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Evolutionary Adaptation in Cactaceae Fruit Trees Photosynthetic Transition and Carbon Metabolism Regulation in Pitaya

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Abstract Pitaya (*Hylocereus* spp.) is a typical crassulacean acid metabolism (CAM) plant in the Cactaceae family, which can effectively utilize water through a special photosynthetic pathway in arid environments. This study reviews the evolutionary mechanism of the conversion of the photosynthetic pathway of pitaya from C3 to CAM, and elaborates on its carbon metabolism regulation characteristics. The taxonomic differences of C3, intrinsic CAM and inducible CAM photosynthesis in Cactaceae plants, as well as the structural and physiological characteristics related to CAM evolution, are compared. The circadian expression characteristics of key enzyme genes of pitaya CAM photosynthesis, the role of transcription factors involved in regulation, and the regulatory mechanisms at the epigenetic and post-transcriptional levels are analyzed. The dynamics of the accumulation and conversion of carbonate assimilates (such as malic acid, sugar and starch) in pitaya during the diurnal cycle, as well as the role of vacuolar carbonate transport in carbon allocation, are discussed. The conservation and novelty of CAM pathway-related genes in Cactaceae plants are explored from the perspective of comparative genomics, and the differences in the genomes of pitaya and its C3 relatives are compared. Through the case of red-fleshed and white-fleshed pitaya varieties, this study analyzed the changes in CAM expression levels and metabolome under drought and high temperature stress, and looked forward to applying CAM characteristics to pitaya stress resistance breeding to improve crop water use efficiency and stress resistance, and promote the efficient use of pitaya in sustainable agricultural development.

Keywords Pitaya (*Hylocereus* spp.); Crassulacean acid metabolism (CAM); Photosynthetic pathway; Carbon metabolism; Drought resistance and adaptation

1 Introduction

Pitaya (*Hylocereus* spp.) belongs to the genus Hylocereus of the Cactaceae family. Its green fleshy stems are responsible for photosynthesis and are typical CAM plants. Unlike traditional C3 fruit trees, pitaya fixes CO_2 at night, significantly reducing water transpiration loss (Chen et al., 2020). Studies have shown that pitaya can survive and no seedlings die under severe drought conditions where the soil moisture supply is only 20% of the field water holding capacity, showing strong drought resistance (Li et al., 2022). This adaptability makes it an important economic fruit in tropical and subtropical arid regions, and it has been widely introduced and cultivated in the Asia-Pacific region and other regions. The rich nutritional value of pitaya (rich in dietary fiber, vitamins and anthocyanins) and high economic benefits have also promoted the expansion of its planting area. As a typical representative of cactus fruit trees, pitaya provides a good model for studying the evolution and application of CAM photosynthetic pathways.

The arid and semi-arid habitats where cacti live are characterized by high temperature, little rainfall, and strong transpiration, which pose severe challenges to plant water use and carbon assimilation. The main stresses faced by pitaya in these environments include severe water shortage, large temperature difference between day and night, and strong light. Under drought conditions, ordinary C3 plants will have limited CO_2 acquisition and stagnation of photosynthesis due to the closure of stomata during the day. However, pitaya cleverly staggers the opening and closing of stomata through the CAM pathway, absorbs CO_2 and fixes it as organic acid during the cool period at night, and can assimilate carbon without opening stomata during the day (Lee et al., 2023). This mechanism prevents excessive water loss during the day and significantly improves water use efficiency. On the other hand, the CAM mechanism also brings certain costs, such as low carbon fixation efficiency at night and the need for additional energy to store and reuse malic acid (Lee and Chang, 2024). However, pitaya has evolved a series of structural and physiological characteristics (such as thick cuticle, developed water storage tissue, nocturnal stomata, etc.) to cope with drought stress (Niechayev et al., 2019). Pitaya plants maintain cellular water balance by reducing stomatal conductance, accumulating osmotic regulating substances such as proline, and enhancing superoxide dismutase (SOD) and peroxidase (POD) activities to reduce oxidative damage under drought stress. These adaptation strategies ensure that pitaya can still maintain basic photosynthetic carbon assimilation and growth functions in a water-limited environment.

This study focuses on the CAM photosynthetic type conversion and carbon metabolism regulation of pitaya, and explains its survival advantage in a drought environment from the perspective of evolutionary adaptation. This study will summarize the special mechanisms and evolutionary advantages of the CAM photosynthetic pathway in pitaya, analyze the molecular network that regulates the key steps of the CAM cycle in pitaya, analyze how pitaya allocates and stores carbon resources at the cellular and overall levels to adapt to environmental changes, and look for genetic factors that may affect CAM efficiency to provide clues for future molecular breeding. It will also propose ideas for applying CAM mechanisms to high-yield and stress-resistant pitaya breeding, providing a scientific basis for expanding the application of CAM in other crops and responding to future drought climate challenges.

2 Photosynthetic Pathways in Cactaceae Evolution

2.1 Classification of C3, CAM, and facultative CAM in cactaceae

Terrestrial higher plants mainly use three carbon assimilation pathways: C3, C4 and CAM to fix CO_2. C3 plants (such as most temperate fruit trees) fix carbon directly through Rubisco in mesophyll cells, while CAM plants separate carbon fixation and carbon release processes into two phases: night and day. Some species exhibit the characteristics of inducible CAM (facultative CAM), that is, C3 metabolism is carried out under sufficient water conditions, but it can be converted to the CAM pathway when encountering drought stress. This type of plant provides an example of the transition state between C3 and CAM. However, pitaya has been confirmed to be an obligate CAM plant, which maintains the CAM mode regardless of sufficient water or drought. The gas exchange experiment of Wang et al. (2019) showed that the CO_2 absorption pattern of pitaya in the diurnal cycle did not change significantly with water supply, and always showed the typical CAM characteristics of net absorption at night and zero absorption during the day. In contrast, inducible CAM plants (such as some Crassulaceae) have stomata open during the day and closed at night when there is plenty of water, showing a C3-type daytime net CO_2 absorption, while switching to nighttime absorption in drought. This flexibility is less common in cactus plants such as pitaya. Evolutionarily, CAM photosynthesis is considered to be the result of multiple independent adaptations of plants to reduced atmospheric CO_2 concentrations and arid environments. CAM plants have been found in hundreds of genera in more than 30 families, and they more or less show consistent characteristics of the CAM cycle. The existence of inducible CAM suggests that some C3 plants may have the potential for partial CAM mechanisms, supporting the hypothesis that the CAM pathway has evolved from C3 to a gradual transition in evolution. As a representative of the intrinsic CAM type, pitaya has a stable and efficient photosynthetic pattern, reflecting the mature evolutionary state of the CAM photosynthetic pathway.

