Slicing the genome of star-fruit (*Averrhoa carambola* L.)

Authors

Yannan Fan^{1#}, Sunil Kumar Sahu^{1#}, Ting Yang¹, Weixue Mu¹, Jinpu Wei¹, Le Cheng², Jinlong Yang², Ranchang Mu³, Jie Liu³, Jianming Zhao³, Yuxian Zhao⁵, Xun Xu^{1,6}, Xin Liu¹, Huan Liu^{1,4*}

#These authors contributed equally

*Corresponding author

Running title: Draft genome of the star fruit

Abstract

The *Averrhoa carambola* is commonly known as star fruit because of its peculiar shape and its fruit is a rich source of minerals and vitamins. It is also used in traditional medicines in countries like India, China, the Philippines, and Brazil for treating various ailments such as fever, diarrhea, vomiting, and skin disease. Here we present the first draft genome of the Oxalidaceae family with an assembled genome size of 470.51 Mb. In total, 24,726 protein-coding genes were identified and 16,490 genes were annotated using various well-known databases. The phylogenomic analysis confirmed the evolutionary position of the Oxalidaceae family. Based on the gene functional annotations, we also discovered the enzymes possibly involved in the important nutritional pathways in star fruit genome. Overall, being the first sequenced genome in the Oxalidaceae family, the data provides an essential resource for the nutritional, medicinal, and cultivation studies for this economically important star-fruit plant.

Keywords: star-fruits, medicinal plant, *Averrhoa carambola* L., whole-genome sequencing, phylogenetic tree

Introduction

The star-fruit plant (*Averrhoa carambola* L.), a member of the Oxalidaceae family, is a medium-sized tree which is distinguished for its unique and attractive star-shaped fruit (Supplementary Fig.1). *A. carambola* is widely distributed around the world, especially in tropical countries, such as India, Malaysia, Indonesia, and the Philippines and is considered as an important species, and thus it is extensively cultivated in South-

east Asia and Malaysia ^{1,2}. Besides, it is also a popular fruit in the United States, Australia, and the South Pacific Islands market ³. Star-fruits have a unique taste with slightly tart, acidic (smaller fruits) and sweet, mild flavor (large fruits). The star-fruit is known as a good source of various minerals and vitamins, and it is also rich in natural antioxidants such as vitamin C and gallic acid. Moreover, the presence of high amounts of fibers in fruits aids in absorbing glucose and retarding the glucose diffusion into the bloodstream and helps in controlling blood glucose concentration.

In addition to the food sources, it is also utilized as herbs in India, Brazil, and Malaysia, and it is widely used in traditional Chinese Medicine preparations ⁴, as a remedy for fever, asthma, headache, and skin diseases ⁵. Several studies have demonstrated the presence of various phytochemicals such as saponins, flavonoids, alkaloids and tannins in the leaves, fruits, and roots of star-fruit plant ^{6,7}, which are known to confer antioxidant and specific healing properties. The study of Cabrini *et al.*, ⁵ indicated that the ethanolic extract from *A. carambola* is highly useful in minimizing the symptoms of ear swelling (edema) and cellular migration in mice. The flavonoid compound (apigenin-6-C- β -fucopyranoside), which isolated from *A. carambola* leaves, showed the anti-hyperglycemic action in rats, and this might be a potential treatment and prevention of diabetes⁸. Moreover, the DMDD (2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione) extracted from the root of *A. carambola* exhibits potential benefits against obesity, insulin resistance, and memory deficits in Alzheimer's disease ^{9,10}.

Even though *A. carambola* plays very significant roles in traditional medicine applications, there are very limited studies at the genetic level on *A. carambola*., mainly due to the lack of genome information. Therefore, filling this genomic gap will help the researchers to fully explore and understand this agriculturally important plant. As a part of 10KP project ^{11,12}, in this study, the draft genome of *A. carambola* collected from Ruili botanical garden, Yunnan, China was assembled using advanced 10X genomics technique to further understand the evolution of Oxalidaceae family. Furthermore, a fully annotated genome of *A. carambola* would serve as a foundation for the pharmaceutical applications and in the improvement of breeding strategies of the star-fruit plant.

