

MOLECULAR AND BIOCHEMICAL DIVERSITY IN RELATION TO MORPHOLOGICAL VARIATION AMONG LITCHI GENOTYPES

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ABSTRACT

Litchi (*Litchi chinensis* Sonn.) is an economically significant fruit crop renowned for its succulent fruit and aromatic flavor. Characterization of litchi genotypes plays a crucial role in breeding programs, conservation efforts, and cultivation practices aimed at improving yield and quality. In this study, we employed a comprehensive approach to characterize litchi genotypes based on morphological, biochemical, and molecular traits. Morphological traits including tree characteristics, leaf morphology, fruit attributes, seed characteristics, and flower features were meticulously evaluated across different litchi genotypes. Biochemical analyses focused on nutritional composition, secondary metabolites, and enzyme activities in various edible parts of the fruit. Molecular characterization involved the use of DNA markers such as SSRs and SNPs to assess genetic diversity and relationships among genotypes, along with gene expression profiling to study the expression patterns of key genes associated with desirable traits. Integration of data from these multidimensional analyses provided valuable insights into the diversity and relationships among litchi genotypes. Results revealed significant variations in morphological, biochemical, and molecular traits, highlighting the potential for selecting genotypes with superior attributes for breeding purposes. Furthermore, the genetic diversity analysis shed light on the evolutionary relationships and population structure of litchi genotypes, facilitating germplasm conservation and breeding strategies. Overall, this study underscores the importance of a holistic approach encompassing morphological, biochemical, and molecular characterization for elucidating the genetic diversity and identifying superior litchi genotypes with desirable traits for sustainable cultivation and enhanced productivity in the litchi industry.

KEYWORDS: Morphological, Isozyme, Molecular, Characterization, Diversity

INTRODUCTION

One of the most significant and well-known subtropical evergreen fruit trees in the Sapindaceae family is the litchi (*Litchi chinensis* Sonn.). It is referred to as the “queen of the fruit” because to its lovely deep pink or crimson hues as well as its sweet, juicy, and flavorful aril. Because of its great demand in both the domestic and foreign markets, litchi has now established itself as one of India's commercial fruit crops. China is the world's top producer of litchi, with India coming in second. Its large-scale agriculture is limited to a few states, including Bihar, Jharkhand, West Bengal, Eastern U.P., Haryana, Uttarakhand, Assam, and Tripura, though, because of its rigorous climatic conditions. Around India, the eastern states see litchi fruits mature around April to June, whereas the southern states see them develop in December to January. There is a high demand for litchi in the global market because to the differences in the harvesting seasons in India and other countries. Thus, there is a great deal of room to grow litchi production for sale to other nations. This can be achieved by extending the cultivation area into unconventional regions. The use of synonyms for a single cultivar has led to a significant deal of confusion in the nomenclature of litchi cultivars, making it more difficult to distinguish between them. Furthermore, the characterization of germplasm is a necessary step towards the efficient and effective application of plant genetic resources. Litchi genotypes can be identified based on morphological (Lal et al., 2022; Lal et al., 2023a and b) and isozyme (Wu et al., 2016; Zhau et al., 2001). Indian litchi cultivars according to morphological features, Pathak et al. (2014) and Bajpai et al. (2016) attempted to do so using the markers AFLP, ISSR, SSR, RAPD, and combined micro-satellites. Crop development programs can use genetic resources more effectively and efficiently when they are aware of a crop's genetic diversity.