2.2 Structural and physiological characteristics associated with CAM evolution

CAM plants have evolved a series of unique structural and physiological characteristics under the selection pressure of long-term drought to adapt to a lifestyle of carbon fixation at night and carbon release during the day. The leaves of pitaya degenerate into scaly areoles, and the main photosynthetic organ is borne by the green fleshy stem. This stem and leaf assimilation tissue can store a large amount of water and support the dilution space required for the accumulation of malic acid at night. Its epidermis has a well-developed cuticle and thick epidermal cells, which reduce water loss during the day. The stomata of pitaya are mostly concentrated in the sunken parts of the stem and have guard hairs, which help to reduce the water vapor pressure gradient around the stomata and reduce water loss when the pores are opened at night (Li et al., 2022). In terms of stomatal morphology, pitaya stomata are small and of moderate density. They can respond quickly at night to open to obtain CO_2 and close tightly during high temperatures during the day. Nie et al. (2022) found that the stomata of the epidermis of pitaya stems close around sunrise and open after sunset, and the diameter changes are highly synchronized with the external light and temperature rhythms. CAM plants have huge vacuoles for storing organic acids. The volume of the central vacuole of pitaya fleshy thin-walled cells can account for more than 80% of the cell volume. The malic acid fixed at night accumulates in the vacuole, causing the acidity of the cell sap to increase significantly, and the pH can drop from about 6 during the day to about 4 at night (Winter, 2019). This "acid night and alkaline day" rhythm is common in CAM plants such as pitaya, and is also an important physiological indicator for CAM identification. Finally, pitaya is rich in chlorophyll and photosynthetic enzymes in stem tissue, similar to the mesophyll of C3 plants, thus ensuring that the Calvin cycle can be efficiently completed when CO_2 is released during the day. Li et al. (2021) found through quantitative inversion of hyperspectral technology that the changes in chlorophyll content in the green stem nodes of pitaya can be accurately monitored by spectral characteristics, proving that its stem tissue has a strong photosynthetic capacity. Structural changes such as fleshy stems, thick cuticles, nocturnal stomata and large vacuoles are highly consistent with the CAM metabolism of diurnal carbon fixation, and are the basis for the successful evolution of the CAM pathway.

2.3 Ecological benefits of CAM adaptation in pitaya

The CAM photosynthetic pathway gives pitaya a significant ecological advantage over C3 plants in arid environments. CAM improves water use efficiency (WUE), allowing pitaya to accumulate sufficient biomass and bloom and bear fruit in areas with scarce rainfall. Studies have shown that under mild (60% field capacity) and moderate drought (40% field capacity), the growth of new branches and the above-ground and underground dry weight of pitaya are not significantly different from those under normal water supply conditions. Even under severe drought (20% field capacity), there was no seedling death in the plants, only growth was inhibited. This shows that the CAM mechanism gives pitaya the ability to maintain life activities when water is extremely scarce, greatly expanding its ecological distribution range. CAM helps pitaya adapt to high temperature and strong light conditions. The closure of stomata during the day avoids excessive transpiration under high temperatures, allowing it to withstand exposure to the sun without wilting. At the same time, the main absorption and fixation of CO_2 at night allows pitaya to avoid the damage of photosynthetic enzymes by strong light at noon. CAM plants usually accumulate a large amount of organic acids and carbohydrates, which is beneficial to their stress resistance. The malic acid accumulated by pitaya at night is converted into sugar during the day, which is not only used for its own metabolism, but also helps to increase the sugar content and flavor of its fruit.

A study comparing red-heart and white-heart pitaya found that the total sugar content of the red-heart variety was about 18.70%, significantly higher than the 14%~15% of the white-heart variety (specifically varies depending on the variety and origin), which may be related to the stronger nighttime carbon fixation ability of the red-heart variety (Wu et al., 2019). The organic acids and osmotic regulating substances (such as proline) accumulated in the tissues of pitaya through the CAM cycle can also improve its salt tolerance and antioxidant capacity, and show strong adaptability under complex adversity. The CAM photosynthetic pathway significantly improves the survival and competitiveness of pitaya in arid and barren environments, enabling it to thrive in areas where other fruit trees are difficult to grow and achieve economic cultivation.

3. Molecular Regulation of CAM Photosynthesis in Pitaya

3.1 Circadian regulation of key CAM genes

The carbon assimilation of CAM plants is highly compartmentalized in time, and this process is finely regulated by the intrinsic biological clock and circadian rhythm. At the molecular level, the key enzyme genes that control the CAM cycle (such as phosphoenolpyruvate carboxylase PEPC, malate dehydrogenase MDH, malic enzyme ME, etc.) show a clear diurnal alternating expression pattern. At night, the gene expression and enzyme activity of PEPC, which is responsible for CO_2 fixation, reach a peak, so that a large amount of CO_2 combines with phosphoenolpyruvate to form malic acid. During the day, malic enzyme (especially NADP-ME) responsible for decarboxylation is activated at the transcriptional and translational levels, decomposing the accumulated malic acid to release CO_2 for use in the Calvin cycle.

For example, in model CAM plants such as pineapples, PEPC proteins are activated by specific phosphorylation at night, and their encoding genes are transcriptionally upregulated from dusk to early night, and rapidly downregulated after dawn. Although relatively few studies have been conducted on CAM gene regulation in pitaya, existing transcriptome results show that its CAM-related enzyme genes also show periodic changes. Comparative genomic analysis by Chen et al. (2020) pointed out that many CAM carbon fixation-related gene promoters are enriched with cis-elements regulated by the biological clock, indicating that these genes are controlled by intrinsic circadian rhythms. Jiao et al. (2021) conducted a joint transcription and metabolomics analysis of pitaya seedlings and found that some enzyme genes involved in organic acid metabolism fluctuated in expression levels during the day and night when they were subjected to diurnal temperature rhythms or heat stress to ensure sufficient carbon fixation at night and timely decarboxylation during the day. Therefore, it can be considered that pitaya achieves precise temporal allocation of carbon flow by circadian regulation of the expression of key CAM enzyme genes. In the future, if the core enzyme genes of the pitaya CAM cycle can be cloned and their promoter characteristics can be analyzed, it will help to further reveal its diurnal regulation mechanism.

