identification of macroscopic variety is hampered by the similarities of the leaves, fruits, and stems among each Annona species. Because of this, molecular identification is required to understand the diversity of Annona sp., which is found all over the world. For researchers, molecular identification makes it simpler to collect reliable data. This journal's goal is to make it easier for researchers to understand the variety of Annona species. This publication compiles data on the variety of plants in Annona sp. Due to restrictions on taxon representation and phylogenetic resolution, a new formal classification based on molecular phylogenetics has yet to be completed. Currently, clades are known by colloquial names that correspond to features of molecular evolution, such as long branch clades (LBC) and short branch clades (SBC). Beginning with a cladistic examination of

macromorphological and palynological traits, phylogenetic reconstruction in the Annonaceae family is undertaken [1].

2. Materials and Methods

We gathered all international papers concerning molecular study on Anonaceace. We look at phylogenetic trees from numerous published literature sources using Google Scholar and keywords like Molecular and Genetic Annonaceae.

3. Result and Discussion

An Annona species known as sweetsop (*Annona squamosa* L.) was introduced to mainland China in the middle of the 17th century. This plant is native to tropical America, India, and Pakistan. This plant is a semi-evergreen shrub or small fruit tree that grows to a height of 6 to 8 meters. Protein, carbohydrates, and polyphenols are more abundant in Srikaya fruit and leaves. An Annona species known as sweetsop (*Annona squamosa* L.) was introduced to mainland China in the middle of the 17th century. This plant is native to tropical America, India, and Pakistan. This plant is a semi-evergreen shrub or small fruit tree that grows to a height of 6 to 8 meters. Protein, carbohydrates, and polyphenols are more abundant in Srikaya fruit and leaves. Isolated DNA samples were extracted using the CTAB technique in research [9]. MseI/EcoRI can be used to digest DNA samples from Annona plants, making them suitable for AFLP analysis. This study used analysis of the amplification fragment length polymorphism to identify the genetic diversity of seven cultivars of sweetsop (*Annona squamosa* L.). There are seven cultivars of sweetsop grown at the Tropical Germplasm Resources Research Institute in Hainan, China: Sweetsop "Fenglishijia" (*Annona atemoya*), Sweetsop "Niuxin" (*Annona reticulate*), Sweetsop "Milu" (A. reticulate), Sweetsop "Yuanhua" (*Annona glabra*), Sweetsop ‘AP’ (A. atemoya).

Marker	Number of characters in aligned matrix	Number of potentially parsimony informative characters (%)	Number of indel characters	CI	RI	Model selected by MrModeltest
rbcL	1376	284 (20.6)	0	0.40	0.80	GTR + I + G
matK	831	247 (29.7)	1	0.61	0.70	GTR + G
ndhF	1956	715 (36.6)	4	0.50	0.70	GTR + I + G
atpB-rbcL	747	206 (27.6)	27	0.70	0.82	GTR + G
trnT-L	673	225 (33.4)	0	0.68	0.73	GTR + G
trnL intron	520	197 (37.9)	23	0.60	0.83	GTR + G
trnL-F	377	214 (56.8)	32	0.56	0.84	GTR + G
psbA-trnH	433	209 (48.3)	24	0.54	0.69	GTR + G
trnS-G	744	241 (32.4)	19	0.70	0.81	GTR + G

Tabel 1. Each locus' specific marker properties have been evaluated using all available information. All nucleotide characters are included in the consistency index (CI) (Lars W Chateau)

According to the research findings from [3], some of their sequencing attempts failed. The link between the two subfamilies Annonoidaea and Ambavioideae is one of the outcomes of the RAxML analysis. These two clades' connecting node has 94% bootstrap support. The yield, probability, the yield from maximal, Bayesian analysis, and various data sets, including larger data sets with more species and sequence data, dramatically disagree with this [1].

A phylogenetic tree is generated from the data matrix of 129 species using RAxML analysis. Malmeoideae and Annoideae, with mean branch lengths of 0.00241 and 0.00724, respectively. According to the t-test, it appears to have a significantly different branch length distribution (p 0.0001, and their data cannot be displayed) [10]. Erkens discovered weak correlations between reduced Miliuseae, nesting in Malmeoideae, and greater diversification rates in big descending clades within Annonoideae within the Annonaceae. They anticipate that further analyses of the phylogenetic patterns in the Annonaceae, based on more thorough taxon and character sampling and a closer look at model assumptions, will enable us to better understand the effects of substitution rate and timing on branch length, which will help us.