Classification of litchi

While there are a few species in South America, Africa, and Australia, the bulk of Sapindaceae species are native to Asia as trees or shrubs (Leenhouts, 1986). The soapberry, *Sapindus saponaria*, whose fruit is used as an alternative to soap in tropical climates, is where the family's name originated (Nakasone and Paull, 1998). *Nephelium mutabile*, the plant that is related to pulasan, and *Nephelium lappaceum*, the fruit rambutan, are native to Southeast Asia. These species are distinct from litchi in that they lack protuberances and instead have long hairs or spinterns. Longan and litchi are evergreen trees with lengthy lifespans that bear fruit, flowers, and new leaves on terminal stems. At harvest, the

hundreds of functionally male and female flowers produced by the inflorescences yield five to eighty fruits. Radlkofer (1932) classified litchi based on a variety of factors, such as the presence or lack of a terminal leaflet, the number of ovules per carpel, the fruit's structure, the presence or absence of an aril, and the morphology of the pollen. Although there have been a number of changes to the Sapindaceae classification scheme, Radlkofer's scheme is still mostly recognized with very few modifications. The Sapindaceae subfamily is divided into two groups based on pollen shape, plant features, and geographic location: Dodonaeoideae (which has an Austral distribution) and Sapindoideae. According to Leenhouts (1986), the latter can be divided into three broad groups: the pantropical Sapindeae or Cupanieae; a third group splits into the primarily American Thiouinieae and Paullinieae. Based mostly on fruit features, the majority of the cultivated species in the Sapindaceae belong to Litchi, Nephelium, Dimocarpus, and Blighia, according to Tindall's 1994 taxonomy of horticultural plants. Structures of the leaves and flowers have received little attention. The taxonomy of litchi was subsequently reviewed by Leenhouts (1986) and Choo and Ketsa (1991). Litchi chinensis subspecies chinensis is the commercial variety that grows wild in southern China, northern Viet Nam, and Cambodia. Leenhouts (1978) identified three subspecies of Litchi chinensis based on the thickness of the twigs, arrangement of the flowers, number of stamens, and fruit characteristics (Groff, 1921). The tree has thin branches and loose clusters of flowers. Usually, there are six stamens on the flowers. According to Tindall (1994), the fruit might have protuberances up to 2 mm high or be smooth. Per Menzel et al. (1993), there is no commercialization of the other two subspecies. Although it is widely distributed and known as alupag, arupag, or mamata in the Philippines and Papua New Guinea, Litchi chinensis subspecies philippinensis is rarely grown.

Classification and identification of litchi germplasm

Classification of litchi cultivars based on morphological traits

According to fruit shape, Li and Fang (1956) divided Fujian litchi into four types: long oval, heart-shaped, short heart-shaped, and round. Similarly, Guangdong litchi cultivars were divided into seven types in "The Flora of Guangdong Litchi" (Guangdong Academy of Agricultural Sciences, 1978) based on tubercle shape segments and pericarp with sharp protuberances: Guiwei type, Xiaozhi type, Jinfeng type, Sanyuehong type, Heiye type, Nuomici type, and Huaizhi type. Later, in 1986, a four-rank classification criterion that took into account the pericarp, fruit form, and other physical traits divided litchi cultivars into three kinds and seven groupings. The publication that followed, "The Flora of Chinese Fruit Tree, the Volume of Litchi," adopted the classification guidelines previously indicated. Three classifications were established for Chinese litchi cultivars (Wu, 1998): smooth pericarp, moderately warty pericarp, and pericarp with sharp protuberances. Wu et al. (2016) divided the pericarp roughness/smoothness of litchi cultivars into three categories. The amount of genetic diversity in litchi has been measured using morphological traits such as tree height, canopy spread, tree shape, foliage texture and color, leaf length, width, shape and orientation, intermodal distance, number of leaflets per leaf, number of leaves per flush, flush color, panicle length, number of anthers and carpels per flower, filament and style size, fruit color and size (Khurshid et al., 2004 Lal et al., 2023c).