3.2 Transcription factors involved in diel gene expression

In addition to the enzyme-encoding genes themselves, many transcription factors (TFs) play an important role in the CAM gene regulatory network. Studies have shown that some transcription factors that regulate the circadian clock, stomatal movement, and stress response may be involved in the expression regulation of key genes in the CAM pathway. For example, the common evening element on the promoter of PPC (PEPC encoding gene) is believed to bind to circadian clock factors (such as LHY/CCA1, TOC1, etc.), thereby giving the PEPC gene the characteristics of post-dusk expression (Ming et al., 2015; Ouyang et al., 2023). A MYB-type transcription factor found in Kalanchoë of Crassulaceae is reported to bind to the PEPC promoter and enhance its nighttime expression, promoting the initiation of the CAM cycle. CAM plants often open their stomata at night, and this process may be controlled by factors that regulate the circadian rhythm of stomatal pores, such as ZEITLUPE (ZTL) and related F-BOX proteins, which affect the guard cell circadian clock and thus indirectly affect the CAM timing.

For example, typical stress response transcription factors such as DREB, bZIP, and WRKY are upregulated under drought, and they may simultaneously regulate osmotic pressure regulation genes in the CAM pathway. A recent molecular study on pitaya cloned a NAC transcription factor (HpNAC1). Although it mainly acts on the pigment synthesis pathway, the study proved that HpNAC1 can activate the promoter of downstream target genes. This suggests that there are also functional transcription factors in pitaya that can affect metabolic pathways. Similarly, if there are TFs that specifically regulate malic acid metabolism or sugar metabolism in pitaya, their activity changes will directly change CAM efficiency. At present, genome analysis of CAM crops such as pineapple has identified dozens of transcription factor genes that are specifically expressed at night, some of which belong to the CAMTA, NAC and GRAS families that are specific to CAM.

3.3 Epigenetic and post-transcriptional regulation mechanisms

In addition to transcriptional regulation, CAM photosynthesis is also finely controlled at the epigenetic and post-transcriptional levels. Epigenetic studies have revealed that DNA methylation and histone modifications can affect the periodic expression of CAM-related genes. Shi et al. (2021) integrated the DNA methylome and transcriptome of the CAM crop pineapple and found that the methylation levels of the promoters of some CAM key enzyme genes changed compared with white non-photosynthetic tissues in the mesophyll green tissue (exercising CAM). In particular, asymmetric methylation differences in the CHH sequence environment may lead to changes in circadian gene expression. It is speculated that CAM plants such as pitaya affect the reading and writing of circadian timing genes by changing the methylation state of specific promoter regions, so that the CAM pathway can adapt to environmental rhythms and long-term stress. Histone modifications such as H3K4me3 (active mark) and H3K9ac (acetylation) may also be involved in the circadian switch of CAM genes.

For example, in Crassulaceae plants, the promoter region of the PEPC gene, which is highly expressed at night, is enriched with active histone marks, suggesting that the biological clock may regulate gene periodicity through chromatin remodeling. At the post-transcriptional level, miRNA and RNA silencing mechanisms have also been shown to affect photosynthesis-related genes. Although there are few studies on miRNAs in pitaya, several miRNAs have been identified in pineapple that are upregulated at night, possibly targeting photosynthesis-related transcripts. The role of these miRNAs or siRNAs may be to help plants quickly reduce enzyme transcripts that are not needed during the day and save energy. For example, malate dehydrogenase mRNA synthesized in large quantities at night may be degraded during the day through the action of specific miRNAs to terminate the nighttime pathway. Epigenetic regulation provides a stable "memory" for the rhythmicity of CAM photosynthesis in pitaya, while post-transcriptional regulation provides a means of rapid response and precise regulation (Lv and Wang, 2024). In the future, intervening in CAM-related genes through means such as methylation inhibition or miRNA manipulation is expected to provide a deeper understanding of the effects of these levels on the CAM cycle. Related research on pineapples has provided us with a model, and research in the corresponding field of pitaya needs to be strengthened.

4. Carbon Metabolism and Storage Dynamics

4.1 Organic acid (malate) accumulation and nocturnal CO_2 fixation

In CAM plants, CO_2 fixed at night is first catalyzed by PEPC enzyme to combine with phosphoenolpyruvate (PEP) to form oxaloacetate, and then reduced to malic acid and accumulated in large quantities in the vacuole. Therefore, malic acid is a core intermediate in CAM carbon metabolism, and its content shows significant diurnal periodic fluctuations: it gradually increases at night, reaches a peak at dawn, and decreases during the day. Pitaya stem tissue accumulates organic acids at night, and the acidity of the juice measured in the early morning is significantly higher than that at dusk, which is consistent with classic CAM plants (such as Sedum and succulents) (Winter, 2019). CAM plants usually measure their nocturnal carbon fixation capacity by measuring the acid content (titratable acidity or malic acid concentration) of the nocturnal mesophyll or stem flesh.

Pitaya has a strong nocturnal acid accumulation capacity, and at a suitable temperature (20 ℃~25 ℃), the malic acid in each kilogram of fresh weight of stem tissue can increase by tens of millimoles at night. If the night temperature is too high (>30 ℃) or too low (<10 ℃), the acid accumulation efficiency decreases because temperature affects enzyme activity and stomatal behavior. Nighttime CO_2 fixation is not only dependent on enzymes, but also limited by stomatal conductance and external CO_2 concentration. When the atmospheric CO_2 concentration increases at night, the carbon fixation rate of CAM plants such as pitaya will also increase. This indicates that the nighttime CO_2 fixation process may still be diffusion-limited, but it only occurs during the dark period. The night phase of the CAM pathway effectively alleviates photorespiration because PEPC is insensitive to CO_2. In pitaya, nighttime CO_2 fixation is also accompanied by some byproducts, such as malate, charge-balanced anion transport, and other processes, which are achieved through organic acid-anion exchange across the vacuole membrane. In general, the nighttime accumulation of malic acid is a hallmark of pitaya CAM metabolism, which ensures sufficient CO_2 supply during the day, so that carbon assimilation can continue in the sun.

4.2 Sugar synthesis and starch turnover during light-dark cycles

During the day, malic acid in the pitaya vacuole is decomposed by malic enzyme to produce CO_2 and pyruvate. CO_2 is assimilated by Rubisco in the chloroplasts and enters the Calvin cycle to produce trisaccharides and further converted into carbohydrates such as sucrose and starch. In CAM plants, starch plays the role of a "diurnal buffer": sugar degradation at night provides PEP for PEPC carbon fixation, while photosynthesis products during the day are resynthesized into starch and stored in chloroplasts. The starch content in the green tissue of pitaya stems is the highest at dusk, and the starch is gradually degraded at night and significantly decreases at dawn; accordingly, the content of soluble sugars (such as sucrose and glucose) increases at night, providing a carbon source for PEP regeneration and basic cell metabolism. Proteomic studies have shown that under drought stress, the expression of multiple starch hydrolases (such as α-amylase, debranching enzymes, etc.) in pitaya is enhanced, resulting in faster starch decomposition and increased supply of soluble sugars at night. Shi et al. (2021) found that under

PEG-simulated drought conditions, key enzymes related to starch and sucrose metabolism in pitaya tissues, such as ISA3 (isoamylase) and DPEP (transglucosidase), were significantly upregulated, promoting starch degradation and sucrose accumulation.

