

## Meta Analysis

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# Genetic Diversity and Breeding Applications of Pitaya Germplasm: A Meta-Analysis Approach

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**Abstract** Pitaya (scientific name *Hylocereus*) is a tropical fruit that has become more popular in recent years and is now cultivated in many parts of the world. This study collects and organizes existing research data to analyze the genetic variation and breeding potential of pitaya. The pitaya varieties that are widely grown today show low genetic diversity, but germplasm resources from different regions still present some genetic differences. Molecular marker tools commonly used in research, such as SSR, ISSR, and RAPD, show that there is a moderate level of genetic diversity and some population structure in pitaya. These tools can help select good parent plants, use marker-assisted selection for target traits, and introduce wild species or germplasm from other areas. However, breeding work still faces problems such as limited sharing of germplasm resources and low application of molecular technology. In the future, building a global germplasm database and using multi-omics and smart breeding technologies may be helpful.

**Keywords** Pitaya; Germplasm resources; Genetic diversity; Meta-analysis; Breeding application

## 1 Introduction

Pitaya, also known as cactus fruit or red dragon fruit, is native to tropical and subtropical forests in Latin America. In recent years, it has become one of the fastest growing tropical fruits in the world (Chen et al., 2021). Pitaya is bright in color, special in appearance, sweet in taste, and nutritious. According to data from the Food and Agriculture Organization of the United Nations, the planting area and production of pitaya are increasing rapidly, especially in Vietnam and China. In Vietnam, the planting area of pitaya has exceeded 50 000 hectares, and 1.3 million tons can be harvested each year, mainly sold to China and the European Union. In China, after more than ten years of development, pitaya has become the fifth largest tropical fruit, second only to lychee, longan, banana and mango (Li et al., 2024). At present, more than 30 countries around the world have begun to commercially grow pitaya.

The genetic diversity of pitaya is relatively rich. In many places, the breeding basis of pitaya is still relatively simple. For fruit trees, the more germplasm resources there are, the more disease-resistant, high-yield and high-quality varieties can be combined (Mastretta-Yanes et al., 2018; Swarup et al., 2021). Like bananas and grapes, in the past, only a few similar varieties were used, resulting in great losses when encountering pests and diseases. To cultivate new pitaya varieties with high yield and stability, it is best to start with seed materials with relatively large genetic differences (Joshi et al., 2023).

Although the pitaya industry has developed rapidly, its genetic basis is still weak, and the existing germplasm resources have not been fully utilized (Shah et al., 2023). In Southeast Asia, most white-fleshed pitayas come from a variety called 'Vietnamese White'. Some red-fleshed pitaya are hybrids of red and white varieties, but their genetic backgrounds are also very close. The study used SSR molecular marker analysis to find that many loci have only 1 to 3 alleles, indicating that the diversity is not high (Rifat et al., 2019). In the origin of Latin America, some local pitayas still retain large genetic differences due to long-term cultivation and farmers' selection. However, these local varieties are rarely used in modern breeding. In the traditional gardens of the Mayans in Mexico, the diversity of pitaya is also decreasing, and many farmers only grow a few varieties with high yields but close genetic relationships.

This study explores the genetic diversity of pitaya and its role in breeding, reviews different types of germplasm resources and their genetic structure, and focuses on research conducted using molecular markers. By integrating data from different sources for meta-analysis, the study evaluates the overall genetic diversity and varietal differences of pitaya, hoping to provide a reference for future research and variety improvement work, and help the long-term development of the pitaya industry.

## 2 Overview of pitaya germplasm resources

### 2.1 Germplasm types and classification characteristics

Pitaya is a plant of the Cactaceae family, belonging to the “*Hylocereus*” category. In traditional classification, they are mainly divided into two genera: *Hylocereus* and *Selenicereus* (Li et al., 2024). In the past, due to inconsistent naming, sometimes the same plant had several names. Later, through molecular biological research, the original *Hylocereus* genus was actually included in the *Selenicereus* genus, so now the two genera have been merged into one, collectively known as the *Selenicereus* genus.

After the merger, this genus has a total of about 33 species, of which 4 are the most common cultivated dragon fruit varieties: *Selenicereus undatus* with red skin and white flesh (formerly known as *Hylocereus undatus*, ‘David Boy’ is a variety of it); *S. monacanthus* with red skin and red flesh (formerly known as *H. monacanthus*, and some people call it *H. polyrhizus*); *S. costaricensis* with red skin and purple red flesh; *S. megalanthus* with yellow skin and white flesh (also known as *H. megalanthus*, both names refer to the same species). Among these species, the red skin and white flesh and red skin and red flesh dragon fruit are the most planted and occupy the largest area (Figure 1) (Chen et al., 2021; Li et al., 2024). In appearance, pitaya is a fleshy climbing plant that has no obvious leaves and relies on flat green stem nodes (also called stem segments) for photosynthesis. Different species have different stem segments and fruit appearances, which can be used to distinguish species (Silva et al., 2017).



