

Advances in Genomic Research and Genetic Improvement of Cactaceae Plants

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Abstract Cactaceae plants have garnered widespread attention due to their unique Crassulacean Acid Metabolism (CAM) pathway and their adaptability to arid environments. This study explores the advancements in genomic research and genetic improvement of Cactaceae, focusing on the integration of traditional breeding and modern molecular breeding techniques. Traditional methods such as selective breeding and hybrid breeding have achieved significant progress in enhancing drought resistance and fruit quality but face challenges such as long breeding cycles and high genetic complexity. Modern techniques, including molecular markers, functional genomics, and gene editing, provide new pathways for more efficient genetic improvement. The study also highlights that the construction of high-density genetic maps and the analysis of gene regulatory networks have significantly facilitated the precise localization of genes associated with target traits. This study underscores that integrating traditional and modern technologies can accelerate the genetic improvement of Cactaceae, supporting sustainable agriculture and ecosystem stability.

Keywords Cactaceae; Genomic research; Genetic improvement; Breeding techniques; Molecular markers; Functional genomics

1 Introduction

The Cactaceae family is a significant branch of succulent plants, originating approximately 35 million years ago during the Eocene-Oligocene period (Arakaki et al., 2011). Its unique Crassulacean Acid Metabolism (CAM) pathway enables Cactaceae plants to absorb carbon dioxide at night and close stomata during the day to reduce water loss, thereby improving water use efficiency and adapting to arid environments (Guerrero et al., 2018). The Cactaceae family is the largest family of succulent plants, comprising 140 genera and over 2 000 species. It is primarily distributed in the Americas, with the highest species richness found in Mexico (Horibe, 2021). Some members of the Cactaceae family can also be found in parts of Asia and Africa.

In recent years, due to their ornamental value, nutritional properties, and medicinal potential, Cactaceae plants have gained widespread attention (Abouseadaa et al., 2020). For instance, pitaya (or Pitahaya) is highly valued for being rich in plant proteins, anthocyanins, vitamins, and dietary fiber; the tender stems of Nopal have been used for reducing blood glucose and cholesterol (Angulo-Bejarano et al., 2019; Holanda et al., 2021). In addition, these plants have been utilized in traditional medicine to treat indigestion, skin infections, and other ailments. However, threats such as habitat destruction, illegal collection, and climate change have put approximately 31% of Cactaceae species at risk of extinction (Gonzaga et al., 2018).

To address the challenges posed by environmental stress and enhance the value of Cactaceae in various applications, genetic improvement is crucial. Traditional breeding methods, such as selective breeding and hybrid breeding, have been used to enhance certain traits, such as fruit yield, drought tolerance, and disease resistance (Paliwal et al., 2021). However, the inherent limitations of these methods necessitate the integration of modern breeding technologies. Advances in molecular genetics offer new opportunities for accelerating breeding efforts and achieving more precise genetic improvement (Cattivelli et al., 2008; Wan et al., 2021). Modern gene-targeting marker technologies, such as Start Codon Targeted (SCoT) polymorphism and Conserved DNA-Derived Polymorphism (CDDP), have proven effective in assessing genetic relationships and variations among species

(Abouseadaa et al., 2020). These technologies not only contribute to the taxonomy and characterization of cactus species but also facilitate the identification of target traits in breeding programs, ultimately cultivating species with enhanced stress resistance, nutritional value, and medicinal properties.

This study systematically analyzes recent advancements in genetic improvement technologies for Cactaceae plants and evaluates their potential in addressing the current challenges faced by these plants. The study explores and understands traditional and modern breeding methods, seeking to identify key genetic markers and traits that can be utilized in breeding programs. Case studies are also integrated to determine future directions that may further advance the genetic improvement of Cactaceae. This study is expected to provide a comprehensive understanding of the genetic patterns of Cactaceae plants, offering strong theoretical support and practical guidance for their future improvement and conservation.

2 Genetic Diversity of Cactaceae

2.1 Current status of genetic diversity

The Cactaceae family, comprising over 140 genera and 2 000 species, exhibits significant genetic variation. Studies have shown that species within this family, such as those in the genera *Mammillaria* and *Notocactus*, display considerable genetic diversity, which is evident through the use of gene-targeting marker techniques like Start Codon Targeted (SCoT) Polymorphism and Conserved DNA-Derived Polymorphism (CDDP) (Abouseadaa et al., 2020). Additionally, the pitaya (*Hylocereus undatus*) genome has revealed extensive genetic variation, including whole-genome triplication and duplication events, which contribute to its genetic diversity (Chen et al., 2021).

Various molecular techniques are employed to assess genetic diversity in Cactaceae. Randomly Amplified Polymorphic DNA (RAPD) markers are commonly used to identify and characterize genetic variations (Ferreira et al., 2023). Other advanced methods include RAD-Seq single-nucleotide polymorphism (SNP) data, which have been utilized to investigate hybridization and genetic architecture in *Melocactus* species (Khan et al., 2019). These techniques provide detailed insights into the genetic relationships and diversity within and between species.

2.2 Factors influencing genetic diversity

Environmental factors play a crucial role in shaping the genetic diversity of Cactaceae. For instance, polyploidy, a significant mechanism in cactus speciation, is influenced by geographical and bioclimatic variables such as temperature and precipitation. Studies have shown that chromosome numbers in Cactaceae are related to temperature variables, while DNA contents are mostly associated with precipitation (Peñas et al., 2023). These findings suggest that environmental conditions drive genetic adaptations in cacti.

Human activities, including habitat destruction and agricultural practices, significantly impact the genetic diversity of Cactaceae. The introduction of new cultivars through breeding programs, often involving somaclonal variation, can lead to genetic changes in crops. While somaclonal variation can generate beneficial traits, it also poses risks of reducing genetic diversity if not managed properly (Ferreira et al., 2023). Additionally, hybridization facilitated by human activities can affect the genetic integrity of species, as seen in *Melocactus*, where hybridization occurs frequently but with low introgression (Khan et al., 2019).

2.3 Importance of genetic diversity conservation

Genetic diversity is vital for the adaptation and resilience of Cactaceae species. It enables populations to withstand environmental changes and resist diseases and pests. For example, the genetic diversity generated through somaclonal variation has supported the development of new genotypes resistant to various stresses (Ferreira et al., 2023). Similarly, the genetic variation observed in pitaya contributes to its adaptability and potential for genetic improvement (Chen et al., 2021).

Conservation strategies are essential to protect the genetic resources of Cactaceae. These strategies include in situ conservation, which involves protecting natural habitats, and ex situ conservation, such as seed banks and botanical gardens. Additionally, molecular techniques can aid in the identification and preservation of genetic

diversity. For instance, gene-targeting marker techniques can classify and characterize species, ensuring the conservation of their genetic integrity (Abouseadaa et al., 2020). Effective conservation strategies must also address the impacts of human activities and promote sustainable practices to maintain genetic diversity.