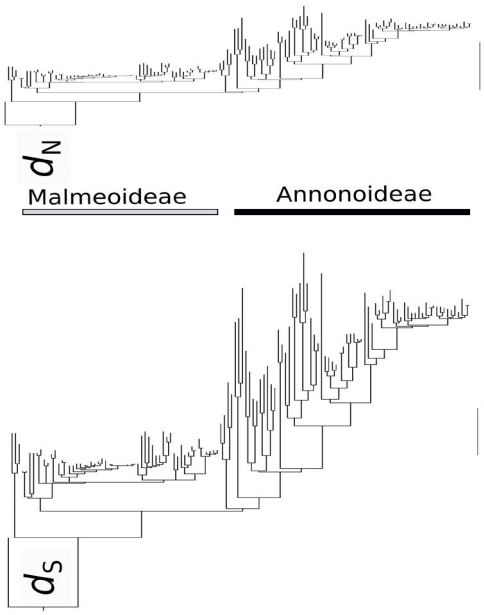

Figure 1. Long coding branches for nonsynonymous (dn) and synonymous (ds) substitutions individually in the RAxMl topology. The scale is the same for these two trees.

Research [10] determined allelic polymorphism using AFLP analysis. AFLP is a method created for the fingerprinting of genomic DNA [11] [12]. The ability to combine restriction endonuclease digestion and polymerase chain reaction (PCR) amplification of restriction fragments is a benefit of this method. Allelic polymorphism analysis using AFLP has a higher resolution and sensitivity than analysis using RAPD [13][14]. Three pairs of primer sets were present in the DNA templates of the seven different kinds used in the AFLP reaction, producing 662 bands that could be measured (size range: 80–550 bp). One person might produce between 80 and 122 bands, whereas a species could produce between 90 and 130. The seven samples' similarity coefficients ranged from 0.572 to 0.818. Yuanhua had the lowest similarity coefficient (0.572) compared to the other types, whereas AP and Feilishijia had the highest similarity coefficient (0.818). (Fig. 1). Between "Niuxin" and "Ciguo," the similarity coefficient was 0.782, while between "Milu" and "Shanci," it was 0.717. It appears that 'AP' and 'Feilishijia' share a tight genetic tie, whereas 'yuanhua' has a more distant relationship.

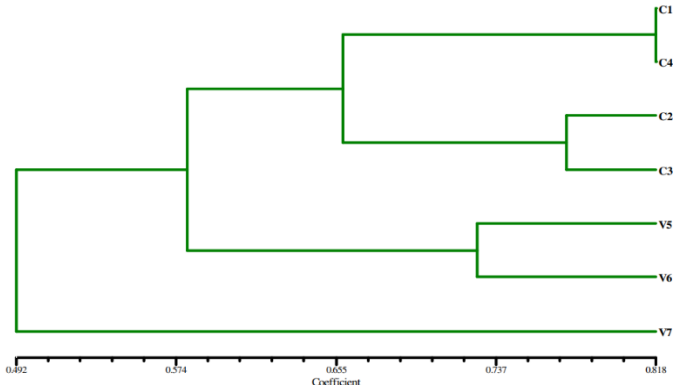

Figure 2. Dendrogram of cluster analysis for sweetsop cultivars based on AFLP markers. C1: ‘AP’ A. atemoya; C2: ‘Niuxin’ A. reticulat; C3: ‘Ciguo’ A. muricata; C4: ‘Fenglishijia’ A. atemoya; V5: ‘Milu’ A. cherimola; V6: ‘Shanci’ A. montana; V7: ‘Yuanhua’ A. glabr [15].

The genetic diversity of the seven kinds and their genetic relationships can be shown through AFLP markers. The findings of this study show that AFLP fingerprint-based distance analysis has high phylogenetic capabilities at the species level. The seven kinds are closely related in terms of fruit characteristics, scale types, and leaf form and smoothness. Both "Niuxin" and "Ciguo" have accurate clustering analysis and good graft compatibility. Theoretically, the breeding, selection, and grafting of sweetsop can be supported by genetic linkages. In South China, the illness of the sweetsop roots severely restricts cultivation and output. Plant breeding for disease

resistance can greatly benefit from understanding the genetic relationships between the seven samples [14].