Figure 1 The phenotype of bud, flower and fruit of four main commercial dragon fruits (Adopted from Li et al., 2024)

Image caption: (a) *S. undatus* has green buds; big, funnel-shaped, and white nocturnal flowers; and oblong fruits with pink peel, and white flesh and covered with green, long, and hard scales. (b) *S. costaricensis* has green buds along with lighter red edges on the outer perianth; big, funnel-shaped and white nocturnal flowers; and ovoid fruit with dark magenta peel and violet-red flesh, and covered with red, soft and short scales. (c) *S. monacanthus* has green buds along with bright red edges on the outer perianth; big funnel-shaped and white nocturnal flowers; and ovoid fruit with crimson peel and flesh covered with red, soft and short scales. (d) *S. megalanthus* has green buds along with dark brown outer perianth; big funnel-shaped (rounder than other species) and white (a little yellow) nocturnal flowers; and ovoid fruit with yellow peel and white (transparent) flesh covered with thorns on tuberculate skin instead of scales (Adopted from Li et al., 2024)

The stem of *S. undatus* is relatively thick, with wavy edges and short triangular thorns. The fruit is oval, with long green scales on the red skin, and white flesh. The edges of the stem segments of *S. costaricensis* are a bit like saw teeth, the fruit is spherical, the skin is dark red, the scales are short and reddish, and the flesh is purple-red. The stem segments of *S. monacanthus* are thinner than the previous two, the distance between the thorns is larger, the fruit is oval, the skin is bright red, the scales are short, and the flesh is red. The stem segments of *S. megalanthus* have small protrusions and short thorns, and the flowers are relatively small and have a yellow tint. Its fruit is yellow and spherical, with obvious protrusions and fine thorns on the outside, and the flesh is white and transparent. Some are new artificially bred varieties, such as Australia's thornless yellow-skinned pitaya 'Aussie Gold'. Although it looks like a yellow-skinned pitaya, it is actually a variation of *S. undatus*.

## 2.2 Global distribution and preservation of pitaya resources

Wild species of pitaya first appeared in tropical areas of Central and South America, such as Mexico, Central America, and some countries in northern South America (Morillo et al., 2022). Now, many countries' planting areas and scientific research institutions have collected and preserved pitaya germplasm resources. In Mexico, national universities and other institutions have established germplasm nurseries, collecting local strains of *H. undatus* from multiple places and some wild *Selenicereus* species (Hernández-Andrade et al., 2019). Colombia has also collected its own yellow-skinned pitaya (*S. megalanthus*), which are mainly used for genetic research and breeding.

Vietnam is the country with the largest export of pitaya. They have established national germplasm nurseries in Long An Province and other places, preserving the country's main varieties, such as the white-fleshed 'Pingshun Longan', and have also introduced red-fleshed varieties from Thailand for hybrid breeding (Xu and Wang, 2024).

In China, germplasm repositories have been established in provinces such as Guangdong, Guangxi, and Hainan. Institutions like South China Agricultural University and Guangxi Academy of Agricultural Sciences have collected many white-, red-, and hybrid-fleshed varieties from Vietnam, Taiwan, and Hainan. Hainan University focuses on conserving the main cultivated types in China and currently maintains close to 100 accessions. Additionally, Ben-Gurion University in Israel has developed a large living germplasm collection with over 200 pitaya accessions introduced from various countries.

## 2.3 Diversity of main agronomic traits of pitaya

In terms of fruit, different varieties have different fruit sizes and shapes. Some fruits weigh only a few dozen grams, while some large ones can exceed 500 grams (Abirami et al., 2021). For example, the yellow-skinned species (*S. megalanthus*) are usually small, generally less than 150 grams. Some red-fleshed varieties, such as 'Okan' cultivated in Israel, can weigh more than 500 grams per fruit. The peel color is red and yellow, and the scales on the peel are long or short, and the color may also be green or red (Li et al. 2024). The flesh color also varies, including white, red, purple-red, and some are pink intermediate colors. Different varieties have different sweetness and taste, and red flesh is usually sweeter than white flesh. For example, among the three types of pitaya grown in the Andaman Islands of India, the red flesh variety has the highest sugar content and the most antioxidants. The nutrients in the pulp, such as vitamin C, anthocyanins, dietary fiber, etc., also vary among different varieties (Shah et al., 2023).

Plant architecture and flowering/fruiting traits also vary. Some cultivars branch more, others less. Flower sizes vary. Certain white-fleshed types are self-incompatible and require cross-pollination. However, newly bred varieties in China, such as 'Guibao' and 'Baiyu Jing', are self-compatible. Spines on cladodes differ too-some varieties are almost spineless, while others have 3~5 long spines. In terms of stress resistance, different pitayas respond differently to pests and diseases and bad weather. Some wild varieties, such as *S. setaceus*, are more drought-tolerant and disease-resistant, but the fruit is smaller. Red-fleshed cultivars are generally more resistant to transportation than white-fleshed ones, but do not perform as well as white-fleshed ones in cold weather (Zhou et al., 2020). Some varieties can activate protective mechanisms, such as "autophagy", after the branches are cold-treated, which can reduce chilling damage (Zhou et al., 2021). Some varieties are more susceptible to blight or anthracnose after picking, but some show better disease resistance (Balendres and Bengoa, 2019; Yao et al., 2022).