3 Advances in Genomic Studies of Cactaceae

3.1 Sequencing and assembly of organelle genomes in Cactaceae

Significant progress has been made in the study of organelle genomes in Cactaceae, particularly in chloroplast and mitochondrial genomes. For instance, Oulo et al. (2020) were the first to sequence the chloroplast genome of *Rhipsalis baccifera*, providing valuable data on the phylogenetic relationships within Cactaceae. Yu et al. (2023) investigated the chloroplast genomes of 35 genera in the subfamily Cactoideae, deepening the understanding of evolutionary relationships. Similarly, Köhler et al. (2023) assembled the chloroplast genomes of 43 species of the tribe *Opuntieae*, one of the most diverse and important lineages of the Cactaceae. Their study revealed the dynamic nature of plastome evolution across closely related lineages. The results showed significant variation in plastome length, structure, and content, particularly with respect to the contraction and expansion of the inverted repeat (IR) region, as well as the pseudogenization or loss of certain genes (Figure 1). This genomic variation is closely related to plant evolution and species identification. Studies by Liu et al. (2021) and Qin et al. (2022) on the chloroplast genomes of pitaya revealed a conserved quadripartite structure but highlighted genome size variations among species, uncovering genetic diversity. These studies also identified phenomena such as gene loss, rearrangement, and pseudogene formation, providing new insights into the evolutionary mechanisms and adaptive strategies of pitaya.

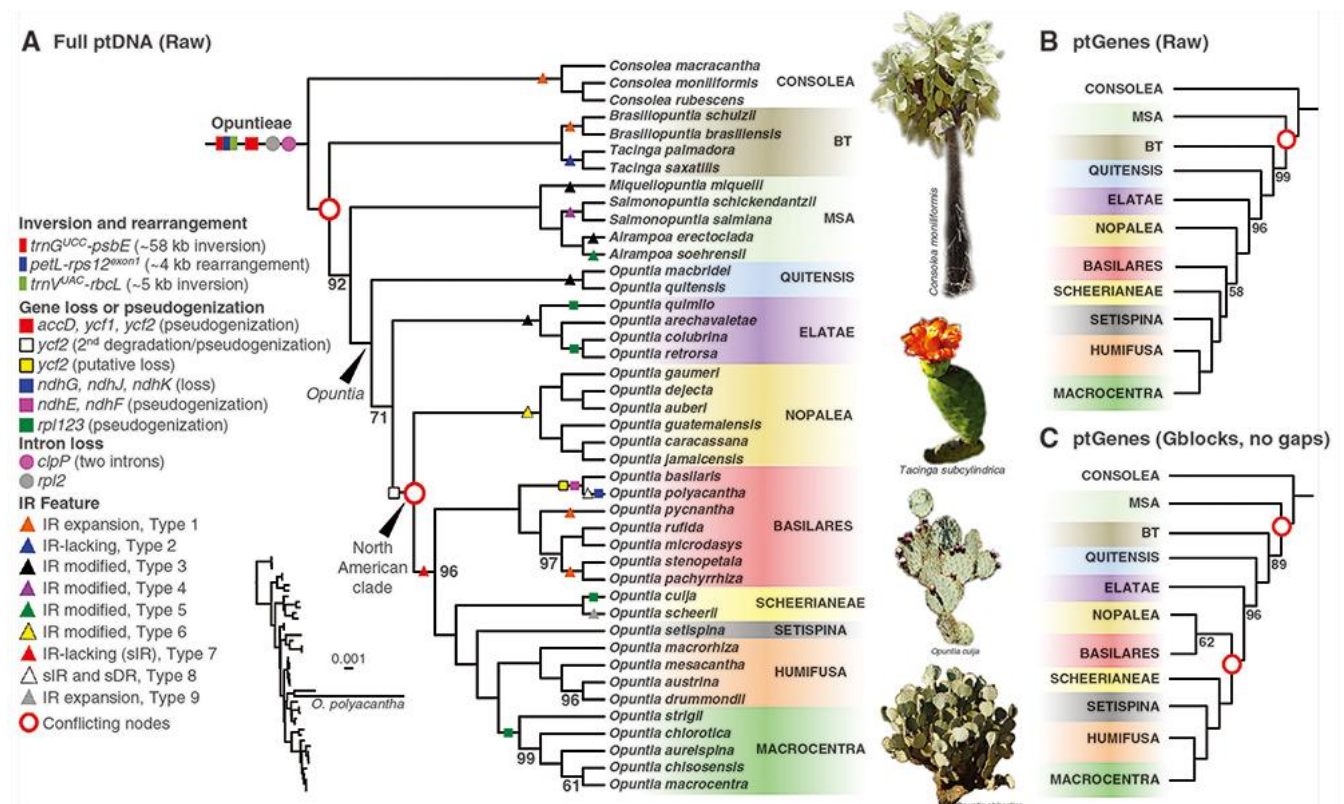


Figure 1 Phylogenetic inference of tribe *Opuntieae* based on plastome sequences with the maximum likelihood (ML) criterion, with the major plastome evolution features mapped on the tree (Adopted from Köhler et al., 2023)

Image caption: (A) Inference based on the full plastome sequences (genes and intergenic regions, raw alignment). (B) Inference based on chloroplast genes (raw alignment). (C) Inference based on plastome genes trimmed with Gblocks (no gaps allowed). Phylogenetic nodes have full bootstrap support (100), except when depicted; incongruent nodes across different datasets are highlighted by red circles (Adopted from Köhler et al., 2023)

The assembly of mitochondrial genomes in Cactaceae has unveiled key aspects of their genetic structure and function. For example, the mitochondrial genome of *Pereskia aculeata* spans 515 187 bp and contains 52 unique genes, including protein-coding genes, tRNA genes, and rRNA genes (Zhang et al., 2023). The genome features a significant number of repetitive elements, which play roles in genome recombination. Lu et al. (2023) assembled the mitochondrial genome of *Selenicereus monacanthus*, revealing a total length of 2 290 019 bp encoding 59 genes. Evolutionary analyses indicated that its mitochondrial genome underwent multiple recombination events, leading to the loss of at least nine protein-coding genes. These studies enhance our understanding of the genetic foundation of pitaya and provide new perspectives on mitochondrial genome evolution in Cactaceae.

3.2 Sequencing and assembly of nuclear genomes in Cactaceae

Compared with other economically important crops, genomic studies of Cactaceae plants are relatively underdeveloped, with only a few nuclear genomes sequenced and assembled. These include two pitaya varieties (Guanhuabai and David Bowie), four North American columnar cacti (*Pachycereus pringlei*, *Lophocereus schottii*, *Stenocereus thurberi*, and *Pereskia humboldtii*), one South American cactus (*Cereus fernambucensis*), and the iconic giant saguaro cactus (*Carnegiea gigantea*) (Sanderson et al., 2020; Amaral et al., 2021; Chen et al., 2021; Tamayo-Ordoñez et al., 2023). These genome assemblies have significantly contributed to understanding cactus evolutionary history, adaptation mechanisms, and the identification of key genes involved in important metabolic pathways.

Despite these advancements, challenges remain due to high heterozygosity, large amounts of repetitive sequences, and difficulties in DNA or RNA extraction. For instance, the genome of *Hylocereus undatus* is approximately 1.41 Gb and contains numerous repetitive sequences, complicating high-quality assembly (Chen et al., 2021). However, improvements in sequencing technologies and bioinformatics tools are expected to advance genomic studies in Cactaceae. Methods such as PacBio-SMRT, Illumina HiSeq paired-end sequencing, 10× Genomics, and Hi-C have enabled chromosome-level genome assemblies of pitaya, achieving an N50 scaffold length of ~127.15 Mb. These advancements provide critical support for understanding the nuclear genomes of Cactaceae.