Results

Genome assembly and evaluation

Based on the k-mer analysis, the total number of 35,655,285,391 kmers were used, with a peak coverage is 75. The *A. carambola* genome was estimated to be ~475 Mb in size (Supplementary Fig. 2). To perform the assembly, a total of 156 Gb clean reads were utilized by Supernova v2.1.1¹³. The final assembly contained 69,402 scaffold sequences with N50 of 2.76 Mb and 78,313 contig sequences with N50 of 44.84 Kb for a total assembly size of 470.51 Mb (Table 1). Completeness assessment was performed using BUSCO (Bench-marking Universal Single-Copy Orthologs) version 3.0.1¹⁴ with

Embryophyta odb9. The result showed that 1327 (92.20%) of the expected 1440 conserved plant orthologs were detected as complete (**Supplementary Table 1**). In order to further evaluate the completeness of the assembled genome, we performed short reads mapping using clean raw data by BWA-MEM software ¹⁵. In total, 943,278,896 (99.12%) reads can be mapped to the genome and 88.13% of them were properly paired (**Supplementary Table 2**).

Table 1. Statistics of genome assembly

	Contig		Scaffold	
	Size(bp)	Number	Size(bp)	Number
N90*	5,420	12,457	7,033	3,988
N80	13,875	7,548	39,210	608
N70	23,325	5,165	34,6109	131
N60	33,460	3,619	1,307,770	60
N50	44,841	2,503	2,757,598	35
Longest	717,770	-	14,768,062	-
Total Size	431,262,337	-	470,508,511	-
Total number (≥2kb)	-	18,820	-	10,777
Total number (≥100bp)	-	78,313	-	69,402

*: Nxx length is the maximum length L such that xx% of all nucleotides lie in contigs (or scaffolds) of size at least L.

Genome annotation

A total of 68.15% of the assembled *A. carambola* genome was composed of repetitive elements (**Supplementary Table 3**). Among these repetitive sequences, the LTRs were the most abundant, accounting for 61.64% of the genome. DNA class repeat elements represented 4.19% of the genome; LINE and SINE classes encoded 0.28% and 0.016% of the assembled genome, respectively. For the gene prediction, we combined homology- and *de novo*- based approaches and obtained a non-redundant set of 24,726 gene models with 4.11 exon per gene on average. The gene length was 3,457 bp on average, while the average exon and intron lengths were 215 bp and 827 bp, respectively. The gene model statistics data compared with the other seven homology species are shown in Supplementary Fig 3. To evaluate the completeness of the gene models for *A. carambola*, we used BUSCO with Embryophyta odb9. There are 1281 (88.9%) complete orthologs were detected by the star fruit predicted gene sets

(Supplementary Table 4).

Functions were assigned to 16,490 (66.69%) genes. These protein-coding genes were then subjected for further exploration against KEGG, NR and COGs protein sequence databases ¹⁶, in addition to SwissProt, and TrEMBL ¹⁷, and then InterProScan ¹⁸ was lastly used to identify domains and motifs (**Supplementary Table 5, Supplementary Fig. 4**). Non-coding RNA genes in the assembled genome were also annotated. We predicted 759 tRNA, 1,341 rRNA, 90 microRNA (miRNA) and 2,039 small nuclear RNA (snRNA) genes in the assembled genome (**Supplementary Table 6**).

Since star fruit is an important cultivated plant, the identification of disease-resistance genes was one of the focus in our study. The NBS (nucleotide-binding site) genes play an important role in pathogen defense and cell cycle. We identified a total of 80 non-redundant NBS-encoding orthologous genes in the star fruit genome (**Supplementary Table 7**). Among those genes, the TIR (encoding the toll interleukin receptor) motif was found to be significantly smaller than other eudicot plants, except cocoa. Unlike other plants, the LRR (Leucine rich repeat) motif in star fruit is not the most or second most type in NBS gene family ¹⁹.