### 3 Analysis of Genetic Diversity of Pitaya Germplasm Resources

#### 3.1 Application of molecular marker technology in genetic diversity research

In studying the genetic diversity of pitaya, scientists have used many molecular marker methods. Among them, SSR markers (also called simple sequence repeat markers) are the earliest and most widely used. Shah et al. (2023) first established the SSR marker system for pitaya, and they analyzed the genetic relationship between 46 pitaya materials. They found a total of 183 different allele variations. On average, 2 to 3 different alleles can be detected at each locus. Later, researchers used SSR markers to study pitaya varieties from different places. Japanese researchers developed 16 new pairs of SSR primers to analyze pitaya collected in Okinawa. They used these primers to “fingerprint” different varieties and also saw the diversity between different varieties. Some primers showed great differences between varieties.

In addition to SSR, there are some easier-to-operate methods, such as RAPD and ISSR. These are also used to study the genetic differences of pitaya. In a study in Bangladesh, scientists used 43 RAPD primers to analyze 15 dragon fruit materials. They found that 86.05% of the loci in these materials showed polymorphism, and the Nei's genetic diversity index was 0.327. According to the results of UPGMA clustering, these materials were divided into 3 groups, which were almost consistent with their geographical origins.

In Colombia, researchers used ISSR markers to analyze 76 yellow-skinned pitaya (*S. megalanthus*) samples. The average heterozygosity was 0.34, showing high genetic diversity and clear genetic differentiation (Morillo et al., 2022). In California, USA, AFLP methods were used to perform cluster analysis of several pitaya varieties, mainly to help identify cultivars.

#### 3.2 Integration of genetic diversity assessment based on meta-analysis

Pitaya grown in Asia (mainly *H. undatus* and its red-fleshed type) is generally at a medium or low level in terms of genetic diversity. Let's look at a commonly used indicator - Nei's genetic diversity index ( $H_e$ ). In China and Southeast Asia, the  $H_e$  of pitaya is mostly between 0.1 and 0.3. For example, Rifat et al. (2019) mentioned that the average  $H_e$  of pitaya varieties introduced in China is about 0.25. In contrast, the  $H_e$  of 15 varieties introduced in Bangladesh is 0.327, which is slightly higher.

Pitaya germplasm in Latin America shows greater genetic diversity. In Colombia, the yellow-skinned pitaya population had an  $H_e$  value of 0.34. In Mexico, red-skinned pitaya had lower overall diversity, with  $H_e$  ranging from about 0.07 to 0.13 (depending on the region). However, genetic differences between regions were still clear. Particularly, the populations in central-western and southeastern Mexico belonged to two distinct genetic groups.

Compared to many outcrossing fruit trees like mango and citrus, pitaya has fewer alleles and lower genetic diversity. However, it has higher diversity than completely self-pollinating crops. Introducing wild species such as *S. setaceus* and crossing them with cultivated varieties may bring in new allelic variations. In such hybrid populations,  $H_e$  can exceed 0.20. The results obtained by different molecular markers like SSR and ISSR are also relatively consistent. In these studies, the average percentage of polymorphic loci was usually between 50% and 85% (Morillo et al., 2022).

#### 3.3 Genetic differentiation and population structure characteristics

After AMOVA analysis of yellow-skinned pitaya from three production areas in Colombia, it was found that the genetic differences within the population accounted for 75%, and the differences between the populations accounted for 25%. The genetic differentiation coefficient  $F_{st}$  is about 0.26 (Morillo et al., 2022). In the pitaya grown in Maya family gardens in Mexico, structural analysis divided the populations in 9 regions into two main genetic groups ( $K=2$ ), but the materials from different places in each group were still mixed together (Shah et al., 2023). Their  $F_{st}$  value is about 0.15, indicating that the degree of genetic differentiation is not high.

Asian pitaya varieties have a complicated history of introduction and propagation, so the population structure is also relatively complex. Some studies have used structural analysis methods to group varieties from multiple countries and found that some patterns can be seen according to the color of the flesh, such as white flesh varieties

are often divided into one group and red flesh varieties are divided into another group. This may be because many red-fleshed varieties come from the same breeding material, such as having a common parent, while white-fleshed varieties may be traditional strains. However, there are exceptions, such as some white-fleshed varieties in Taiwan and Vietnam, which are mixed with red-fleshed varieties in SSR cluster analysis. This may be because these white-fleshed varieties are mixed with genes of red-fleshed varieties during breeding.

Some newly bred self-compatible varieties in southern China are mostly selected from the offspring of the varieties introduced by ‘Vietnamese White’ (Nashima et al., 2021). The hybrid offspring bred with the Brazilian wild species *H. setaceus* as the father are very different from the general cultivated varieties in appearance and genetic analysis (Li et al., 2024).