This shows that pitaya maintains the carbon skeleton supply and osmotic pressure balance required for CAM by accelerating starch-sugar conversion under stress. Diurnal carbohydrate dynamics are very important in CAM plant physiology: if starch reserves are insufficient at night, PEP supply will be limited, thereby weakening CO_2 fixation; if sugar utilization or output is not smooth during the day, excessive sugar feedback inhibits photosynthesis and hinders starch resynthesis. The development of pitaya fruit will also compete for photosynthetic assimilates. When more sugar is transported to the fruit, the starch in the stem may decrease, which may affect the intensity of the CAM cycle in the long run. Therefore, in production, the nighttime carbon fixation of pitaya can be promoted by appropriately increasing the starch content of the mesophyll (for example, enhancing photosynthesis or lowering the temperature at night). On the contrary, under conditions such as continuous rain and overcast weather, the decline in starch reserves will weaken the function of CAM, which needs to be restored by increasing light and other measures. Pitaya achieves a balance between carbon supply and carbon storage through the alternating conversion of starch and sugar during the diurnal cycle, thus ensuring the smooth operation of CAM photosynthesis and the supply of carbon sources required for organ growth.

4.3 Role of vacuolar transporters and carbohydrate partitioning

The smooth progress of CAM photosynthesis is highly dependent on the storage and release function of the vacuole for organic acids. This process is precisely controlled by various transport proteins on the vacuole membrane, including organic acid-anion exchange proteins, proton pumps and ion channels. At night, malic acid is actively transported to the interior of the vacuole in the form of malic acid anions through dicarboxylate transporters in the vacuole membrane (probably belonging to the ALMT or tDT protein family), while proton pumps (V-ATPase and V-PPase) pump H^+ into the vacuole to maintain electrical neutrality. During the day, malic acid in the vacuole needs to be released into the cytoplasm through specific channels or antiporters for decomposition. According to research, in the typical CAM plant Sedum, there are two types of malic acid channels on the vacuole membrane: fast voltage-dependent channels and slow regulatory channels, which together mediate the transmembrane movement of malic acid. The capacity and activity of these transporters will directly affect the efficiency of CAM. If the storage capacity of the vacuole for malic acid is limited, CO_2 fixation at night will be prematurely inhibited by feedback; on the contrary, if the vacuole release is not smooth, the raw materials for the Calvin cycle during the day will be insufficient.

Chen et al. (2020) proposed that the evolution of CAM is partly due to the new spatiotemporal combination of existing central metabolic enzymes and membrane transporters. This means that pitaya may achieve efficient malic acid storage and mobilization by changing the expression timing or localization of existing vacuolar transporter genes. For example, pitaya may have CAM-specific aluminum-activated malic acid channel (ALMT) family members, which are activated at night to accelerate the entry of malic acid into the vacuole, and closed during the day to prevent premature leakage. There is evidence that in pineapples, some membrane transport-related genes are transcribed more during the dark cycle, and it is speculated that the proteins they encode play a role in the CAM cycle. In terms of carbon allocation, CAM plants often need to balance carbon resources between vegetative growth and reproductive growth. During the fruiting period, a large amount of photosynthetic carbon assimilates are allocated to fruit sugar and biomass accumulation, which may affect the carbohydrate reserves of the stem itself. Appropriate agricultural measures can alleviate this contradiction, such as providing sufficient potassium fertilizer in the late fruiting period to promote the transport of photosynthetic products to the fruit and maintain carbon and water storage in the stem. Li et al. (2022) found that in pitaya orchards with medium and low fertility soils, increasing the amount of potassium fertilizer significantly increased the plant's absorption of nitrogen and phosphorus and fruit yield. Potassium, as the main cation in the vacuole, helps neutralize malate anions and maintain turgor pressure, so sufficient potassium nutrition is also beneficial to the vacuolar acid storage function. It can be seen that vacuolar transport proteins and ion balance are crucial in the diurnal dynamics of pitaya carbon metabolism, affecting both CAM efficiency and carbon allocation pattern.

Regulation of these transport processes (such as increasing vacuolar acid storage capacity through breeding or optimizing the ion environment through fertilization) will help to give full play to the CAM advantages of pitaya and achieve high and stable yields.

5 Comparative Genomic Insights into CAM Evolution

5.1 Expansion of gene families related to CAM regulation

With the help of genomic technology, researchers have begun to understand the genetic basis of CAM photosynthesis from a macroscopic level. Comparison of the genomes of multiple CAM plants and C_3 related species revealed that gene families of some key enzymes and regulatory factors have expanded or functionally diverged in CAM plants. For example, the PEPC gene exists in multiple copies in some CAM species, and homologous genes may be specialized for metabolic needs during the day and at night. The study by Heyduk et al. (2022) focused on plants of the genus Yucca in the Agave family. The results showed that a copy of the PEPC gene called PPC2 in the genus Yucca evolved nocturnal expression in CAM species and had temporal structured expression that existed before the origin of CAM. This suggests that some gene family members were "recruited" to assume new functions during the evolution of CAM. For example, the gene encoding malic enzyme (ME) has different isozymes in some CAM plants. Among them, NADP-ME type is often used for C3 photorespiration, while NAD-ME type is more suitable for dark decarboxylation under CAM conditions. The two may achieve functional differentiation through gene duplication and expression regulation. Gene family expansion is also reflected in ion transport and stomatal regulation. Analysis of the pineapple genome found that genes related to stomatal rhythm and tonoplast transport have been expanded. For example, some protein kinases and channel protein genes that regulate stomatal movement have been repeated in CAM evolution, which is conducive to strengthening nighttime stomatal control and organic acid transmembrane transport capabilities. CAM plants are often rich in fleshy stem water storage tissues, which may be related to genetic changes such as cell wall relaxation proteins and water channel proteins to adapt to tissue expansion and water storage. Pitaya is rich in calcium oxalate crystals, which may also be related to changes in calcium ion transport and metabolic genes (Huang and Li, 2024). Comparative genomics reveals that CAM plants have strengthened the genetic basis related to carbon assimilation and water utilization through the expansion and functional innovation of gene families, providing a higher upper limit and redundancy for the realization of CAM functions.