Genome evolution

The characterization of star fruit genome can provide the necessary features to further analyze the evolutionary history of Oxalidaceae. The γ whole genome triplication event represents over 75% of extant angiosperms, and is associated with the early diversification of the core eudicots. In order to investigate the evolutionary events at the genomic level in star fruit, we identified 1,134 paralogous gene families on the basis of the 24,726 gene models. The synonymous substitution rates (Ks) in the duplicated genes (Ks = 1.9) suggested that an ancient γ event occurred in star fruit (**Fig. 1a**). Furthermore, we performed an intergenomic collinearity between *Arabidopsis* ²⁰, Poplar ²¹, and grape ²² genomes and identified relationships among star fruit orthologous. The mean Ks for the one-to-one orthologous analysis of star fruit to *Arabidopsis*, Poplar, and grape was 1.8, 1.0, and 1.2, respectively (**Fig. 1b-d**). The result confirmed the shared γ WGD event between the four species. Moreover, we also generated the whole-genome syntenic dotplots of star fruit based on the Ks value (**Fig. 1e**). Over 50% of the syntenic blocks shared the Ks rate between 1.0 to 2.0, and only about 10% of the gene pairs shared the Ks under 1.0, which indicated that no recent WGDs occurred in the star fruit genome.

Figure 1. The *A. carambola* genome duplication. The distribution of synonymous substitution rate (K_s) distance values observed for (a) *A. carambola*-paralog, (b) *A. carambola*-*A. thaliana* ortholog, (c) *A. carambola*-*V. vinifera* ortholog, and (d) *A. carambola*-*P. trichocarpa* ortholog. (e) The colinear blocks of *A. carambola* paralog colored by K_s value.

Gene family analysis and phylogenetic tree

We performed *A. carambola* gene family analysis using OrthoMCL software²³, with protein and nucleotide sequences from *A. carambola* and ten other plant species (*A. thaliana*, *C. sinensis*, *F. sylvatica*, *G. max*, *K. fedtschenkoi*, *M. domestica*, *P. granatum*, *P. trichocarpa*, *T. cacao*, *V. vinifera*) based on an all-versus-all BLASTP alignment with an E-value cutoff of $1e-05$. The predicted 24,726 protein-coding genes in *A. carambola* were assigned to 9,731 gene families consisting of 15,301 genes, while 9,425 were not organized into groups, which were unique for *A. carambola* (**Supplementary Table 8, Fig. 2b**). In total, 163 single-copy orthologs corresponding to the eleven species were extracted from the clusters and were used to construct the phylogenetic tree. The constructed tree topology supported the APG IV²⁴ system that Oxalidales (*A. carambola*) and Malpighiales (*P. trichocarpa*) are sister clades which belong to the same cluster Rosids. Based on the phylogenetic tree, *A. carambola* was estimated to separate from *P. trichocarpa*, *V. vinifera* and *K. fedtschenkoi* approximately 94.5, 110.2 and 126.3 Mya, respectively (**Supplementary Fig. 5**).

We also analyzed the expansion and contraction of the gene families between species using CAFÉ. The result showed that 888 gene families were substantially

expanded and 15,724 gene families were contracted in *A. carambola* (Fig. 2a). In total, 2,916 and 6,057 genes of *A. carambola* were identified from expanded and contracted families, respectively. which the contraction was about 2 times more than expansion.

Later, the Gene Ontology (GO) and KEGG functional enrichment analyses were performed for all expansion gene families. The KEGG pathway enrichment analysis results are shown in Table 2 and the GO enrichment are listed in Supplementary Table 9. In a previous study, researchers had isolated the flavonoids from the fresh fruit of *A. carambola*, which are known to reduce the harmful inflammation²⁵. In our study, the flavonoid biosynthesis pathway was found to be significantly enriched in the expanded families. Terpenoids are yet another important type of compound which has been isolated from star fruit²⁶, which has been proven to exhibit anti-inflammatory activities. *A. carambola* likely synthesize terpenoids via the Diterpenoid biosynthesis pathway.

Figure 2. Gene family analysis and phylogenetic tree construction. (a) The phylogenetic tree with the expansion and contraction size of gene families. Pungra: *P. granatum*; Poptri: *P. trichocarpa*; Avecar: *A. carambola*; Thecac: *T. cacao*; Aratha: *A. thaliana*; Citsin: *C. sinensis*; Fagsyl: *F. sylvatica*; Maldom: *M. domestica*; Glymax: *G. max*; Vitvin: *V. vinifera*; Kalfed: *K. fedtschenkoi*. (b) The Venn diagram for the number of shared gene families within *A. carambola*, *V. vinifera*, *T. cacao* and *P. trichocarpa*.

Table 2. Enriched KEGG pathways of unique genes of *A. carambola* with expansion.