## 4 Application Value of Pitaya Genetic Diversity in Breeding

### 4.1 Parent selection strategy guided by diversity information

In traditional breeding, we often encounter a problem: good varieties are too similar, and the hybrid offspring do not change much. In order to make the offspring more different, breeding experts now use a method called “molecular markers” to look at the genetic differences between different materials. In this way, varieties with relatively far different genes can be selected as parents. Because many commercial varieties are developed from several old varieties, if the parents are too close, the genes of the offspring may be more single. Some studies have used tools like SSR to analyze this. They found that when two varieties fall into different branches on a “clustering tree,” they likely have more genetic differences and are better for crossbreeding.

Some researchers in China have used molecular markers to perform cluster analysis. They found that some red-fleshed strains that can bear fruit on their own are quite different from traditional white-fleshed strains. Such red-fleshed strains can be paired with white-fleshed strains, so that the new generation may have the advantages of “self-flowering” and “high-sugar red meat” (Li et al., 2024).

In Vietnam, local breeders hybridized a local white-fleshed strain with red-fleshed materials introduced from Colombia. In the end, a new red-fleshed strain, Longding No.1, was obtained, which can also bear fruit on its own (Mitra, 2024). It is also not good if the selected parents are too closely related. In Israel, breeders created a large database of over 200 dragon fruit accessions with “molecular fingerprints”. Before pairing the parents, they can check whether they are too closely related.

### 4.2 Integration of excellent trait-related markers and breeding utilization

Through a variety of genetic diversity analyses, researchers have found gene markers related to some important traits of pitaya (such as flesh color, sweetness, and self-compatibility). For example, using the transcriptome comparison method, a glycosyltransferase gene that is only expressed in red-fleshed pitaya was found, and a molecular marker was developed based on this. This marker can help us distinguish between red-fleshed and white-fleshed varieties (Figure 2) (Le Bellec et al., 2006; Mou et al., 2022).

Self-incompatibility of pitaya has always been a breeding problem. In recent years, some studies have found some key genes related to self-compatibility. Wang et al. (2023) compared the expression of styles of compatible and incompatible varieties and found several candidate genes with higher expression levels in incompatible varieties, such as *S-RNase* and *F-box* genes. Molecular markers for S-RNase have been developed, which can be used to determine whether a variety is self-compatible. During breeding, plants with certain mutations that cause self-compatibility (such as loss of function) can be selected through these markers.

Some dragon fruit varieties are also resistant to stem blight, and related RAPD molecular markers have also been found. In a breeding program in Hainan, researchers used high-density SNP molecular markers to establish a genetic map. They used this map to locate QTLs in an F1 population and found several important loci related to fruit sweetness. Then, they used molecular markers to select individual plants with excellent genes for self-pollination and offspring selection. As more functional molecular markers were discovered, such as the HpDof1.7 and 5.4 markers that regulate sugar accumulation, there are also markers related to flowering time.

### 4.3 Pre-breeding approaches to broaden genetic bases

Pre-breeding is a preparation step before formal breeding. It often uses distant hybridization or mutation to introduce useful traits from wild species or underused germplasm into breeding materials (Silva et al., 2017). Many wild relatives of dragon fruit and some underutilized germplasms contain valuable trait genes. For example, the wild species *S. setaceus* is drought- and disease-resistant and grows vigorously, but its fruits are small and it has low self-pollination success. However, its resistance genes can be introduced into cultivated dragon fruit through hybridization.