3.3 Advances in transcriptomic studies of Cactaceae

Transcriptomic studies have also made remarkable progress in understanding gene expression and the regulation of vital biological processes in Cactaceae. RNA sequencing (RNA-Seq) has proven instrumental in analyzing gene expression, identifying novel transcripts, alternative splicing, and developing molecular markers. Key transcriptomic studies have focused on various aspects of Cactaceae physiology, from root development to stress responses. Hua et al. (2016) conducted the first transcriptomic study on pitaya, marking a new chapter in functional genomics research of this fruit.

In the case of the saguaro cactus (*Pachycereus pringlei*), transcriptomic sequencing identified genes related to root development, providing insights into growth regulation (Rodriguez-Alonso et al., 2018). Additionally, transcriptomic data from pitaya have been used to elucidate the molecular mechanisms underlying its growth, stress responses, and development (Chen et al., 2021). These studies enhance the understanding of cactus biology and support breeding programs aimed at developing superior cultivars. With continuous advancements in sequencing technologies, transcriptomic studies in Cactaceae are expected to expand significantly. This will ultimately enrich our understanding of the genomes, gene functions, and evolutionary processes in Cactaceae, thereby promoting their genetic improvement.

4 Traditional Breeding Methods for Cactaceae Plants

4.1 Hybrid breeding

Hybrid breeding is one of the primary traditional breeding methods for Cactaceae plants. By combining desirable traits from different varieties or species, offspring with multiple favorable characteristics can be obtained (Oliveira et al., 2020; Tel-Zur, 2022). For example, in the breeding of dragon fruit (*Hylocereus* spp.), hybrid breeding is often used to improve fruit size, color, and flavor (Ding, 2021). Through artificial pollination between different types of dragon fruit, breeders can combine the high disease resistance of red-fleshed dragon fruit with the high

yield of white-fleshed dragon fruit to develop new varieties with superior comprehensive traits.

In yellow pitaya (*Selenicereus megalanthus*), hybrid breeding has also yielded positive results. Through hybridization, breeders have enhanced its adaptability and fruit quality, such as improving sweetness and appearance to better meet market demand (Morillo-Coronado et al., 2021). Although hybrid breeding as a traditional method requires substantial time and labor, it plays a significant role in maintaining the diversity of Cactaceae plants and improving their traits.

4.2 Selection breeding

Selection breeding is a method of improving varieties by selecting naturally occurring variants and reproducing them over multiple generations. In the breeding of cactus (*Opuntia* spp.), selection breeding is widely used to select individuals with higher cold resistance or better drought tolerance (De Jesus et al., 2023). As an important plant in desert areas, cactus has enhanced its survival ability in harsh environments and increased its nutritional value as a forage plant through selection breeding. After multiple generations of selection, the cold and drought tolerance of cactus has significantly improved, adapting to more diverse cultivation conditions.

As an ornamental Cactaceae plant, queen of the night (*Epiphyllum oxypetalum*) is primarily bred for improving flower shape and color (Sampaio and De Almeida, 2021). Breeders select individuals with higher flowering frequency and more attractive flower colors to enhance the plant's appeal in the horticultural market. This selection breeding method effectively improves the traits of ornamental plants to meet the aesthetic needs of gardening enthusiasts.

4.3 Limitations of traditional methods

Although hybrid and selection breeding have played important roles in the improvement of Cactaceae plants, traditional methods also have certain limitations. On the one hand, breeding cycles are long and unpredictable. Cactaceae plants grow relatively slowly, and the breeding process may take several years or even decades, making it challenging for traditional breeding methods to respond quickly to market changes and pest threats (Yusop et al., 2020). For example, the hybrid breeding of dragon fruit requires several generations of backcrossing and selection to stabilize genetic traits, limiting the speed of new variety development (Tel-Zur, 2022).

Furthermore, traditional methods rely on natural variation and cannot precisely control target traits. In selection breeding, breeders often need to screen a large number of individuals to find variants that meet the target traits. This randomness not only consumes human and material resources but also makes it difficult to ensure that all desirable traits are stably passed on. Additionally, hybrid breeding may result in hybrid disadvantage, where some hybrid offspring perform worse than the parents, which is especially evident in breeding yellow pitaya and other species (Morillo-Coronado et al., 2021; Tel-Zur, 2022).

5 Biotechnological Advances in Cactaceae Breeding

5.1 Construction of high-density genetic maps based on molecular markers

High-density genetic maps are indispensable tools in modern plant breeding, enabling precise localization of genes associated with target traits and assisting breeders in making efficient selections. In Cactaceae plants, genome-wide resequencing (WGrS) technology has facilitated the development of numerous single nucleotide polymorphism (SNP) markers and the construction of high-resolution genetic maps. For example, in *Hylocereus undatus*, high-throughput sequencing identified 6,434 polymorphic SNP markers distributed across 11 linkage groups, corresponding to the chromosome number of dragon fruit. The total map length reached 14 128.7 cM, with an average marker interval of 2.2 cM, providing a reference for gene mapping of traits such as disease resistance, drought tolerance, and fruit quality (Wu et al., 2021). Another study employed a hybrid assembly method combining long-read sequencing (PacBio) with short-read data to reassemble the genome of *Carnegiea gigantea*, significantly improving genome contiguity and accuracy (Copetti et al., 2023). This advancement lays the foundation for further genetic research on Cactaceae plants.

Additionally, the integration of linkage and association analyses allows breeders to identify quantitative trait loci

(QTLs) with higher precision, accelerating the breeding process. These high-density genetic maps facilitate marker-assisted selection (MAS) in Cactaceae breeding, improving efficiency and accuracy (Chen et al., 2021).

5.2 Genetic diversity analysis and hybrid breeding using molecular markers

Molecular marker technologies, especially inter-simple sequence repeat (ISSR) markers, play a critical role in analyzing the genetic diversity of Cactaceae plants. Studies have shown that out of 16 ISSR primers screened, 14 primers produced 178 reproducible amplification bands with polymorphism percentages ranging from 20% to 92.8%, and the polymorphic information content (PIC) values reached as high as 0.91 (Abirami et al., 2021; Nashima et al., 2021). These markers effectively distinguish the three major cultivars of dragon fruit, clustering them into different genetic groups based on flesh color and geographic origin. For instance, DGF1 and DGF3 showed 52% genetic similarity, while DGF2 and DGF4 exhibited a higher genetic similarity of 76%. These findings elucidate genetic relationships among cultivars, providing scientific insights for the classification, identification, and breeding of dragon fruit germplasm resources.

Moreover, combining morphological and molecular marker analyses enhances the reliability of germplasm studies. ISSR marker analysis has revealed significant differences among dragon fruit cultivars in traits such as fruit morphology, peel, and flesh color, which are highly consistent with molecular data (Abirami et al., 2021). This integrative approach effectively supports the development and utilization of germplasm resources, offering valuable references for the conservation and genetic improvement of Cactaceae plants such as dragon fruit.