Pathway ID	KEGG description	Adjusted P-value (≤ 0.05)	Number of genes	Total genes in the pathway
map03020	RNA polymerase	3.51E-09	30	137
map00904	Diterpenoid biosynthesis	1.00E-05	18	63
map00240	Pyrimidine metabolism	3.16E-05	34	215

map01100	Metabolic pathways	7.17E-05	234	2621
map00901	Indole alkaloid biosynthesis	7.17E-05	15	55
map00230	Purine metabolism	7.17E-05	36	244
map00565	Ether lipid metabolism	0.00014092	14	52
map01110	Biosynthesis of secondary metabolites	0.00038039	142	1478
map02010	ABC transporters	0.00080624	20	119
map00902	Monoterpenoid biosynthesis	0.00116462	9	29
map00460	Cyanoamino acid metabolism	0.00270665	18	110
map00941	Flavonoid biosynthesis	0.03063434	17	120
map00190	Oxidative phosphorylation	0.0354555	19	142
map00940	Phenylpropanoid biosynthesis	0.0354555	32	280
map00062	Fatty acid elongation in mitochondria	0.0354555	7	32
map00563	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0.03937265	8	41
map00040	Pentose and glucuronate interconversions	0.0426558	21	167

186

187 Genes specially involved in star fruit nutrition pathways

188 The star-fruit is an excellent source of various minerals and vitamins, especially for
189 natural antioxidants such as L-ascorbic acid (vitamin C) and riboflavin (vitamin B₂)^{1,25}.
190 Through the ortholog search in the KEGG pathway, we identified the enzymes which
191 are potentially involved in the vitamin C and vitamin B₂ biosynthesis pathways in *A.*
192 *carambola*.

193 In a previous report, a major component of plant ascorbate was reported to
194 synthesize through the l-galactose pathway²⁷, in which GDP-d-mannose is converted
195 to l-ascorbate by four successive intermediates, as summarized in Fig. 3a. Laing *et al.*
196²⁸ reported the identification of l-galactose guanylyltransferase encoding homologous
197 genes from *Arabidopsis* and kiwifruit which catalyzes the conversion of GDP-l-
198 galactose to l-galactose-1-P. In this study, five necessary enzymes - GalDH, GalLDH,
199 GGalPP, GalPP, and GME were identified, which are involved in the vitamin C pathway,
200 suggesting the possibility of ascorbic acid synthesis in the star-fruit (**Table 3**). For the
201 L-galactose dehydrogenase (GalDH) gene, we identified 4 paralogous genes in the star
202 fruit genome. The copy number of the GalDH genes in star fruit is close to the tomato
203 (5) and papaya (4), but nearly half of other species (10 in poplar, 11 in orange, 8 in
204 *Arabidopsis*, and 13 in grape, **Supplementary Table 12**). Further evolutionary analysis
205 showed three clusters in the phylogenetic tree, and the most ancient cluster comprised
206 all the grape genes. In the other two sister clusters, one is ancient from poplar including
207 4 genes, the other is closer to orange including 7 genes. The 4 genes in star fruit are
208 divided into two clusters and were recently separated from their ancestors (**Fig. 3b**).

209

210

Figure 3. Genes involved in vitamin C metabolism. (a) A proposed model for L-ascorbic acid biosynthesis pathways in star fruit. Genes identified as being involved in the pathways shown in the blue circle. (b) Phylogenetic analysis of the GalDH gene family in *A. carambola* (rose red), *A. thaliana* (purple), *C. sinensis* (yellow), *C. papaya* (blue), *P. trichocarpa* (sky blue), *S. lycopersicum* (orange), and *V. vinifera* (green).

GalDH: L-galactose dehydrogenase; GalLDH: L-galactono-1,4-lactone dehydrogenase; GGalPP: GDP-L-galactose phosphorylase; GalPP: L-galactose-1-phosphate phosphatase; GME: GDP-D-mannose-3',5'-epimerase.

We also identified the possible enzymes involved in riboflavin (vitamin B₂) biosynthesis pathway in the star-fruit (**Fig. 4a, Table 3**). Through the catalyzation by RIB3, RIB4, and RIB5, the D-Ribulose 5-phosphate compound can finally produce the riboflavin. Furthermore, to investigate the possible biosynthesis pathway for the special product oxalate in star fruit, we identified three enzymes – CS (citrate synthase), aceA (isocitrate lyase), and ACO (aconitate hydratase) which can potentially catalyze oxalacetic acid to glyoxylate within the glyoxylate and dicarboxylate metabolism pathway (**Supplementary Table 10**).