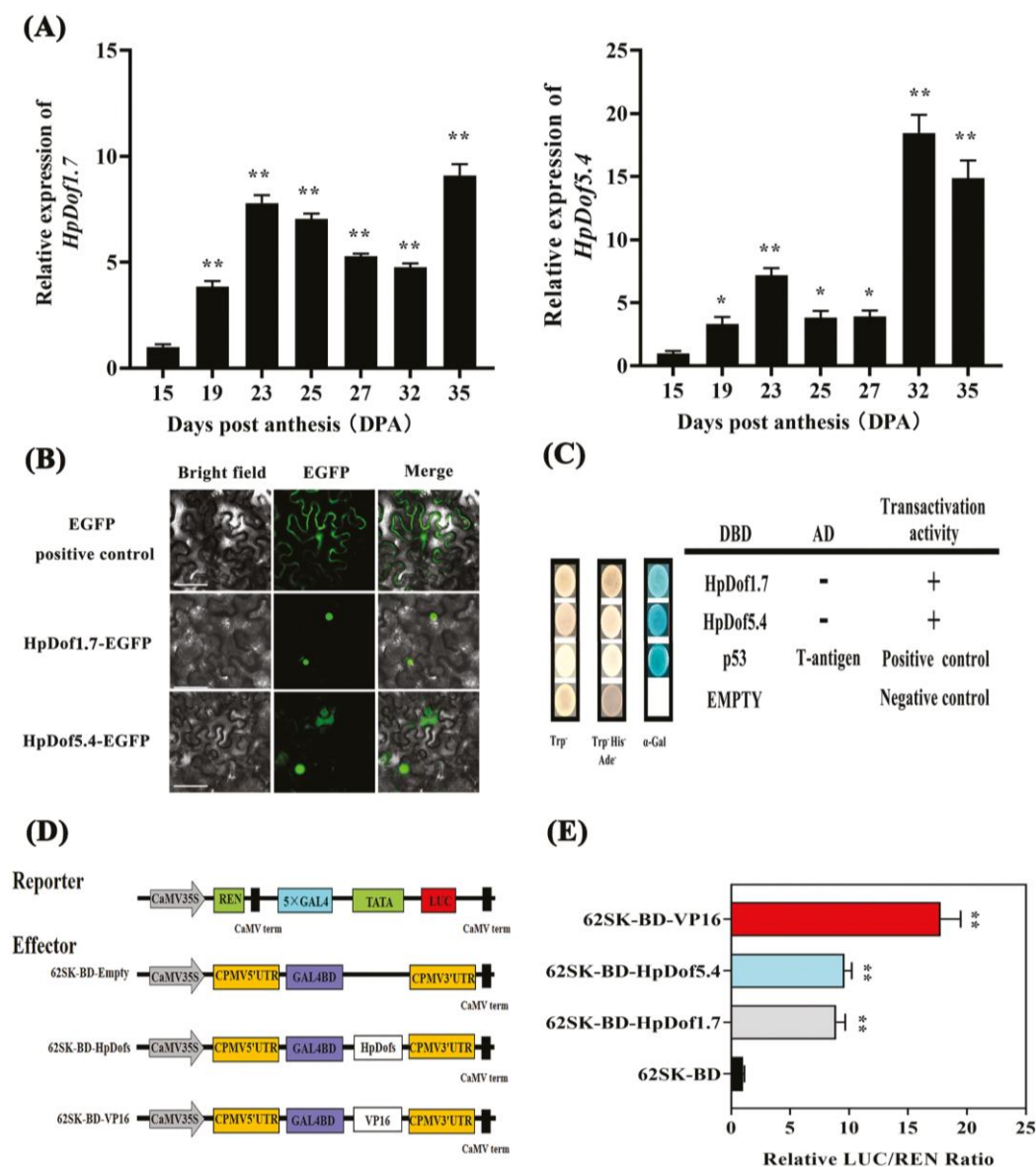


Figure 2 Molecular characterization of HpDof1.7 and HpDof5.4 (Adopted from Mou et al., 2022)

Image caption: (A) Expression patterns of *HpDof1.7* and *HpDof5.4* during pitaya maturation. (B) Subcellular localization assay of *HpDof1.7* and *HpDof5.4*. Bars=50  $\mu$ m. (C) Transcriptional activation assay of *HpDof1.7* and *HpDof5.4* in yeast cells. (D) Schematic representation of reporter and effector vectors constructed for transcriptional activity analysis. (E) Transcriptional activity analysis of *HpDof1.7* and *HpDof5.4* *in vivo*. The data are normalized to a value of 1 for the 62SK-BD group. 62SK-BD-VP16 served as positive control. Each value represents means $\pm$ SE of six biological replicates (\* $P$ <0.05, \*\* $P$ <0.01, compared to 62SK-BD) (Adopted from Mou et al., 2022)

The Tel-Zur team in Israel crossed *H. polyrhizus* with *S. setaceus* and obtained the first-generation (F1) hybrids. They then backcrossed them with cultivated types and developed new varieties with larger fruits and some resistance. In South America, a cactus genus called *Stenocereus* produces fruits with excellent flavor and high

anthocyanin content, which is good for health. Crossing it with *Hylocereus* dragon fruit may improve taste and nutrition. Although intergeneric hybridization between *Selenicereus* and *Stenocereus* is still difficult, some unique hybrid markers have been detected in a few seedlings.

#### 4.4 Potential for whole-genome breeding and precision selection

Breeding has now entered the genomics era. New technologies such as genome-wide selection (GS) and gene editing are already used in fruit tree breeding. Chen et al. (2021) sequenced the genome of a variety called ‘Guangzhou White’ and found it has a genome size of 1.41 Gb, with 27 753 annotated genes. Comparative analysis showed that dragon fruit has undergone triploidization and gene duplication, and some gene families have expanded. These changes are related to traits like fruit color (Zheng et al., 2021).

Researchers also used resequencing to build a high-density genetic map of dragon fruit and tried genome-wide association studies (GWAS). Wu et al. (2021) analyzed 109 F1 individuals and found candidate markers related to fruit length, diameter, sweetness, and other traits. Because dragon fruit takes two to three years from seed to fruit, the breeding cycle is long. GS technology may help shorten this time significantly. In gene editing, several genes affecting fruit redness (such as *CYP76AD1*, *DODA*, and *ADH*) have been found to differ across varieties.