5.3 Trait improvement through gene regulation and functional genomics

Functional genomics, which deciphers transcriptome data and gene regulatory networks, provides essential support for trait improvement in Cactaceae plants. In the study of pitaya (*Selenicereus* spp.), the transcription factor HmoWRKY40 was identified as a key regulator of betacyanin synthesis, activating the biosynthesis of secondary metabolites by binding to the W-box region of the CYP76AD1 gene promoter (Zhang et al., 2021). Silencing of *HmoWRKY40* resulted in a significant reduction in betacyanin content, further demonstrating the importance of this transcription factor in betalain biosynthesis (Figure 2). Additionally, multi-omics analyses have shown that genes related to stress resistance, such as those encoding antioxidant enzymes (POD and APX), and signal transduction genes are significantly upregulated under stress conditions. These genes, along with transcription factors such as WRKY and MYB, participate in stress responses and metabolic regulation.

Through functional enrichment analyses and weighted gene co-expression network analysis (WGCNA), researchers have identified key gene modules involved in antioxidant defense, signal transduction, and metabolic regulation. Functional genomic studies have positioned transcription factor networks as a significant research direction for dragon fruit breeding. For example, the silencing of HmoWRKY40 using virus-induced gene silencing (VIGS) confirmed its role in enhancing fruit coloration and betacyanin content, demonstrating that its overexpression significantly improves these traits (Khokhar et al., 2024). In the future, combining functional genomics with molecular breeding technologies will further enhance the quality and stress resistance of dragon fruit while providing potential applications for the genetic improvement of other crops.

6 Case Studies

6.1 Study on root structure and genetic mechanism of *Pachycereus pringlei*

Cactaceae species often thrive in arid or semi-arid environments, and their root structure and growth patterns are crucial for adapting to extreme conditions (Martino et al., 2018). *Pachycereus pringlei*, as a representative species, exhibits determinate growth of its primary root due to the exhaustion of root apical meristem (RAM). Rodriguez-Alonso et al. (2018) constructed the transcriptome of *P. pringlei* root apex at three developmental stages through RNA sequencing, revealing specific gene expression patterns and their relationship to RAM maintenance. The results indicated that the RAM of *P. pringlei*'s primary root becomes exhausted at specific developmental stages, leading to determinate growth (Figure 3). This growth pattern is possibly an adaptive strategy to extreme arid environments, enhancing water absorption efficiency and promoting lateral root development.

Additionally, by comparing the transcriptomes of root zones in Arabidopsis, it was found that after RAM exhaustion, *P. pringlei* root tip cells retain some functionality, similar to the differentiated cells in Arabidopsis. The study also identified several lineage-specific transcripts potentially related to RAM exhaustion, which may play a role in root development regulation and warrant further exploration. This study provides new insights into the molecular mechanisms of Cactaceae species' adaptation to arid environments and serves as a reference for understanding the evolution and genetic regulation of root system development in plants.

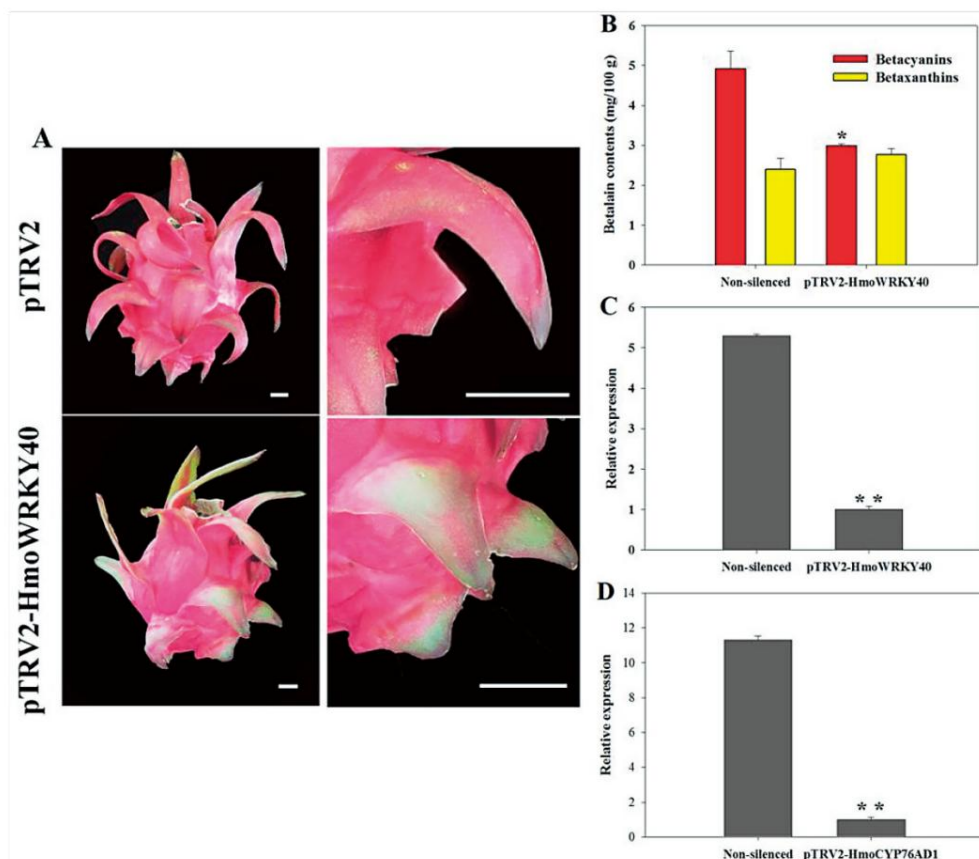


Figure 2 Silencing of *HmoWRKY40* inhibits betalain production (Adopted from Zhang et al., 2021)

Image caption: (A) Virus-induced gene silencing of *HmoWRKY40* in red scales. Bars = 2 cm, (B) Betalain contents in pitaya scales after virus-induced silencing of *HmoWRKY40* (* indicates $p < 0.05$). Three independent experiments were conducted ($n = 3$). The error bars indicate one standard error, (C) RT-qPCR analyses of *HmoWRKY40* in virus-induced gene silencing (VIGS) treatment scales. The expression level of pTRV2-*HmoWRKY40* was used as the calibrator (set as 1). The data represent mean values from three biological replicates (\pm S.D.). ** indicates significant differences at p value < 0.01 using a two-tailed t-test and (D) RT-qPCR analyses of *HmoCYP76AD1* in VIGS treatment scales. The expression level of pTRV2-*HmoCYP76AD1* was used as the calibrator (set as 1). The data represent mean values from three biological replicates (\pm S.D.). ** indicates significant differences at p value < 0.01 using a two-tailed t-test (Adopted from Zhang et al., 2021)

6.2 Pitaya genome analysis and betalain regulation study

Pitaya, an important tropical fruit tree of the Cactaceae family, has gained significant attention for its unique nutritional value and abundant betalains. However, the lack of a reference genome has greatly limited its breeding and evolutionary studies. Chen et al. (2021) constructed a chromosome-scale genome, revealing the evolutionary trajectory of the pitaya genome and the regulatory mechanisms of betalain biosynthesis. The study utilized multiple sequencing methods, including PacBio, Illumina, 10 \times Genomics, and Hi-C, successfully assembling a 1.41 Gb genome and annotating 27 753 protein-coding genes. Comparative genomic analysis revealed that pitaya has undergone whole-genome triplication (WGT) and recent whole-genome duplication (WGD) events, which are major drivers of its genome expansion.