5.2 Conserved vs. novel CAM genes across Cactaceae

Within the Cactaceae, the CAM mechanisms between different genera may have both commonalities and individualities. In terms of conservation, core CAM enzymes such as PEPC, MDH, ME, PPT (phosphopyruvate transporter), etc. are highly homologous in various species of Cactaceae, and are very conservative in sequence and structure, indicating that these genes have been tested by evolution for a long time before being incorporated into the CAM pathway, and their catalytic functions do not need to be greatly modified to meet the requirements of CAM (Shu et al., 2022). For example, although columnar cacti and Crassulaceae plants are distantly related, their key sites of PEPC proteins are almost the same, indicating that the carbon fixation function of CAM has the characteristics of convergent evolution at the molecular level. On the other hand, different CAM species have also evolved novel genes and regulatory patterns to optimize CAM efficiency. Some metabolic pathway genes unique to the Cactaceae have been incorporated into the CAM network, such as the abundant mucilage metabolism-related genes in pitaya stems, which may assist in ion balance and water retention, and support CAM operation from the side. In the genome of pitaya ‘David Bowie’, the 11 longest pseudochromosomes account for 88.7% of the total genome length, and 87.8% of protein-coding genes, 72.6% of non-coding RNA genes, and 58.0% of tRNA genes are distributed on them, showing a significant concentration of functional genes. Whole genome sequencing showed that compared with cacti such as Carnegiea, Hylocereus has a gene cluster expansion that is significantly enriched in drought resistance and sugar metabolism functions. These gene clusters include some dehydration response proteins and cell wall degrading enzymes. It is speculated that in order to grow rapidly and cope with seasonal drought, climbing cacti such as pitaya have evolved CAM regulatory gene modules that are different from those of large columnar cacti. Some genes required for CAM processes in the Cactaceae family may not be newly created, but derived from a common ancestor. For example, Cactaceae plants generally experienced a whole genome duplication (WGD) event, and many duplicated metabolic enzyme genes were retained, which provided genetic raw materials for the later independent origin of CAM (Figure 1) (Zheng et al., 2021). Analysis of the Hylocereus genome supports this view: the redundant genes provided by its ancestral WGD, including carbon metabolism and water channel protein genes, were likely reused when pitaya evolved CAM.

5.3 Evolutionary divergence between CAM and C3 relatives in pitaya

Some of the closest relatives of pitaya may not fully adopt CAM metabolism. For example, some primitive groups of the Cactaceae family (such as the genus Pereskia) still retain the C3 mode. Comparing pitaya with these C3 relatives can provide insights into genomic changes during the origin of CAM. Chen et al. (2020) proposed that the evolution of CAM is more of a change in the regulatory level rather than the generation of completely new structural genes. This view is supported by genetic comparisons: compared with some C3 cacti, the gene coding regions of pitaya are not significantly different, and the main difference lies in the expression level and timing of key genes.

For example, the promoter of the pitaya PEPC gene has more circadian clock elements, so its expression is enhanced at night; while C3 relatives may lack these promoter modifications, so PEPC still maintains daytime expression and does not participate in carbon fixation at night. For example, the tonoplast carrier gene responsible for organic acid storage may have evolved higher expression or dark induction in pitaya, which may be achieved through promoter mutation or duplication, but the gene itself already exists in C3 species. Gene co-expression network analysis also showed that the genes involved in CAM in pitaya interact more closely with each other and show modular characteristics, while in C3 related species, these genes belong to different network modules. This suggests that the formation of CAM is accompanied by the reorganization of gene regulatory relationships. Another example is that the chloroplast genome of pitaya is not particularly different from that of C3 related species, but pitaya highly expresses the expression of some chloroplast proteins (such as NDH complex) at night to help adapt to dark respiration and photosynthetic electron flow. This may also be a difference at the transcriptional regulation level.

It is worth mentioning that some basic metabolic genes may even be suppressed in pitaya to adapt to CAM, such as reducing the expression of photorespiration-related genes during the day to avoid competition with C2. In summary, the evolutionary differentiation of the dragon fruit genome relative to its C3 relatives is mainly reflected in "new uses for old parts": that is, by adjusting the spatiotemporal expression patterns of existing genes, efficient CAM pathways are gradually assembled, rather than evolving completely new metabolic pathways. This strategy is more evolutionarily economical, and it also explains why CAM can appear independently in multiple lineages-many plants already have the potential elements to implement CAM, and only need to change the regulation appropriately. From the perspective of modern breeding, this means that by adjusting the regulatory elements of key genes through gene editing and other means, it may be possible to introduce CAM-like functions into related species.

6. Case Studies in Pitaya Cultivars

6.1 Differences in CAM expression between white- and red-fleshed cultivars

Pitaya can be divided into white-fleshed and red-fleshed varieties according to the color of the flesh, and there are some differences in physiological metabolism between the two. Generally speaking, red-fleshed pitaya varieties accumulate more sugars and pigments such as betacyanin in the flesh, while white-fleshed varieties have slightly higher fiber and water ratios (Zhou et al., 2018). This difference may be partly due to the different activities of the CAM photosynthetic pathway and carbon allocation patterns. Since red-fleshed varieties need to synthesize a large amount of sugars and pigments, their photosynthetic products flow more to the fruit, so during the growth period, they may need stronger nighttime carbon fixation capacity to meet the carbon supply of the fruit.

The survey showed that under the same conditions, the total soluble sugar content of red-fleshed pitaya was significantly higher than that of white-fleshed varieties, and the titratable acid content was also slightly higher. The difference in the sugar-acid ratio between the two makes the red-fleshed variety more flavorful. This indicates that red-fleshed varieties may accumulate more malic acid and convert it into sugar, suggesting that their CAM cycle may be more fully operated. On the other hand, white-fleshed varieties usually have slightly higher yields and larger fruits, which may be because they use more carbon for biomass growth, while red-fleshed varieties invest more carbon into secondary metabolites.