### 5 Case Studies

#### 5.1 Vietnam’s national germplasm bank and breeding with high diversity parents

In Binh Thuan Province in southern Vietnam, there is a national dragon fruit germplasm bank. It holds many types of dragon fruit, including local white-fleshed varieties, red-fleshed ones introduced from Central America, and a few yellow-skinned types (Mitra, 2024). Researchers analyzed these and found many differences in their traits. The traditional white-fleshed variety ‘Binh Thuan’ has high yield and good disease resistance, but the pale flesh color is less attractive. The red-fleshed variety from Colombia has bright color, but its fruit set rate in Vietnam is very low.

To combine the strengths of both, breeders at the Long An Agricultural Research Center did a hybridization experiment. They used ‘Binh Thuan’ as the female parent and crossed it with the red-fleshed line with good color but poor self-pollination. After several rounds of selection, they developed a new red-fleshed variety named ‘Long Định 1’.

‘Long Định 1’ keeps the high yield and adaptability of ‘Binh Thuan’, has red-purple flesh, and is very sweet. Most importantly, it can self-pollinate, solving the problem of poor fruit set in red-fleshed varieties. Genetic tests also confirmed that it inherited key genes from both parents based on SSR marker analysis (<https://www.kingelong.com.vn/blogs/market-news/why-do-red-dragon-fruits-intended-to-be-exported-to-japan-have-to-be-s>). This new variety is now widely grown in Vietnam. It is well accepted because it sets fruit easily and has good quality. Besides this, Vietnam also tried other crosses, like combining Thailand’s self-pollinating red-flesh types with local varieties. One result is the ‘Dragon Pearl’ series, which also performs well.

#### 5.2 Molecular marker-assisted breeding case of Hainan research team in China

The research team of the College of Tropical Agriculture of Hainan University established a germplasm resource garden, which collected the main varieties planted in China and some introduced germplasms. They used molecular markers to analyze the genetic diversity of these materials and screened out core germplasms (Li et al., 2024). The team focused on several important traits and carried out molecular marker-assisted breeding.

White meat varieties usually require artificial pollination, which is not very convenient. To solve this problem, the Hainan team introduced self-fertile varieties from Israel, used them as parents, and hybridized with local high-yield white meat varieties to obtain a batch of offspring. In order to improve the screening efficiency, they used the previously developed S-RNase molecular marker technology to detect whether these seedlings carry self-compatible genes (Wang et al., 2023). In this way, plants that cannot self-fertilize can be eliminated at the seedling stage, leaving single plants that may be self-fertile to be verified in the field. Finally, the new varieties bred, such as ‘Bai Yulong’, can produce stable fruits without artificial pollination. They also used high-density

genetic maps to find QTLs linked to sweetness (Figure 3) (Chen et al., 2021). Based on this, they developed SNP markers to identify seedlings with favorable sweetness genes in F1 populations. After two generations of selection, the average sugar content of new varieties increased by about 2 Brix.