Furthermore, the study used transcriptome and weighted gene co-expression network analysis (WGCNA) to reveal regulatory networks between genes and transcription factors involved in betalain biosynthesis. The results showed that betalains (betacyanin and betaxanthin) gradually increased in the pulp of 'GHH' and were significantly higher compared to 'GHB'. Betalain biosynthesis-related genes such as *ADH*, *CYP76AD1*, and *DODA* were highly expressed during the maturation of GHH pulp, indicating that their high expression is directly related to betalain accumulation, highlighting the crucial role of gene expression in fruit color changes (Figure 4). This research provides valuable genomic resources for the molecular breeding of pitaya and offers new insights into understanding pitaya genome evolution and betalain regulation.

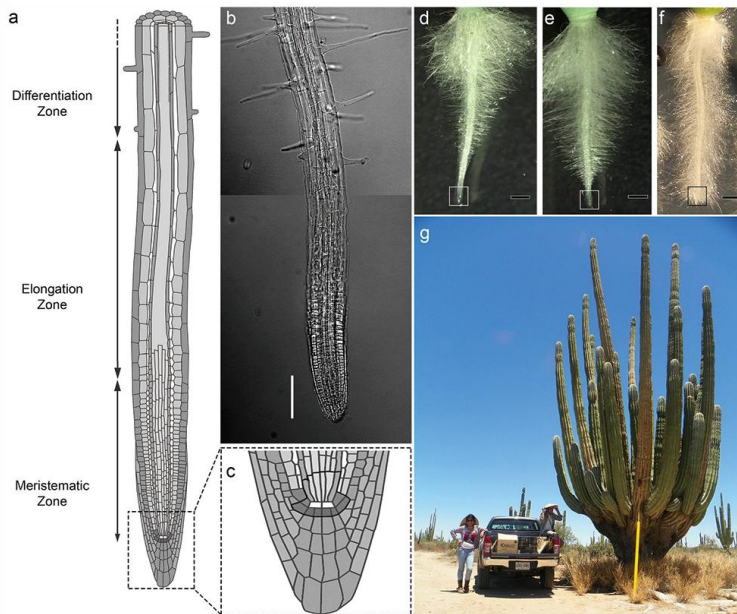


Figure 3 Indeterminate and determinate root growth (Adopted from Rodriguez-Alonso et al., 2018)

Image caption: (a) Growing roots can be divided in three developmental zones along the longitudinal axis as illustrated here for the *Arabidopsis thaliana* primary root; the root apical meristem (RAM) is located in the meristematic zone and it is present and active in most angiosperm roots for long periods. (b) The developmental zones in an *A. thaliana* lateral root can be distinguished by the cell features. Scale bar: 100 μ m. (c) The RAM contains a quiescent centre composed of cells with very low mitotic rate (white cells in the scheme). The cells adjacent to the quiescent centre, delimited with a thick line, are called stem (initial) cells, and are a source of the dividing cells for the meristem. (d–f) The *Pachycereus pringlei* primary root exhibits determinate growth. In this work, 1 mm of the primary root apex, delimited with a white or black box, was collected at three developmental stages: initial, when the RAM is present and fully active (d); intermediate, when the RAM is smaller and the differentiation zone is closer to the root apex (e); and terminal, when the RAM is exhausted and all the cells in the root, including those at the root apex, are differentiated (f). A mature *P. pringlei* is shown in (g). (a) and (c) were taken and modified from Peret, Benjamin (2017): doi:10.6084/m9.figshare.5143987.v4, originally deposited on FigShare as open access content under a CC BY 4.0 license (Adopted from Rodriguez-Alonso et al., 2018)

7 Challenges and Future Directions in Cactaceae Breeding

7.1 Limitations of current breeding techniques

The genetic improvement of Cactaceae is often limited by the biological characteristics of these plants, including their slow growth rates, long generation times, and complex reproductive mechanisms. Traditional breeding methods, such as hybridization and selection, are labor-intensive and time-consuming, which makes significant progress difficult to achieve within a reasonable timeframe (Khan et al., 2019; Ramanauskas and Igić, 2021). Cacti exhibit a high degree of genetic diversity, which, while beneficial for resilience, poses challenges for achieving consistency in desired traits across cultivars (Da Silva et al., 2021).

Moreover, the incompatibility between species and the occurrence of polyploidy in certain Cactaceae often complicate crossbreeding efforts. The chromosomal analysis of *Pachycereus pringlei* indicates that polyploidy (tetraploid) is widely distributed, affecting its reproductive isolation and breeding system (Gutiérrez-Flores et al.,

2018). The lack of comprehensive genomic information for many Cactaceae species further hampers the ability to effectively target specific genes for improvement, leading to limitations in both genetic gains and breeding efficiency (Tamayo-Ordoñez et al., 2023).