The CTAB approach from [15] and [16] was utilized in research [6] to isolate DNA by altering detergents with sodium sulfite, sodium carbonate, and sodium hydrogen phosphate. Because of its potent buffering and detergent characteristics, sodium carbonate has been proven to be helpful in both fresh and dried Annona squamosa leaves. In both fresh and dry leaves, Method [15] produced high-quality and high DNA concentrations more successfully. Additionally, it was discovered that using this approach reduced the price of DNA isolation [15]. This is a result of the leaves' high polysaccharide A. squamosa concentration as well as other secondary metabolites that other researchers have also identified.

In research [8], dATP, dTTP, dGTP, and dCTP were utilized as random primers with RAPD analysis. The CTAB extraction method was used to obtain genomic DNA [17] [8]. There are 4 populations in Central Java and East Java represented by 40 samples of Annona muricata. A total of 58 bands were generated, averaging 14.5 bands per primer and ranged from 9 to 20 bands per primer. Every single one of the chosen RAPD primers showed greater than 90% polymorphism. The DNA sequence determines the polymorphisms that RAPD can identify. The population under study contained many polymorphic loci and showed a high level of genetic variety, with more than 90% polymorphism. Heterozygosity or the level of polymorphism is used to gauge the genetic diversity within a population. Individual gene variation is referred to as genetic variability (polymorphism). Four populations of A. muricata in Central Java and East Java can be distinguished genetically using RAPD analysis [8].

In studies [18] A seed vegetable that grows in both the dry and wet seasons is Annona senegalensis Press., a member of the Annonaceae family. From Senegal through Nigeria and the Central African Republic, this plant is common [19] [18]. Pure DNA must be produced by a successful extraction process for DNA isolation. The cell membrane releases the DNA into the extraction buffer once the cell wall is destroyed in order to liberate other cellular components. Using detergents like sodium dodecyl sulfate (SDS) or cetyl-methyl ammonium bromide is to blame for this (CTAB). Large quantities of RNA, proteins, polysaccharides, tannins, and pigments found in DNA extracts can interfere with DNA extraction and are challenging to separate [20] [18]. By denaturing the extract and precipitating it with phenol or chloroform, proteins can be eliminated. RNase A. can remove RNA normally by extraction treatment, however it is difficult to separate DNA retrieved from inside the cell from impurities that make up the components of the cell. Polysaccharides have been known to be removed using CTAB and NaCl at a concentration of greater than 0.5 M [21] [18]. The literature mentions concentration ranges ranging from 0.7 M [23] [19] to 6 M [24] [18].

One of approximately 110 genera in the Annonaceae family is Annona L. [1] [14]. This plant is a member of the Magnoliid clade of the angiosperm family within the Magnoliales [24] [5]. Due of the angiosperms' varied phylogenetic status, this family is very interesting from both a taxonomic and evolutionary standpoint. On the basis of nuclear and chloroplast sequences, various molecular markers have been utilized to infer evolutionary connections between species families. Studies [25] [1] [26] have used the chloroplast sequence between rbcL, matK, ndhF, trnL, trnT-L, trnL-F, trnS-G, atpB-rbcL, psbA-trnH, ycf1, rpl32-trnL, or ndhFrpl32.

In studies [5] we developed species-specific primers by separating the seven most agronomically interesting Annona species present in Central and South America (Annona cherimola, A. reticulata, A. squamosa, A. muricata, A. macroprophyllata, A. glabra, and A. purpurea) based on the new data sequences. Most popular plant stem-coding genes discriminator (rbcL and matK). Based on certain markers, 42 sequences—21 matK and 21 rbcL—were chosen to create the seven Annonas. The 3′ ends of each primer in the polymorphic area were located using a specific set of primers, and their sequences were compared to those in all Annona species. 42 sequences were found, ranging in size from 470 to 528 bp for rbcL and 752 to 820 bp for matK. Seven different species of Annona may be distinguished by the matK barcode gene's nucleotide sequence, however A. cherimola and A. squamosa cannot be distinguished by rbcL. Because there were just a few samples examined by Locus rbcL, this locus could only distinguish between 5 of the 7 species, whereas Locus matK could distinguish between all of them. The CBOL Plant Working Group has suggested rbcL and matK as key crop barcodes [5].