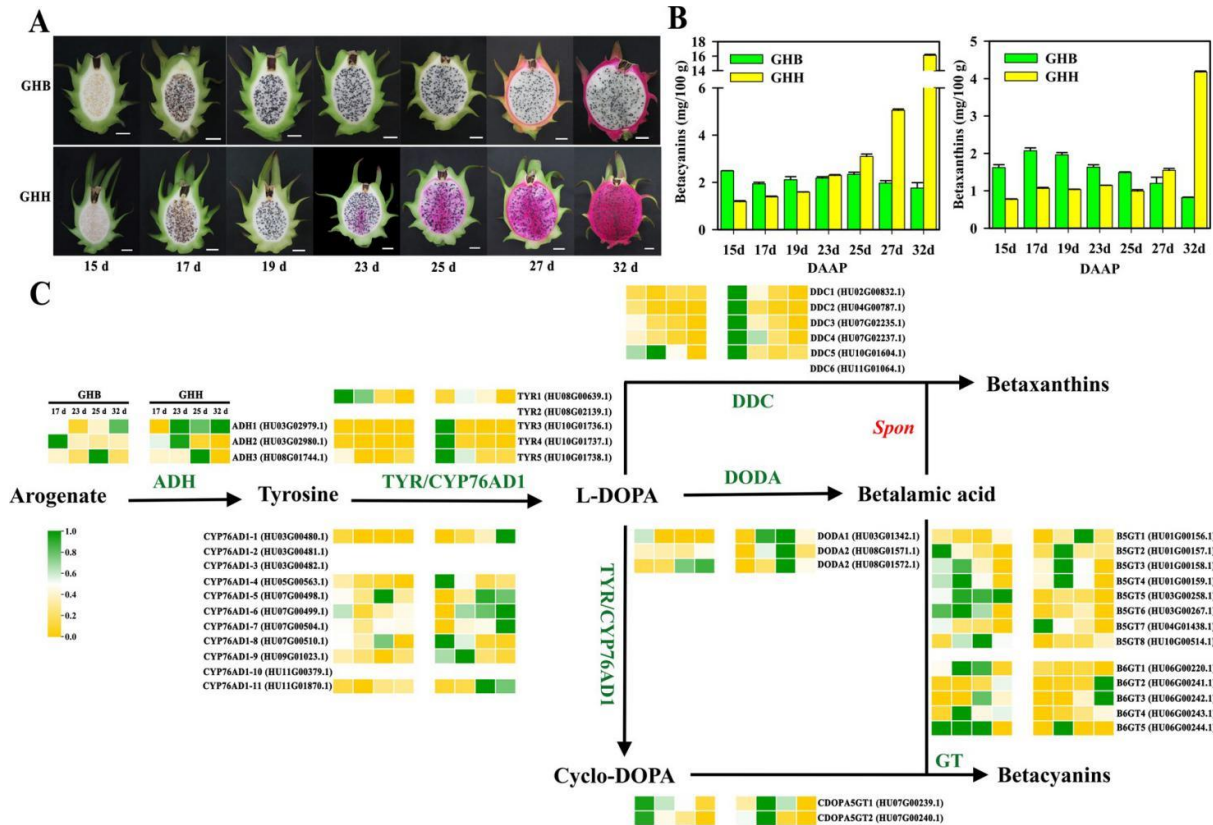


Figure 3 Genes involved in the betalain biosynthesis cascade (Adopted from Chen et al., 2021)

Image caption: A The development of 'Guanhuabai' (GHB) and 'Guanhuahong' (GHH) pitaya pulp. B The betacyanin and betaxanthin contents of 'GHB' and 'GHH' pitaya pulp. C The expression profiles of genes related to betalain biosynthesis according to the RNA-Seq datasets of 'GHB' and 'GHH' pitaya pulp. The gene IDs are in brackets. Spon, spontaneous. Bar = 2 cm (Adopted from Chen et al., 2021)

The team also used SSR fingerprinting technology to confirm whether the hybridization was successful. For example, when doing a "three-line" hybridization experiment, they used SSR molecular markers to confirm whether the hybrid offspring came from the predetermined parents, and promptly eliminated those self-pollinated seedlings or hybrid seedlings. The results show that molecular marker technology is indeed very useful in pitaya breeding (Shah et al., 2023). At present, they have used this method to breed several new varieties, such as 'Zixialong' and 'Xinghui No.1'. These varieties have performed well and have entered the stage of regional trials and promotion of planting.

### 5.3 Hybrid germplasm innovation using wild dragon fruit from Mexico

In Mexico, breeders used local wild dragon fruit species to develop new varieties. Their goal was to introduce wild traits into cultivated types. Since the late 1990s, a team from the National University of Mexico collected local wild relatives of dragon fruit. These included species from *Hylocereus* (also known as *Selenicereus*) and other edible cacti. One special wild type, *Hylocereus purpusii*, has rare orange-yellow flesh and a strong aroma. However, its fruit is small and its vines are hard to manage.

Researchers crossed *H. purpusii* with a cultivated red-flesh variety (Hernández-Andrade et al., 2019). They used embryo rescue to produce the first-generation hybrids. These hybrids had larger fruit than the wild type, with orange-red flesh and good flavor. After more backcrossing and selection, they created lines with fruit sizes close to commercial types but retained the orange color and aroma. One of these is named 'Xiítek', meaning "rainbow"

in the local language. ‘Xfitek’ is now tested in semi-arid regions. It grows well under drought and poor soil conditions, likely thanks to its wild ancestor.

Though Mexico doesn’t have native yellow-skinned types (*S. megalanthus*), researchers imported seeds from Colombia. They grew seedlings and crossed them with local red-skinned varieties. Most hybrids failed to produce fruit or looked unattractive, but a few gave light pink-skinned, white-fleshed fruit. This is considered a breakthrough- a new intermediate type between yellow and red. These hybrids aren’t yet commercial, but they pave the way for future varieties like thornless yellow types or high-sugar white flesh.

#### **5.4 Multi-country collaborative project on genetic diversity (Asia-Pacific and Latin America)**

Countries in the Asia-Pacific and Latin American regions joined a project to assess dragon fruit genetic diversity. The program is organized by the Asian Tropical Fruits Association and the Tropical Fruits Network of the Americas. Participants include China, Vietnam, Thailand, Australia, Mexico, Colombia, and Ecuador.

In the first phase, each country selected 10 representative germplasm materials, including both cultivated and wild types. DNA samples were extracted and sent to a single lab for testing. The lab used 24 pairs of SSR primers provided by Prof. Tel-Zur from Israel, which have high polymorphism. Interestingly, genetic differences did not always match geography. For example, two Asian red-flesh varieties were genetically closer to some Latin American lines than to local white-flesh ones.

In the second phase, the project aims to build an open genetic diversity database. This database will store fingerprint data, trait records, and images. Breeders can search it to find germplasm with traits different from their current breeding materials and reach out for collaboration. For example, a Colombian anthracnose-resistant line was sent to Thailand for testing. Also, a red-flesh self-compatible variety from Australia was sent to Mexico for hybridization.