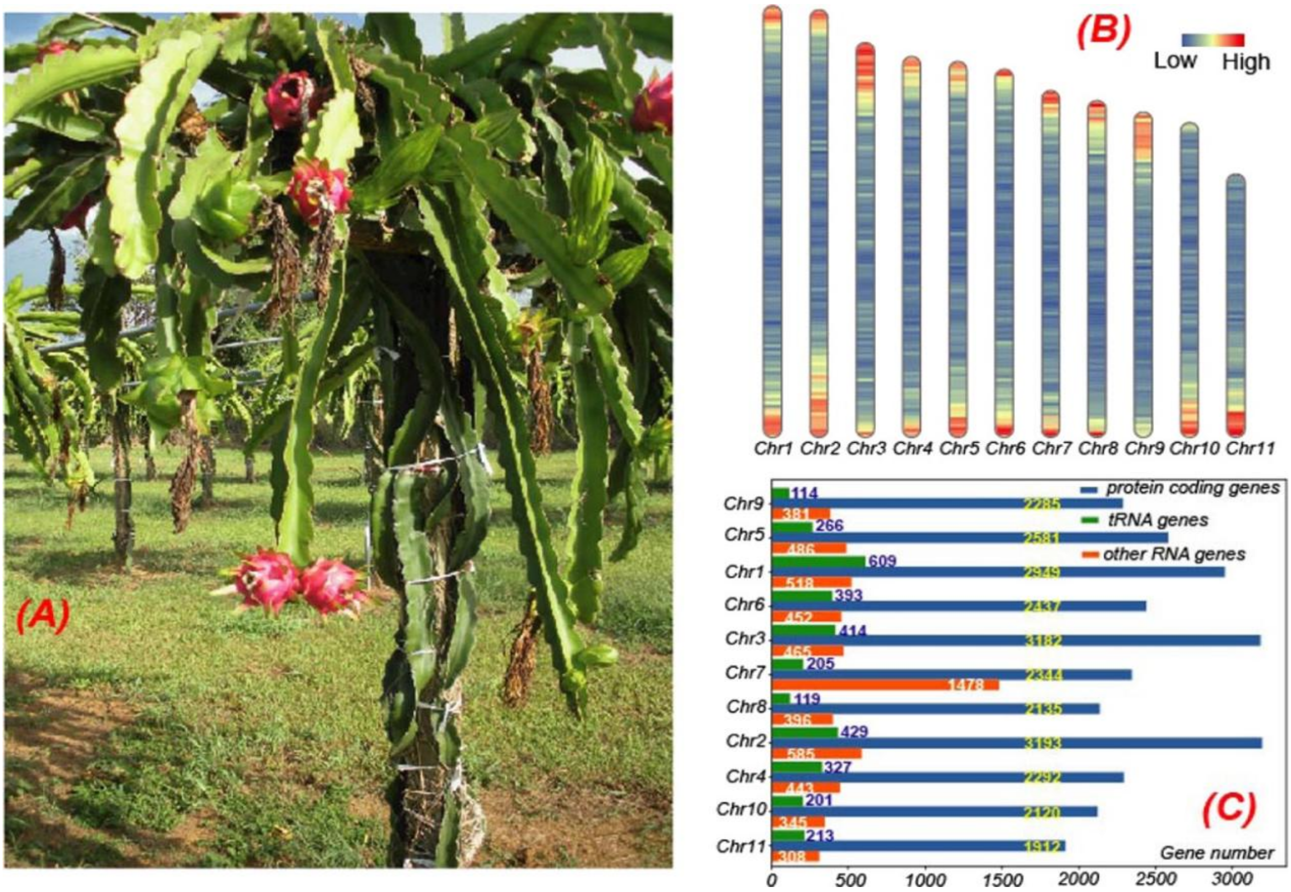

Figure 1 Gene distribution in the 11 longest scaffolds (pseudochromosomes) which account for 88.7% of the dragon fruit draft genome (Adopted from Zheng et al., 2021)

Image caption : Protein-coding genes, noncoding RNA gene, and tRNA genes resided in these scaffolds account for 87.8%, 72.6%, and 58.0% of all these genes, respectively. A A photo of the whole plant of Hylocereus undatus cultivar "David Bowie" from the USDA-ARS Tropical Agriculture Research Station in Mayaquez, Puerto Rico. B Protein-coding gene density of dragon fruit in the 11 longest scaffolds/pseudochromosomes with a window size 100,000 bp, which is plotted by Rldeogram111. C Distribution of protein-coding genes (blue), noncoding RNA genes (including rRNAs, orange), and tRNA genes (green) on the 11 longest scaffolds. The Chr7 (Scaffold 33675) has the most (1478) noncoding RNAs, including 1125 5S rRNAs. The mapping of scaffolds and pseudochromosomes is as follows: Chr1: Scaffold 33678, Chr2: Scaffold 19641, Chr3: Scaffold 33676, Chr4: Scaffold 10417, Chr5: Scaffold 33679, Chr6: Scaffold 33677, Chr7: Scaffold 33675, Chr8: Scaffold 33673, Chr9: Scaffold 33680, Chr10: Scaffold 3410, Chr11: Scaffold 2055 (Adopted from Zheng et al., 2021)

Red-fleshed varieties may have higher PEPC activity and malic acid peak concentrations than white-fleshed varieties at night, thereby supporting their higher sugar accumulation and antioxidant synthesis; while white-fleshed varieties may be more inclined to use fixed carbon for tissue construction (such as cell walls and structural carbohydrates) during the day, which may be reflected in higher fiber content and slightly lower pulp sweetness. It is worth noting that the CAM patterns of red and white-fleshed varieties are essentially the same, and the difference is only in degree. These varietal differences provide natural experimental materials for studying CAM regulation. For example, the differences in CAM enzyme gene expression or malic acid accumulation between red and white-fleshed varieties under drought stress can be compared to find out the varietal factors that affect CAM efficiency. In breeding, it is possible to consider combining the high carbon fixation and high sugar production characteristics of red-fleshed varieties with the high yield characteristics of white-fleshed varieties to cultivate new varieties with both high sweetness and high yield.

6.2 Metabolomic shifts under drought and high temperature

Pitaya shows flexible responses of CAM pathway and carbon metabolism under different adverse conditions. Metabolomics studies can analyze the changing patterns of each metabolite in these responses. Under drought stress, pitaya generally accumulates organic acids, soluble sugars and osmotic regulating substances to enhance drought resistance. Li et al. (2022) showed that as drought intensified, the content of proline (Pro) in pitaya stems and branches increased significantly to help maintain cell osmotic pressure, while malondialdehyde (a product of membrane lipid peroxidation) increased in moderate to severe drought, indicating a certain degree of oxidative stress. At the same time, the activity of antioxidant enzymes such as SOD and POD increased to remove excess reactive oxygen and protect photosynthetic machinery. In terms of metabolites, the malic acid content in pitaya stems may increase during moderate drought, because CO_2 fixation at night is not significantly inhibited, and the plant stores limited carbon sources by accumulating more acid; however, when drought reaches extremes (20% soil water holding capacity), malic acid accumulation may decrease because, on the one hand, the supply of PEPC substrate PEP is limited (starch degradation is reduced), and on the other hand, the plant may enter a conservative mode (CAM-idling), that is, the stomata are almost completely closed, and only the CO_2 released by respiration is recycled in the mesophyll. The effect of heat stress on pitaya CAM is also very significant. When the night temperature continues to be high (>30 ℃), the activity and specificity of PEPC decrease, resulting in a decrease in the CO_2 fixation rate at night.