In the *A. carambola* gene family analysis, the KEGG pathway enrichment analysis for the expanded gene families revealed that 17 genes participate in flavonoid synthesis pathway (P-value = 0.03, **Table 3**). The previous studies proved that flavonoids can be isolated from *A. carambola* and other plants from the Oxalidaceae family^{1,9,25,29-32}. Here, we identified 11 enzymes that could be potentially involved in the flavonoid biosynthesis pathway (**Fig. 4b, Supplementary Table 11**) The most two enzymes in the pathway which contain 23 and 21 genes are HCT (shikimate O-

hydroxycinnamoyltransferase) and CHS (chalcone synthase), respectively. Among the end-point products, apigenin, cyanidin, epicatechin, and quercetin has been extracted from the leaves, fruits or barks of *A. carambola* in previous studies ^{6,33-35}.

Table 3. List of genes involved in vitamin C and Vitamin B₂ pathway

pathway	Enzyme	Description	Copy numbers	Gene ID	Protein (AA)
Vitamin C pathway	GalDH	L-galactose dehydrogenase	4	Aca.sc093668.g0.5	332
				Aca.sc000054.g1.6	334
				Aca.sc000055.g24.2	351
				Aca.sc000055.g24.3	594
	GalLDH	L-galactono-1,4-lactone dehydrogenase	5	Aca.sc000059.g19.35	601
				Aca.sc000229.g0.29	603
				Aca.sc000078.g43.5	598
				Aca.sc100684.g0.3	99
				Aca.sc102574.g0.3	99
	GGalPP	GDP-L-galactose phosphorylase	3	Aca.sc150475.g0.5	275
				Aca.sc150564.g0.4	288
				Aca.sc098705.g0.4	410
	GalPP	L-galactose-1-phosphate phosphatase	2	Aca.sc006151.g1	181
				Aca.sc000246.g13.57	360
	GME	GDP-D-mannose-3',5'-epimerase	2	Aca.sc096116.g0.5	383
				Aca.sc000061.g13.42	383
Vitamin B ₂ pathway	RIB3	3,4-dihydroxy 2-butanone 4-phosphate synthase	3	Aca.sc000063.g38.15	545
				Aca.sc000056.g65.27	572
				Aca.sc000071.g11.34	590
	RIB4	6,7-dimethyl-8-ribityllumazine synthase	1	Aca.sc023103.g76.2	185
	RIB5	riboflavin synthase	1	Aca.sc000036.g2.16	343
	RFK	riboflavin 5'-phosphotransferase	1	Aca.sc000058.g77.42	539
	FLAD1	FMN adenylyltransferase	1	Aca.sc000058.g25.58	520

Figure 4. Identification of genes involved in the (a) riboflavin and (b) flavonoid biosynthesis pathways. Genes identified as being involved in two pathways shown in the blue circle.

FLAD1: FMN adenylyltransferase; RIB3: 3,4-dihydroxy 2-butanone 4-phosphate synthase; RIB4: 6,7-dimethyl-8-ribityllumazine synthase; RIB5: riboflavin synthase; RFK: riboflavin 5'-phosphotransferase; ANR: anthocyanidin reductase; ANS: leucoanthocyanidin dioxygenase; CCOAMT: caffeoyl-CoA O-methyltransferase; CHI: chalcone isomerase; CHS: chalcone synthase; CYP75A: flavonoid 3',5'-hydroxylase; CYP75B1: flavonoid 3'-monooxygenase; CYP98A: coumaroylquinic acid 3'-monooxygenase; DFR: flavanone 4-reductase; F3H: naringenin 3-dioxygenase; HCT: shikimate O-hydroxycinnamoyltransferase.

Discussion

This study presents the first draft genome in the Oxalidaceae family. The sequenced species *A. carambola* (star fruit) is a widely cultivated and utilized as an edible fruit and serves as an important source of minerals, vitamins, and phytomedicinal properties. The genome size was assembled to be 470.51 Mb with scaffold N50 of 2.76 Mb. Since we cannot compare the genome size with other species in this family, we have found a similar genome size, which is 434.29 Mb of *Populus trichocarpa* and 350.62 Mb of *Ricinus communis* in the closest order Malpighiales. However, the genome of the chromosome level will be further required to better understand the diploid character of this species.