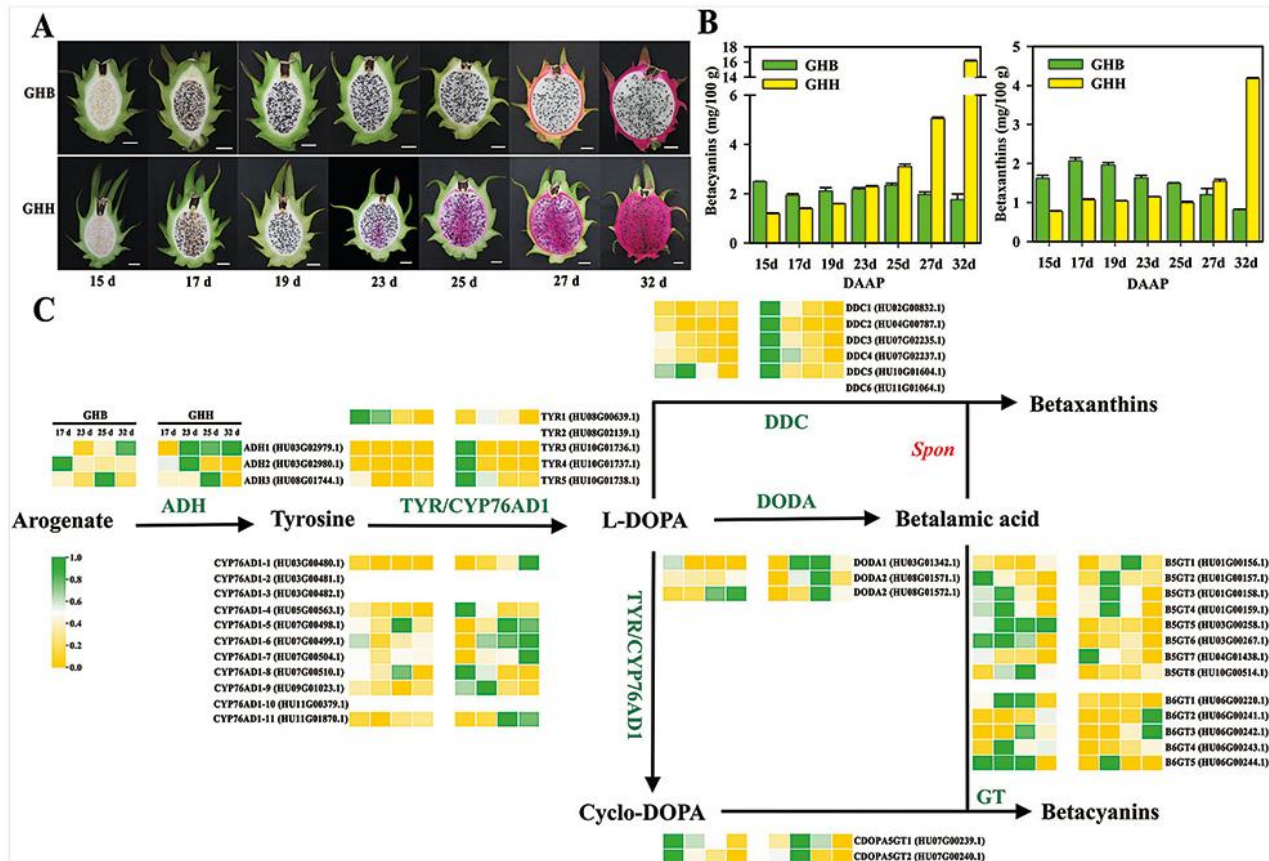


Figure 4 Genes involved in the betalain biosynthesis cascade (Adopted from Chen et al., 2021)

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)

7.2 Potential for biotechnology integration

The integration of biotechnological tools in Cactaceae breeding holds considerable potential for overcoming many of the limitations of traditional breeding techniques. Biotechnology can facilitate the development of desirable traits, such as enhanced disease resistance, improved water-use efficiency, and faster growth rates (Oltehua-Lopez et al., 2023). Techniques such as marker-assisted selection (MAS), tissue culture, and genetic transformation can significantly accelerate the breeding process and improve the precision of trait selection. Tissue culture techniques, for instance, can help bypass the long reproductive cycles by enabling rapid clonal propagation of elite genotypes.

Advances in genomic sequencing and CRISPR/Cas9 gene-editing technology offer new avenues for precise manipulation of key genes involved in stress tolerance, growth, and fruit quality (Liu et al., 2021). However, the application of biotechnology to Cactaceae breeding remains in its nascent stages, and more research is required to fully harness these tools for improving breeding outcomes.

7.3 Sustainable breeding approaches for climate change resilience

In the face of climate change, breeding for resilience is becoming an increasingly important focus for Cactaceae improvement. Cacti are naturally adapted to arid environments, but the growing unpredictability of climatic conditions necessitates the development of more robust and adaptive varieties. Sustainable breeding approaches that leverage both traditional knowledge and modern science are essential to achieve this goal (Hultine et al.,

2023). Techniques such as participatory breeding, where local farmers and breeders collaborate to develop climate-resilient cultivars, can help ensure that the new varieties meet the specific needs of different regions (Ceccarelli and Grando, 2022).

Furthermore, integrating agroecological principles into breeding programs can promote the conservation of genetic diversity, thereby enhancing the overall resilience of Cactaceae species to biotic and abiotic stresses. A greater emphasis on selecting traits such as drought tolerance, heat resistance, and pest resilience will be critical for ensuring that cacti can thrive in increasingly challenging environments while contributing to ecosystem stability and agricultural sustainability.

8 Concluding Remarks

It was found that traditional methods such as selective breeding and hybridization have made significant contributions to improving traits like drought resistance and fruit quality. However, their long breeding cycles and the genetic complexity of polyploids have limited the pace of rapid improvement. The integration of molecular genetics and biotechnological approaches has opened new pathways for achieving more precise and efficient genetic improvement. Marker-assisted selection, genomic studies, and gene editing are considered promising tools that can address the challenges posed by traditional breeding. The successful application of genomic information and functional genomics in improving key traits provides valuable insights for the future of Cactaceae breeding.

The advancements in Cactaceae breeding have significant implications for sustainable agriculture, particularly in arid and semi-arid regions. By improving traits such as drought tolerance, disease resistance, and water-use efficiency, the genetic enhancement of Cactaceae plants can contribute to more resilient agricultural systems capable of withstanding the growing challenges posed by climate change. Additionally, the use of biotechnological tools, such as tissue culture and gene editing, allows for the development of cultivars that are well-suited to specific environmental conditions, reducing the reliance on chemical inputs and promoting ecological stability. The integration of traditional knowledge with modern biotechnological techniques ensures that breeding efforts align with the goals of sustainability, enhancing the overall adaptability and productivity of Cactaceae species in diverse climatic regions.

The genetic improvement of Cactaceae holds great potential in addressing environmental stresses and promoting sustainable agricultural practices. Traditional breeding methods provide a foundation, but the adoption of modern genomic tools is crucial for achieving more precise and rapid breeding progress. The integration of molecular markers, functional genomics, and gene editing technologies offers a powerful strategy to enhance stress resistance and productivity in Cactaceae. However, the genomics of Cactaceae is still in its early stages of development, characterized by high genomic heterozygosity and a significant proportion of repetitive sequences, which makes it difficult to accurately align sequencing fragments to the reference genome, thereby increasing the complexity of genome assembly. In the future, closer collaboration among researchers, local farmers, and breeders is necessary to ensure that these advances can be effectively translated into practical applications. By utilizing both traditional and modern breeding techniques, the future cultivation of Cactaceae can be ensured, ultimately contributing to agricultural sustainability and the conservation of these valuable species.

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Conflict of Interest Disclosure