Jiao et al. (2021) detected that some core carbon metabolic pathways in the seedlings were disturbed after high temperature treatment at 40 ℃: gene expression of enzymes related to sucrose and starch metabolism was downregulated, while pathways related to stress defense such as proline and thermogenic protein synthesis were upregulated. Metabolomics results show that high temperature can lead to a decrease in soluble sugars in pitaya tissues (due to increased respiratory consumption and obstructed transport to the whole plant), while some organic acids (such as fumaric acid and citric acid) may accumulate abnormally, suggesting that the tricarboxylic acid cycle is affected (Figure 2). High temperature may prompt pitaya to enter a partial C3 state to dissipate excess energy, that is, slightly open stomata during the day for transpiration and heat dissipation, but this will increase water loss. Therefore, pitaya often enters a dormant or semi-dormant state under the combined stress of high temperature and drought, reducing the overall metabolic level until the environment improves. Metabolomics can also reveal varietal differences. For example, red-fleshed varieties may accumulate more antioxidant secondary metabolites (such as polyphenols) to protect cells under stress, which has been reflected in the determination that the antioxidant activity of red-fleshed pitaya is stronger than that of white-fleshed pitaya. In the future, through non-targeted metabolomics methods, the metabolic change profiles of pitaya under different stresses (drought, high temperature, salt stress, etc.) can be systematically compared, and then associated with the regulation of the CAM pathway. For example, if the ratio of malic acid to sucrose changes significantly under certain stress conditions, it can be inferred that the carbon fixation and sugar conversion in the CAM cycle are affected. This information is also instructive for formulating cultivation management strategies (such as adjusting irrigation frequency and heatstroke prevention and cooling measures).

6.3 Implications for breeding drought-tolerant, high-sugar varieties

As an economic crop, pitaya has breeding goals including increasing yield, enhancing stress resistance and improving quality (sugar content, nutritional value, etc.). The existence of CAM pathway provides new ideas for achieving these goals. In terms of drought-resistant breeding, the drought resistance of plants can be improved by selecting germplasm with stronger CAM ability. Those pitaya genotypes that can still maintain high malic acid accumulation and CO_2 fixation under drought conditions are undoubtedly excellent drought-resistant materials. Studies on inducible CAM plants have shown that some genotypes can strengthen the CAM mode more quickly when under stress. Corresponding to pitaya, if a variety increases carbon fixation at night in advance under mild drought, its drought resistance and yield stability will be better. Breeders can simulate drought to screen a batch of lines with more acidity increase and less photosynthesis decrease under stress for hybridization or promotion. Breeding to increase the sugar content (sweetness) of fruits can also start from the CAM perspective. The sweetness of the fruit depends on the accumulation and distribution of photosynthetic products. Enhancing the nighttime carbon fixation capacity of pitaya plants (e.g., higher PEPC and MDH activity) will provide more carbon skeletons, potentially increasing the sugar supply of the fruit. Improving the utilization efficiency of CO_2 assimilation during the day and reducing ineffective respiratory losses can also allow more carbohydrates to be delivered to the fruit. CAM plants have lower daytime respiration, which is an advantage, but respiration still exists at night. It is possible to consider breeding types with lower nighttime respiration intensity to save carbon. Furthermore, regulating carbon allocation is also critical, ensuring that the stems have sufficient CAM circulation storage and timely carbon transfer to the fruit. In practice, experienced growers often use water control measures to increase the sweetness of the fruit: moderate water stress before the fruit matures can stimulate the plant to accumulate more sugar and acid and enhance the flavor. The principle behind this is the result of enhanced CAM action and osmotic regulation (Qu et al., 2020; Liu et al., 2024). This experience can be used in breeding selection: screening genotypes whose fruit sugar content significantly increases under water stress as sweet fruit breeding materials. In the longer term, modern molecular breeding such as CRISPR gene editing can also be used for the targeted improvement of pitaya's CAM characteristics. If a transcription factor is known to increase the expression of CAM enzymes, the genome of the cultivated variety can be edited to enhance the function of the factor, thereby achieving the plant's "built-in drought resistance" property without changing the external environment. The many advantages that CAM confers on pitaya provide strategic inspiration for breeding work: through a combination of traditional hybridization and modern biotechnology, high CAM activity, high stress resistance, high yield, high quality and other traits can be integrated into new varieties to meet the needs of agricultural production under future extreme climates.

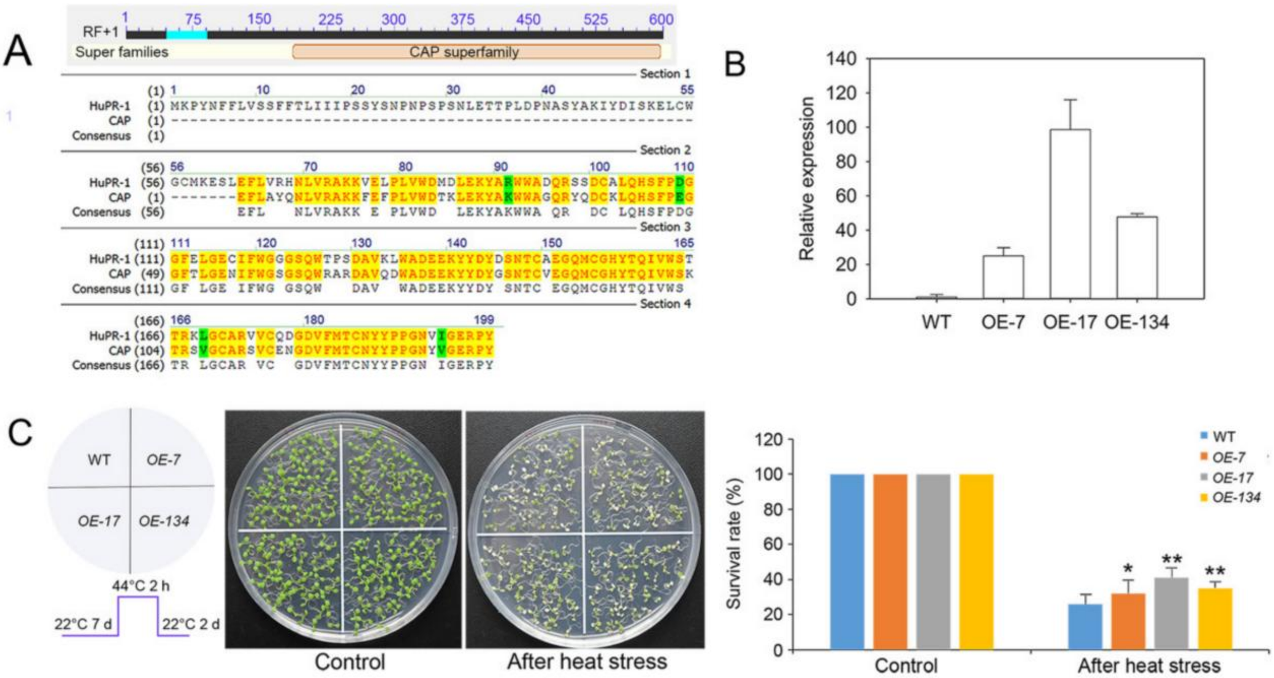

Figure 2 *HuPR-1* gene is involved in the heat stress response of pitaya and its functional verification of enhanced heat tolerance in transgenic Arabidopsis (Adapted from Jiao et al., 2021)