Totally, 24,726 gene models were identified from *A. carambola*. The gene number is relatively smaller than the earlier reports in *A. thaliana*, *P. trichocarpa*, or *T. cacao*. The length distribution of exons of prediction star fruit proteins was consistent with

other species, although its intron and CDS length tended to be shorter than other species in the comparison (**Supplementary Fig. 3**). The proportion of predicted genes that contain InterPro functional domain is 52.3%, and that can be aligned with NCBI nr database (66.4%) are the highest among all databases. It is likely that *A. carambola* is so far the only species which has been assembled in the Oxalidaceae family; there might be some evolutionarily unique genes in this family remaining to be annotated.

Later we performed gene family analysis together with the other ten species and identified the significant expansion of 888 gene families contains 2,916 unique genes in *A. carambola*. These genes have significantly enriched (P-value ≤ 0.05) within 28 GO classes, including 18 biological processes, 2 cellular components, and 8 molecular functions (**Supplementary Table 6**). The DNA binding biological process contained the most genes (60) within the expanded families. Within the significantly enriched biological processes, defense response to fungus might be related to the anti-microbial property of star fruits in the previous studies¹. On the other hand, we found that oxidoreductase activity is enriched in the molecular function GO class, which could be one of the potential reasons for the antioxidant activity in star fruits.

The genome evolution analysis indicated that star fruit only shared an ancient γ event, and no recent WGD was observed. In the genome evolution study of poplar which belongs to Malpighiales, the presence of a hexaploidization event and recent duplication have been reported²¹. This result could partially explain why star fruit has half of the gene sets compared to poplar.

Moreover, within the enriched KEGG pathway, we identified the enzymes involved in nutrition metabolic pathways – vitamin C, vitamin B₂, oxalate, and flavonoid pathways. Although the potential functional enzymes have been identified from the genome, those functional pathways should be verified by the experimental studies in the future.

In the summary, it can be expected that this draft genome will facilitate in understanding the formation of specific and important traits in the star-fruit plant, such as the discovery of biosynthesis pathways of pharmacologically active metabolites, and in the improvement of breeding of strategies of the star-fruit plant.

Materials and methods

Plant materials and sequencing

The leaf samples of *A. carambola* were collected from the Ruili Botanical Garden, Yunnan, China. The genomic DNA was extracted by using CTAB (cetyl-triethylammonium bromide) method³⁶. The 10X Genome sequencing was performed to obtain high-quality reads. The high-molecular-weight (HMW) DNA was loaded onto a Chromium Controller chip with 10X Chromium reagents and gel beads, and the rest of the procedures were carried out as per the manufacturer's protocol³⁷. Then, BGISEQ-500 platform to produce 2 × 100 bp paired-end sequences, generating a total

of 183 Gb raw data. The raw reads were filtered using SOAPfilter v2.2 with the following parameters “-q 33 -i 600 -p -l -f -z -g 1 -M 2 -Q 20”. After filtering low-quality reads, around 157 Gb of clean data (high-quality reads >Q35) remained for the next step.

Estimation of *A. carambola* genome size

All the 157 Gb clean reads obtained from the BGISEQ-500 platform were subjected to the 17 kmer frequency distribution analysis with Jellyfish³⁸ using the parameters “-k 17 -t 24”. The frequency graph was drawn, and the *A. carambola* genome size was calculated using the formula: genome size = k-mer_Number/Peak_Depth.

***De novo* genome assembly**

The Linked-Read data were assembled using the Supernova v2.1.1 software¹³ using the “--localcores=24 --localmem=350 --max reads 280000000” parameter. In order to fill the scaffold gaps, GapCloser version 1.12³⁹ was used with the parameters “-t 12 -l 155”. Finally, the total assembled length of *A. carambola* genome was 470.51 Mb, with a scaffold N50 of 275.76 Kb and contig N50 of 44.84 Kb, respectively.

Repeat annotation

For the transposable element annotation, RepeatMasker v3.3.0⁴⁰ and RepeatProteinMasker v3.3.0⁴⁰ were used against Repbase v16.10⁴¹ to identify known repeats in the *A. carambola* genome. Tandem repeats were identified using software Tandem Repeats Finder v4.07b⁴². *De novo* repeat identification was conducted using RepeatModeler v1.0.5⁴³ and LTR_FINDER v1.05⁴⁴ programs, followed by RepeatMasker v3.3.0⁴⁰ to obtain the final results.