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References

- Abirami K., Swain S., Baskaran V., Venkatesan K., Sakthivel K., and Bommayasamy N., 2021, Distinguishing three Dragon fruit (*Hylocereus* spp.) species grown in Andaman and Nicobar Islands of India using morphological, biochemical and molecular traits, *Scientific Reports*, 11(1): 2894.
<https://doi.org/10.1038/s41598-021-81682-x>
- Abouseadaa H., Atia M., Younis I., Issa M., Ashour H., Saleh I., Osman G., Arif I., and Mohsen E., 2020, Gene-targeted molecular phylogeny, phytochemical profiling, and antioxidant activity of nine species belonging to family Cactaceae, *Saudi Journal of Biological Sciences*, 27: 1649-1658.
<https://doi.org/10.1016/j.sjbs.2020.03.007>
- Amaral D.T., Bombonato J.R., da Silva Andrade S.C., Moraes E.M., and Franco F.F., 2021, The genome of a thorny species: comparative genomic analysis among South and North American Cactaceae, *Planta*, 254(3): 44.
<https://doi.org/10.1007/s00425-021-03690-5>
- Angulo-Bejarano P.I., Gómez-García M.D.R., Valverde M.E., and Paredes-López O., 2019, Nopal (*Opuntia* spp.) and its effects on metabolic syndrome: new insights for the use of a millenary plant, *Current Pharmaceutical Design*, 25(32): 3457-3477.
<https://doi.org/10.2174/1381612825666191010171819>
- Arakaki M., Christin P., Nyffeler R., Lendel A., Egli U., Ogburn R., Spriggs E., Moore M., and Edwards E., 2011, Contemporaneous and recent radiations of the world's major succulent plant lineages, *Proceedings of the National Academy of Sciences*, 108: 8379-8384.
<https://doi.org/10.1073/pnas.1100628108>
- Cattivelli L., Rizza F., Badeck F., Mazzucotelli E., Mastrangelo A., Francia E., Marè C., Tondelli A., and Stanca A., 2008, Drought tolerance improvement in crop plants: An integrated view from breeding to genomics, *Field Crops Research*, 105: 1-14.
<https://doi.org/10.1016/J.FCR.2007.07.004>
- Ceccarelli S., and Grando S., 2022, Return to agrobiodiversity: Participatory plant breeding, *Diversity*, 14(2): 126.
<https://doi.org/10.3390/d14020126>
- Chen J.Y., Xie F.F., Cui Y.Z., Chen C.B., Lu W.J., Hu X.D., Hua Q.Z., Zhao J., Wu Z.J., Gao D., Zhang Z.K., Jiang W.K., Sun Q.M., Hu G.B., and Qin Y.H., 2021, A chromosome-scale genome sequence of pitaya (*Hylocereus undatus*) provides novel insights into the genome evolution and regulation of betalain biosynthesis, *Horticulture Research*, 8: 164.
<https://doi.org/10.1038/s41438-021-00612-0>
- Copetti D., Búrquez A., Shimizu K.K., Wing R.A., Sanderson M.J., and Wojciechowski M.F., 2023, An improved genome assembly of the saguaro cactus (*Carnegiea gigantea* (Engelm.) Britton & Rose), *bioRxiv*, 2023-04.
<https://doi.org/10.1101/2023.04.11.536419>
- Da Silva G., De Santana Lopes A., Pacheco T., De Godoy Machado K., Silva M., De Oliveira J., De Baura V., Balsanelli E., De Souza E., De Oliveira Pedrosa F., and Rogalski M., 2021, Genetic and evolutionary analyses of plastomes of the subfamily Cactoideae (Cactaceae) indicate relaxed protein biosynthesis and tRNA import from cytosol, *Brazilian Journal of Botany*, 44: 97-116.
<https://doi.org/10.1007/s40415-020-00689-2>
- De Jesus M.S., Passos A.R., and Diniz R.P., 2023, Selection indexes and principal components for agronomic and bromatological traits in forage cactus, *Revista Caatinga*, 36: 189-198.
<https://doi.org/10.1590/1983-21252023v36n120rc>
- De Oliveira M., Shuhua L., Kumbha D., Zurgil U., Raveh E., and Tel-Zur N., 2020, Performance of *Hylocereus* (Cactaceae) species and interspecific hybrids under high-temperature stress, *Plant Physiology and Biochemistry*, 153: 30-39.
<https://doi.org/10.1016/j.plaphy.2020.04.044>
- Ding J., 2021, Floral morphology and pollination process of red-fleshed dragon fruit (*Hylocereus polyrhizus*) grown in an open field, *Korean Journal of Horticultural Science and Technology*, 39(3): 277-293.
<https://doi.org/10.7235/HORT.20210025>
- Ferreira M., Rocha A., Nascimento F., Oliveira W., Soares J., Rebouças T., Lino L., Haddad F., Ferreira C., Santos-Serejo J., Fernández J., and Amorim E., 2023, The role of somaclonal variation in plant genetic improvement: A systematic review, *Agronomy*, 13(3): 730.
<https://doi.org/10.3390/agronomy13030730>
- Gonzaga D., Moraes L., De Vasconcelos L., and Reis R., 2018, Rediscovery of *Rhipsalis ewaldiana* Barthlott & N.P. Taylor (Cactaceae): notes of morphology and conservation of an endemic and threatened species from the Brazilian Atlantic Forest, *Phytotaxa*, 344: 291-294.
<https://doi.org/10.11646/PHYTOTAXA.344.3.10>
- Guerrero P., Majure L., Cornejo-Romero A., and Hernández-Hernández T., 2018, Phylogenetic Relationships and Evolutionary Trends in the Cactus Family, *The Journal of Heredity*, 110(1): 4-21.
<https://doi.org/10.1093/jhered/esy064>
- Gutiérrez-Flores C., León-de la Luz J.L., García-De León F.J., and Cota-Sánchez J.H., 2018, Variation in chromosome number and breeding systems: implications for diversification in *Pachycereus pringlei* (Cactaceae), *Comparative Cytogenetics*, 12(1): 61.
<https://pmc.ncbi.nlm.nih.gov/articles/PMC5904372/>
- Holanda M., Lira S., Da Silva J., Marques C., Coelho L., Lima C., Costa J., Silva G., Santos G., Zocolo G., Dionísio A., and Guedes M., 2021, Intake of pitaya (*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose) beneficially affects the cholesterol profile of dyslipidemic C57BL/6 mice, *Food Bioscience*, 42: 101181.
<https://doi.org/10.1016/J.FBIO.2021.101181>