The initial stage of sexual reproduction is flowering, and in agriculture and horticulture, the interval between vegetative development and flowering is a crucial characteristic [27] [28]. Numerous transcription factor (TF) genes are specifically expressed during the flowering process, including Suppressor Of Overexpression Of Co 1 (SOC1), Flowering Locus T (FT), Constans (CO), Aagamous-Like 24, Flowering Locus D, Flowering Locus E, Flowering Wageningen, and Protodermal Factor 2 [29] [30]. This suggests that a complex gene regulatory network underlies the process of flower development. Additionally, the LEAFY and APETALA1 genes that are located in the floral meristem tissue will be activated by the integrator in flowers to promote flowering [31].

Measurements of endogenous hormones were made in research [30]. IAA, ABA, GA, and cytokinin levels in sugar apple blossoms were evaluated at different phases of flower development to investigate the function of endogenous hormones during floral development. For the evaluation of endogenous hormones, separate samples taken from flowers at various developmental phases (IM, FB, FL1, and FL2) were employed. During the flower growth process, the contents of IAA, GA, and ZRs dropped while the contents of ABA increased. The GA content dropped dramatically from 236.55 during flowering. The MYB108 and MYB24 gene homologs work

downstream of MYB21, TF R2R3-MYB, on the transcription process of this gene that regulates stamen and pollen development, according to transcriptional profiles and have similar roles [32] [33] [30]. Interestingly, srikaya also had gene homologs for MYB24 and R2R3-MYB. There are a lot of bHLH homologs in srikaya (717 were found), and blooming increased the gene's average level of expression. The development of flowers and plants is similarly influenced by NAC [34].

296 bZIPs were found in the study [30] and displayed diverse expression patterns during the srikaya flowering cycle. The ARF and Aux/IAA genes demonstrate the critical role of the ARF-Aux/IAA regulatory pathway in srikaya flower formation. IAA10 displayed the highest expression in srikaya throughout the flower development period. In srikaya, flower growth peaked during the FL2 stage, while IAA3 expression increased during the floral transition. In this study, we discovered the AtMLO4 homolog and the auxin transporter gene (PIN7) (MLO4). It's interesting to see that PIN7 and MLO4 both reduced as the srikaya flower developed (Figure 2).

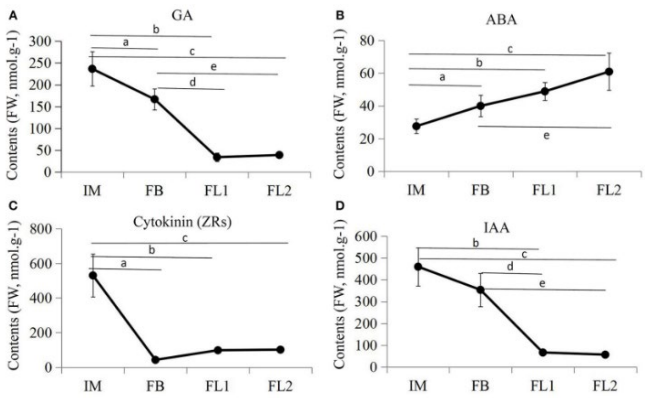

Figure 3. Endogenous hormones measurements in various flowering stages. The differences in endogenous (A) GA contents, (B) ABA contents, (C) ZA contents, and (D) IAA contents during four flower developmental stages in sugar apple were measured. The data were analyzed by three independent repeats, and standard deviations were shown with error bars. Significant differences in expression level between IM and FB were indicated by “a”; Significant differences in expression level between IM and FL1 were indicated by “b”; Significant differences in expression level between IM and FL2 were indicated by “c”; Significant differences in expression level between FB and FL1 were indicated by “d”; Significant differences in expression level between FB and FL2 were indicated by “e” [30].

The proton pump encoded by AVP1 uses the energy released during pyrophosphate's breakdown into two phosphate molecules to acidify vacuoles [35] [30]. According to research [31], sugar apples' expression of AVP1 during flower development may be induced by the need for phosphate. In addition to auxins, GA, ABA, and cytokinins are significant factors in blooming promotion.

4. Conclusion

The AFLP marker reveals the genetic variety of the seven types and their genetic relationships, demonstrating the tremendous genetic diversity of the genus Annona. The findings of this study show that AFLP fingerprint-based distance analysis has high phylogenetic capabilities at the species level. More than 90% polymorphism was present in all of the chosen RAPD primers, indicating a high level of genetic variety. The soursop populations' high genetic variety enables Annona muricata to maintain its evolutionary potential and adapt to changing environmental conditions.

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