Image caption: (A) Protein sequence alignment result of HuPR-1 in NCBI and protein alignment by Vector NTI Advance 11. (B) The qRT-PCR was used to measure the expression levels of HuPR-1 in transgenic Arabidopsis plants. (C) Survival rates (%) of WT and transgenic seedlings after heat treatment. Seven-day-old seedlings were heat-treated at 44 °C for 2 h and returned to 22 °C to grow for 2 days, and then photographed; following this, the survival rates were calculated. More than 50 plants of each line were analyzed. Bars represent standard deviations. Asterisks indicate statistically significant differences compared with wild type by Student’s t-test (* $p<0.05$, ** $p<0.01$) (Adopted from Jiao et al., 2021)

7 Conclusion

As a representative fruit tree of the Cactaceae family, pitaya has completed the functional transformation of photosynthetic pathway from C3 type to CAM type during the evolution process, and successfully adapted to the extreme environment of arid and semi-arid. The studies reviewed in this article show that pitaya has achieved efficient water utilization and stable carbon acquisition through the CAM mechanism of fixing CO_2 at night and releasing and assimilating it during the day. Its special structure of fleshy stem not only acts as a "leaf" for photosynthesis, but also as a "reservoir" to store water and organic acids, so that photosynthesis and water storage can be achieved at the same time. At the molecular level, pitaya retains most of the basic photosynthetic metabolic genes of ancestral plants, but by changing their expression time and regulation mode, it is assembled into a unique CAM biological clock network. This combination of conservation and innovation has enabled CAM photosynthesis to converge in multiple lineages, and has also given pitaya a significant adaptive advantage. The metabolic regulation of pitaya CAM photosynthesis is exquisite and complex: a large amount of fixed CO_2 is stored in the vacuole in the form of malic acid at night, and is released by a series of transport and enzymatic steps during the day for Calvin recycling, and the carbon supply balance is maintained through dynamic starch/sugar conversion. Pitaya ensures that this circadian rhythm process is coordinated with environmental changes through its internal biological clock and multi-level gene regulation. Because of this, even in the face of periodic drought stress, pitaya can still regulate its own metabolism with the CAM mechanism to maintain basic growth and reproduction functions. Pitaya cleverly combines the evolution of photosynthetic pathways with metabolic regulation, and its successful experience reflects an extreme strategy for plants to adapt to adverse environments.

CAM photosynthesis is not only a weapon for pitaya in competition in nature, but also provides a reference for improving crop resistance and resource utilization efficiency in agricultural production. CAM's extremely high water use efficiency is of great significance for agricultural water conservation. Introducing CAM crops such as pitaya into arid areas can greatly reduce irrigation water requirements and achieve "water-based production". In actual planting, pitaya has been proven to be more drought-resistant than many C3 fruit trees, and its yield is almost unaffected under mild water stress. Therefore, the promotion of CAM economic crops such as pitaya will help expand the utilization of arid marginal land and increase farmers' income. At the same time, for other crops, CAM provides a reference for improvement ideas: introduce CAM key genes or regulatory elements through molecular breeding methods to cultivate "CAM-ized" water-efficient varieties. For example, some studies have envisioned introducing PEPC genes into some C3 crops and inducing their expression under artificial control, so that they can partially perform nighttime carbon fixation during drought, thereby reducing transpiration losses during the day. Although this idea is still in the experimental stage, it reflects the potential of the CAM concept in crop improvement. For pitaya itself, the full play of the CAM mechanism is also directly related to yield and quality. Appropriate water and fertilizer management and environmental control can help pitaya better operate the CAM cycle. For example, moderate cooling at night is conducive to increasing CO_2 fixation, and potassium supplementation is helpful for malic acid storage and balance, thereby ultimately increasing fruit yield and sugar content. Therefore, taking CAM as the entry point for optimizing agronomic measures and improving varieties can simultaneously achieve the goals of high yield and high stress resistance of pitaya.

Looking to the future, the research and application of CAM photosynthesis will play a greater role in the response of pitaya and other crops to climate change. With the development of genome editing and synthetic biology, cross-species CAM engineering is becoming increasingly feasible. Perhaps in the near future, we can accurately modify the biological clock genes or metabolic enzyme genes of pitaya to cultivate new pitaya varieties with higher CAM efficiency and stronger carbon fixation ability at night. These varieties can still maintain high photosynthetic rates and yields under extreme drought conditions, which not only ensures the safety of food and fruits, but also reduces dependence on irrigation water resources. The CAM pathway can be combined with other stress resistance mechanisms (such as antioxidants, thick skin tissue, etc.) to construct comprehensive stress-resistant crops.

The example of pitaya shows that changes in photosynthetic pathways are often accompanied by the co-evolution of multiple traits. For example, the evolution of pitaya CAM also acquired adaptations such as fleshy stems and superoxide dismutase systems. This suggests that breeding work should comprehensively consider the coordinated improvement of multiple stress resistance traits, rather than optimizing a single aspect in isolation. Against the backdrop of global warming, CAM plants may become more important as carbon sinks in ecosystems because they can continuously fix CO_2 in hot and arid environments. Cash crops such as pitaya can also serve as "carbon fixation pioneers" while providing food, so encouraging their cultivation is also conducive to carbon farming and the exploration of climate change mitigation. Finally, gene editing and traditional breeding of pitaya should be combined with local knowledge, such as using the rich wild relatives of pitaya in Southeast Asia and Central America to explore CAM-related alleles evolved in special environments and introduce them into cultivated varieties. This will greatly expand the genetic diversity basis of pitaya to cope with various adversities (drought, flooding, heat, and cold). CAM photosynthesis is not only the secret to the success of pitaya's evolution in the past, but will also become an important tool for us to cultivate "climate-smart" crops in the future. Through interdisciplinary collaboration and innovation, we hope to fully tap the potential of CAM and help agriculture prosper in the era of drought.

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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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