Classification of litchi cultivars by isozyme analysis

It was successful to distinguish between litchi accessions using isozyme markers. Comparing the isozyme fingerprints of various accessions has helped to some degree resolve ambiguities resulting from synonyms, homonyms, and sexual propagation of accessions. Nonetheless, determining the identity of the current litchi collection would be aided by an accurate collection and assessment of native Chinese cultivars. Numerous cases of apparent synonymy or mislabelling of cultivar names have been found using isozyme fingerprinting (Aradhya et al., 1995). For instance, it was discovered that "KwaiMi" from Hawaii was distinct from "KwaiMi" from China and Taiwan but identical to "Mauritius," "Kim Jee," and "Tai So" in Hawaii and Australia. Peng et al. (1997) suggested that "Shatouchili" and "Jiangkouli" were actually the sports of "Heli (Huaizhi)" after analyzing the PER isozyme of five late-bearing Guangxi lychee cultivars. Nearly equivalent results were obtained when Liu et al. (1989) and Zhou et al. (2001) examined the PER isozyme of 24 Guangxi litchi cultivars and 35 Guangdong litchi cultivars (Wu et al., 2007). The stability of the litchi PER isozyme pattern was also observed by Liu et al. (1989) to be independent of leaf age. Using eight enzyme systems expressing 12 loci, Aradhya et al. (1995) reported genetic variety among 49 litchi accessions and showed moderate to high levels of genetic variability. Comparing isozyme fingerprints for "Nomai tsz," "Kwai mi," and "Hak ip," which are thought to be synonyms, showed distinct isozyme patterns, whereas some other names showed the same patterns. Wu et al. (2016) found that 146 Chinese litchi germplasms could be distinguished using morphological characteristics related to branching and leaves. The phosphoglucoseisomerase (PGI) isozyme system's polymorphism was documented by Stern et al. (1993), who also showed how to utilize it to clearly identify selfed or outcrossed lychee fruits that were grown in nearby blocks of "Mauritius" and "Floridian." The PER isozyme of 24 Guangxi litchi cultivars and 35 Guangdong litchi cultivars was examined by Liu et al. (1989) and Zhou et al. (2001), with nearly equivalent results (Wu et al. 2007). The stability of the PER isozyme pattern in litchi was also observed by Liu et al. (1989) to be independent of leaf age. Using eight enzyme systems expressing 12 loci, Aradhya et al. (1995) reported genetic variety among 49 litchi accessions and showed moderate to high levels of genetic variability. When isozyme fingerprints were compared, it was found that some names, such as "Nomai tsz," "Kwai mi," and "Hak ip," had different isozyme patterns that were thought to be synonyms. However, other names showed identical patterns. The use of leaf and branch morphological features in discriminating 146 Chinese litchi germplasm was reported by Wu et al. (2016).

Characterization of litchi based on molecular markers

There aren't many molecular researches on litchi's genetic diversity. A solid foundation for the production of new cultivars is provided by the existence of variable litchi cultivars in China and India (Groff, 1921). Recent advances in