### **6 Current Problems and Challenges**

#### **6.1 Insufficient resource integration and uneven research areas**

There is no global unified pitaya germplasm information system yet. The germplasm resources preserved by each country are relatively scattered, and there is not much communication between them. Some widely planted varieties are repeatedly preserved in resource banks in multiple countries, while some wild close relatives cannot be found in many countries. This situation of poor resource integration has forced many breeding experts to use local materials.

At present, the main countries doing pitaya research in the world are China, Vietnam, and Israel. However, new countries like the Philippines, India, and Africa that have begun to grow pitaya have relatively weak scientific research levels. For example, some African countries have begun to grow pitaya on a large scale, but they have basically not done much variety improvement and scientific research. When faced with problems such as pests and diseases, there is also a lack of scientific methods to deal with them. While some countries focus on cultivation techniques or nutritional analysis, few have invested in genetic and breeding studies. Although genomic and transgenic technologies are advancing rapidly, studies on pitaya’s stress resistance mechanisms or feasibility of wide hybridization are still limited.

#### **6.2 Barriers to the application of molecular breeding**

In terms of technology, the whole genome selection and gene editing technology of pitaya is just getting started. Compared with major food crops such as rice and wheat, pitaya does not have mature technology in molecular breeding, nor many successful examples. Many breeders are not familiar with these new technologies and need time to learn and master them. Some traditional breeding units are not strong enough in analyzing molecular markers and genomic data (Xu and Wang, 2024). Now if you want to use genomic selection on pitaya, there is still a big problem that there is too little data for training the model, especially the need to accumulate a large amount of phenotypic and genotypic data.

In terms of economy, the cost of molecular breeding is relatively high. Many pitaya breeding companies are small and medium-sized, and do not have much money to build laboratories or buy sequencing equipment. They are more likely to choose the lower-cost traditional hybrid breeding method. From the perspective of approval and promotion, new varieties selected by molecular technology must undergo a series of tests before they can be put on the market, and the whole process takes a long time. Moreover, if a certain nutrient is changed through gene editing, consumers may not accept it, and may even worry about whether it is a genetically modified product and dare not purchase it.

## **7 Outlook and Suggestions**

### **7.1 Build a global pitaya germplasm sharing platform and database**

The current pitaya germplasm resources are scattered and not well organized. In the future, we can consider establishing a global unified platform and database (Li et al., 2024), led by some international organizations, and resource conservation units from various countries will join. This platform can be called the “Global Pitaya Germplasm Information System”. Each germplasm is assigned a unique number, and its name, origin, flesh color, whether it can self-pollinate, where it is stored, and other basic information are recorded.

In addition, the molecular marker data in some scientific research literature should also be integrated to help each germplasm establish a DNA archive. Like the Asia-Pacific and Latin American cooperation projects, a set of commonly used SSR or SNP markers can be selected to classify pitaya in different countries, and the results can be uniformly entered into the system.

The database interface should be simple, multilingual, and regularly updated. The IRRI rice database serves as a good example. This initiative can be led by CGIAR or FAO in cooperation with agricultural departments of major producing countries like China and Vietnam, who could initially provide some datasets. Chinese universities such as Hainan University have already begun efforts in this direction.

### **7.2 Promote the construction of multi-omics joint breeding system**

The current new technology makes pitaya breeding more refined. In the future, multiple omics data can be used to guide breeding. For example, we need to continue to improve the genomic data of pitaya, sequence different varieties, and establish a comprehensive gene information library (Zheng et al., 2021). We can also compare the fruit development process of different varieties to find out which genes affect characteristics such as sweetness, aroma and color, and then use metabolomics data to verify (Mou et al., 2022).

There is currently a pitaya omics database PGMD, which contains genome, transcriptome and metabolome data (Chen et al., 2022). These data can be used for association analysis between genotypes and traits, so as to establish a predictive model for selecting offspring. Through genomic information, it is also possible to determine in advance which two parents may produce good offspring after hybridization, as well as possible trait performance.

In order to promote this type of breeding method, talents from different disciplines need to work together. Traditional breeding experts should form a team with bioinformaticians and molecular biologists. In addition, breeding units in various regions should also build their own laboratories and introduce some new sequencing and analysis equipment.

### **7.3 Building a smart dragon fruit breeding path**

Smart breeding is to use modern technology to improve every breeding link. Take dragon fruit as an example. You can build a data platform to collect information such as germplasm resources, test results, and climate. This data can be used for analysis, such as using machine learning to find useful patterns. During hybridization, artificial intelligence can recommend suitable parent combinations based on previous data. During the breeding process, drones can also be used to take photos and quickly measure traits such as fruit size and stem length.

Smart greenhouses and precision planting can also help speed up breeding. By planting multiple generations of dragon fruit in a controlled environment, the breeding cycle can be shortened. At the same time, sensors can monitor the growth of plants in real time and synchronize this data to the breeding database. New technologies

such as gene editing can also be used in this system. For example, a platform is built to allow the model to recommend the gene position to be modified based on the target trait, and breeders can use this information to make more precise genetic improvements.

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## Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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