- Horibe T., 2021, Cactus as crop plant—physiological features, uses and cultivation—, *Environmental Control in Biology*, 59(1): 1-12.
<https://doi.org/10.2525/ECB.59.1>
- Hua Q.Z., Chen C.J., Chen Z., Chen P.K., Ma Y.W., Wu J.Y., Zheng J., Hu G.B., Zhao J.T., and Qin Y.H., 2016, Transcriptomic analysis reveals key genes related to betalain biosynthesis in pulp coloration of *Hylocereus polyrhizus*, *Frontiers in Plant Science*, 6: 1179.
<https://doi.org/10.3389/fpls.2015.01179>
- Hultine K.R., Hernández-Hernández T., Williams D.G., Albeke S.E., Tran N., Puente R., and Larios E., 2023, Global change impacts on cacti (Cactaceae): current threats, challenges and conservation solutions, *Annals of Botany*, 132(4): 671-683.
<https://doi.org/10.1093/aob/mcad040>
- Khan G., Franco F., Silva G., Bombonato J., Machado M., Alonso D., Ribolla P., Albach D., and Moraes E., 2019, Maintaining genetic integrity with high promiscuity: Frequent hybridization with low introgression in multiple hybrid zones of *Melocactus* (Cactaceae), *Molecular Phylogenetics and Evolution*, 142: 106642.
<https://doi.org/10.1016/j.ympev.2019.106642>
- Khokhar A.A., Hui L., Khan D., You Z., Zaman Q.U., Usman B., and Wang H.F., 2024, Transcriptome Profiles Reveal Key Regulatory Networks during Single and Multifactorial Stresses Coupled with Melatonin Treatment in Pitaya (*Selenicereus undatus* L.), *International Journal of Molecular Sciences*, 25(16): 8901.
<https://doi.org/10.3390/ijms25168901>
- Köhler M., Reginato M., Jin J.J., and Majure L.C., 2023, More than a spiny morphology: plastome variation in the prickly pear cacti (Opuntieae), *Annals of Botany*, 132(4): 771-786.
<https://doi.org/10.1093/aob/mcad098>
- Liu J., Liu Z.Y., Zheng C., and Niu Y.F., 2021, Complete chloroplast genome sequence and phylogenetic analysis of dragon fruit (*Selenicereus undatus* (Haw.) D.R. Hunt), *Mitochondrial DNA Part B*, 6(3): 1154-1156.
<https://doi.org/10.1080/23802359.2021.1903356>
- Lu G., Wang W., Mao J., Li Q., and Que Y., 2023, Complete mitogenome assembly of *Selenicereus monacanthus* revealed its molecular features, genome evolution, and phylogenetic implications, *BMC Plant Biology*, 23(1): 541.
<https://doi.org/10.1186/s12870-023-04529-9>
- Martino P.A., Bauk K., Ferrero M.C., Gurvich D.E., and Las Peñas M.L., 2018, Ecological significance of determinate primary root growth: inter- and intra-specific differences in two species of *Gymnocalycium* (Cactaceae) along elevation gradients, *Flora*, 248: 70-75.
<https://doi.org/10.1016/J.FLORA.2018.09.001>
- Morillo-Coronado A., Hernández E., and Forero-Mancipe L., 2021, Phenotypic Diversity of Morphological Characteristics of Pitahaya (*Selenicereus megalanthus* Haw.) Germplasm in Colombia, *Plants*, 10: e12255.
<https://doi.org/10.3390/plants10112255>
- Nashima K., Hosaka F., Shimajiri Y., Matsumura M., Tarora K., Urasaki N., Shoda M., Nishitani C., Sawamura Y., and Yamamoto T., 2021, SSR marker development and genetic identification of pitaya (*Hylocereus* spp.) collected in Okinawa Prefecture, Japan, *The Horticulture Journal*, 90(1): 23-30.
<https://doi.org/10.2503/hortj.UTD-220>
- Oltehua-López O., Arteaga-Vázquez M.A., and Sosa V., 2023, Stem transcriptome screen for selection in wild and cultivated pitahaya (*Selenicereus undatus*): an epiphytic cactus with edible fruit, *PeerJ*, 11: e14581.
<https://doi.org/10.7717/peerj.14581>
- Oulo M.A., Yang J.X., Dong X., Wanga V.O., Mkala E.M., Munyao J.N., Onjolo V.O., Rono P.C., Hu G.W., and Wang Q.F., 2020, Complete chloroplast genome of *Rhipsalis baccifera*, the only cactus with natural distribution in the old world: genome rearrangement, intron gain and loss, and implications for phylogenetic studies, *Plants*, 9(8): 979.
<https://doi.org/10.3390/plants9080979>
- Paliwal R., Singh G., Mir R., and Gueye B., 2021, Genomic-assisted breeding for abiotic stress tolerance in horticultural crops, in *Genomics in Horticultural Crops*, 91-118.
<https://doi.org/10.1016/B978-0-12-822849-4.00007-3>
- Peñas M., Bauk K., Rossi P., and Bernardello G., 2023, Correlations among chromosome numbers, nuclear DNA contents and climatic variables of South American Cactaceae in a phylogenetic context, *Biological Journal of the Linnean Society*, 141(1): 87-101.
<https://doi.org/10.1093/biolinnean/blad070>
- Qin Q., Li J., Zeng S., Xu Y., Han F., and Yu J., 2022, The complete plastomes of red fleshed pitaya (*Selenicereus monacanthus*) and three related *Selenicereus* species: insights into gene losses, inverted repeat expansions and phylogenomic implications, *Physiology and Molecular Biology of Plants*, 28(1): 123-137.
<https://doi.org/10.1007/s12298-021-01121-z>
- Ramauskas K., and Igić B., 2021, RNase-based self-incompatibility in cacti, *The New Phytologist*, 231(5): 2039-2049.
<https://doi.org/10.1111/nph.17541>
- Rodríguez-Alonso G., Matvienko M., López-Valle M.L., Lázaro-Mixtco P.E., Napsucially-Mendivil S., Dubrovsky J.G., and Shishkova S., 2018, Transcriptomics insights into the genetic regulation of root apical meristem exhaustion and determinate primary root growth in *Pachycereus pringlei* (Cactaceae), *Scientific Reports*, 8(1): 8529.
<https://doi.org/10.1038/s41598-018-26897-1>

- Sampaio P., and De Almeida O., 2021, Without Moths or Bats, Cacti form Friendships with Bees: Reproductive Biology of the Queen of Night *Selenicereus setaceus* (Cactaceae) in São Thomé Das Letras, Brazil, Preprint.
<https://doi.org/10.21203/rs.3.rs-690841/v1>
- Sanderson M.J., Búrquez A., Copetti D., McMahon M.M., Zeng Y., and Wojciechowski M.F., 2020, A new (old) approach to genotype-based phylogenomic inference within species, with an example from the saguaro cactus (*Carnegiea gigantea*), bioRxiv.
<https://doi.org/10.1101/2020.06.17.157768>
- Tamayo-Ordoñez Y.J., Tamayo-Ordoñez M.C., Rosas-García N.M., Sosa-Santillán G.J., and Ayil-Gutiérrez B.A., 2023, Assembly of the *Cereus fernambucensis* genome, gene annotation, and tertiary structure of secondary metabolism enzymes in *Carnegiea gigantea*, *Lophocereus schottii*, *Pachycereus pringlei*, *Pereskia humboldtii*, *Selenicereus undatus*, and *Stenocereus thurberi*, Journal of the Mexican Chemical Society, 67(3): 284-304.
<https://doi.org/10.29356/jmcs.v67i3.1969>
- Tel-Zur N., 2022, Breeding an underutilized fruit crop: a long-term program for *Hylocereus*, Horticulture Research, 9: uhac078.
<https://doi.org/10.1093/hr/uhac078>
- Wan L., Wang Z., Tang M., Hong D., Sun Y., Ren J., Zhang N., and Zeng H., 2021, CRISPR-Cas9 gene editing for fruit and vegetable crops: strategies and prospects, Horticulturae, 7(7): 193.
<https://doi.org/10.3390/HORTICULTURAE7070193>
- Wu Z., Deng H., Liang G., Ye X., Qin Y., and Huang L., 2021, Construction of a high-density genetic map for pitaya using the whole genome resequencing approach, Horticulturae, 7(12): 534.
<https://doi.org/10.3390/horticulturae7120534>
- Yu J., Li J., Zuo Y., Qin Q., Zeng S., Rennenberg H., and Deng H., 2023, Plastome variations reveal the distinct evolutionary scenarios of plastomes in the subfamily Cereoideae (Cactaceae), BMC Plant Biology, 23(1): 132.
<https://doi.org/10.1186/s12870-023-04148-4>
- Yusop M., Sukaimi J., Amiruddin M., Jalloh M., Swaray S., Yusuff O., and Chukwu S., 2020, Genetic improvement of oil palm through recurrent selection, In: Ithnin M., Kushairi A. (eds) The Oil Palm Genome. Compendium of Plant Genomes. Springer, Cham, PP.35-46.
https://doi.org/10.1007/978-3-030-22549-0_4
- Zhang L.L., Chen C.B., Xie F.F., Hua Q.Z., Zhang Z.K., Zhang R., Chen J.Y., Zhao J.T., Hu G.B., and Qin Y.H., 2021, A novel WRKY transcription factor HmoWRKY40 associated with betalain biosynthesis in pitaya (*Hylocereus monacanthus*) through regulating HmoCYP76AD1, International Journal of Molecular Sciences, 22(4): 2171.
<https://doi.org/10.3390/ijms22042171>
- Zhang X., Shan Y., Li J., Qin Q., Yu J., and Deng H., 2023, Assembly of the Complete Mitochondrial Genome of *Pereskia aculeata* Revealed That Two Pairs of Repetitive Elements Mediated the Recombination of the Genome, International Journal of Molecular Sciences, 24: 98366.
<https://doi.org/10.3390/ijms24098366>

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