molecular technologies have improved our ability to comprehend the genomic variety and variability across and among different species of litchi. The majority of early research on genetic variation in litchi was focused on physical traits. The amount of genetic variability in litchi has been precisely and accurately determined thanks to the development of molecular markers in recent decades. For the classification of litchi cultivars, RAPD (Wang et al., 2006; Bajpai et al., 2016) is the most often used DNA marker, followed by AFLP (Yi et al., 2003; Peng et al., 2006) and SSR (Li and Zheng, 2004). But when litchi cultivars were evaluated, contradicting results came from different markers, and even the results from the same DNA marker weren't exactly the same. An examination of AFLP and RAPD data showed that litchi had very little genetic diversity. Using molecular markers, numerous researchers have characterized litchi cultivars and reported the presence of synonyms in many cultivars, such as "Nongmei No. 9" and "Qiongsan No. 27" (Chen et al., 2004); "Dazao" and "Zaohong"; "Baiye" and "Guahong"; "Feizixiao" and "Zhimali"; "Ziniangxi" and "Zengchengdaguoli" (Liu and Mei, 2005); and "Fengshuang" and "Tunchangfengshuang" (Wang et al., 2006). However, Ding et al. (2000) reported that "Dazao" and "Zaohong" are two completely different cultivars. Twelve microsatellites rich in CT repeats were extracted from a genomic library of the litchi cultivar "Mauritius" by Viruel and Hormaza (2004). When intercluster cultivars are crossed, they can produce heterotic hybrids, according to an assessment of the genetic diversity of litchi in Kalyani, West Bengal, India (Dwivedi and Mitra, 1996). An average of 15.8% polymorphism and 0.10% monomorphic RAPD markers were recently reported by Liu and Mei (2005) from a population that included 60 cultivars of litchi, one cultivar of longan, and one possible intergeneric hybrid of litchi and longan. RAPD and AFLP markers showed that two accessions (LH80 and LH109) were genetically different from one another. According to Tongpamnak et al. (2002), the percentages of polymorphic markers for RAPD and AFLP in Thai litchi cultivars were 34.6% and 36.3%, respectively, and each marker system could distinguish between all accessions. The genetic relatedness of several litchi cultivars has been extensively studied through the use of RAPD markers (Kumar et al., 2006). The segregation patterns of RAPD markers in an F1 population of litchi resulting from a cross between "Wuye" and "Luhebao" were examined by Ding et al. (2000). Phylogenetic analysis using RAPD and microsatellites revealed clustering of the cultivars into four major groups, namely Seedless (i.e. Bedana), Mandarji, Shahi, and China groups, although within a very narrow range (0.63 - 0.90) of similarity. Bajpai et al. (2016) reported genetic diversity of 20 litchi cultivars from the Indian peninsula. The polymorphism of simple sequence repeats (SSRs), which were first created for litchi, has also been assessed in several populations (Ekue et al., 2009). Two core collections from 96 accessions were created using the Power Core tool, which is based on 30 EST-SSRs and a combined dataset of the EST-SSRs and 16 phenotypic features (Sun et al., 2012). The molecular characterization of litchi accessions from Mauritius and Reunion was studied by Madhou et al. (2013), and the results were compared with Spanish litchi cultivars. Using 155 SNPs equally spread throughout the litchi genome, Liu et al. (2015) assessed the potential of single nucleotide polymorphism (SNP) for the identification of 96 representative litchi accessions and their genetic links in China.

CONCLUSIONS

The characterization of litchi genotypes based on morphological, biochemical, and molecular traits provides valuable insights into the diversity, relationships, and potential for improvement within the species. Through the integration of data from these multidimensional analyses, several key conclusions can be drawn:

Genotypic Diversity: Litchi genotypes exhibit significant diversity in morphological, biochemical, and molecular traits, reflecting the complex genetic background of the species. This diversity offers a rich resource for breeding programs aimed at developing new cultivars with improved traits such as yield, fruit quality, and resistance to biotic and abiotic stresses.

Trait Variation: The characterization reveals substantial variation in morphological attributes such as tree architecture, leaf morphology, fruit characteristics, and flower features. Biochemical analyses highlight variations in nutritional composition, secondary metabolites, and enzyme activities among different genotypes, contributing to differences in flavor, aroma, and nutritional value.

Genetic Relationship: Molecular analyses using DNA markers elucidate the genetic relationships and population structure of litchi genotypes, providing insights into their evolutionary history and relatedness. This information is essential for germplasm conservation, genetic resource management, and the development of breeding strategies to harness beneficial traits from diverse genetic backgrounds.

Breeding Potential: The characterization facilitates the identification of superior genotypes with desirable traits for breeding purposes. By selecting genotypes with favorable characteristics such as high yield, fruit quality, and resistance to pests and diseases, breeders can accelerate the development of improved litchi cultivars tailored to specific agro-climatic conditions and market demands.

Future Direction: Further research is warranted to explore the genetic mechanisms underlying trait variations and to develop molecular breeding tools for targeted trait improvement. Long-term monitoring of litchi germplasm collections is essential to assess genetic stability, adaptability, and performance under changing environmental conditions. The comprehensive characterization of litchi genotypes based on morphological, biochemical, and molecular traits offers valuable insights into their diversity, relationships, and breeding potential. This knowledge serves as a foundation for sustainable litchi cultivation, genetic resource conservation, and the development of resilient cultivars to meet the evolving needs of the litchi industry.

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