Gene prediction

Prior to the gene prediction analysis, we masked the repetitive regions of the genome. The MAKER-P v2.31⁴⁵ was utilized to predict the protein-coding genes based on homologous, and *de novo* prediction evidence. For homologous-based prediction, protein sequences of *Theobroma cacao*, *Prunus persica*, *Prunus mume*, *Prunus avium*, *Populus trichocarpa*, *Populus euphratica*, and *Arabidopsis thaliana* were aligned against *A. carambola* genome using BLAT⁴⁶. Then, the gene structure was predicted using GeneWise⁴⁷. In order to optimize different *ab initio* gene predictors, we constructed a series of training set for *de novo* prediction evidence. Complete gene models by homologous evidence were picked for training in Augustus tool⁴⁸. Genemark-ES v4.21⁴⁹ was self-trained using the default criteria. The first round of MAKER-P was also run using the default parameters on the basis of above evidences, with the exception for “protein2genome” which was set to “1”, yielding only protein-supported gene models. SNAP⁵⁰ was then trained with these gene models. Default parameters were used to run the second and the final rounds of MAKER-P, generating the final gene models.

Functional annotation

The predicted gene models were functionally annotated by aligning their protein sequences against the Kyoto Encyclopedia of Genes and Genomes (KEGG)⁵¹, the Clusters of Orthologous Groups (COG)¹⁶, SwissProt¹⁷, TrEMBL, and the National Center for Biotechnology Information (NCBI) non-redundant (NR) protein databases with BLASTP (E-value $\leq 1e-05$). Protein motifs and domains were identified by comparing the sequences against various domain databases, including PFAM, PRINTS, PANTHER, ProDom, PROSITE, and SMART using InterProScan v5.21¹⁸. For ncRNA annotation, tRNA genes were identified by tRNAscan-SE v1.23⁵². For the identification of rRNA genes, we aligned the assembled data against the rRNA sequences of *A. thaliana* using BLASTN (E-value $\leq 1e-05$). The miRNAs and snRNAs were predicted by using INFERNAL⁵³ software against the Rfam database⁵⁴.

To classify the NBS domain in the star fruit protein sequences, we used hidden Markov models (HMM) to search for the Pfam NBS (NB-ARC) family PF00931 with E-value cutoff of 1.0. To detect TIR domains, the amino acid sequences were also screened by HMM model Pfam TIR PF01582 (E-value ≤ 1.0). Moreover, to identify LRR motifs, we performed the HMM search for Pfam LRR models (E-value ≤ 1.0) against star fruit NBS-encoding amino acid sequences.

In order to compare the ortholog genes in vitamin C, vitamin B₂, flavonoid and oxalate pathways between other plant species (*P. trichocarpa*, *C. sinensis*, *A. thaliana*, *S. lycopersicum*, *C. papaya*, and *V. vinifera*), we also annotated the protein sequences by aligning them against the KEGG database⁵¹ with BLASTP (E-value $\leq 1e-05$) and then filtered by Pfam domains which annotated using InterProScan v5.21¹⁸.

Genome duplication

The genome sequences for orthologous analysis were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov>) for *A. thaliana* (GCA_000001735.2), *V. vinifera* (GCA_000003745.2), and *P. trichocarpa* (GCA_000002775.3). Next, we used wgd software⁵⁵ to perform the Ks distribution analysis. The paralogous gene families and one-to-one orthologs were performed using “wgd mcl” command. Then, a Ks distribution for a set of paralogous families or one-to-one orthologs was constructed using the “wgd ksd”. Next, we performed “wgd kde” for fitting kernel density estimates (KDEs). Finally, the colinear blocks of *A. carambola* paralog were identified by I-ADHoRe⁵⁶, and colored by their median Ks value.

Gene family construction and phylogenetic analysis

For gene family analysis, the OrthoMCL²³ software was utilized to construct orthologous gene families on all the protein-coding genes of *A. carambola* and 12 sequenced plant species (*B. napus*, *G. raimondii*, *H. brasiliensis*, *J. curcas*, *L. usitatissimum*, *M. esculenta*, *P. euphratica*, *P. trichocarpa*, *P. mume*, *R. communis*, *T. cacao*, *V. vinifera*). Before OrthoMCL, the BLASTP was used to find similar matches

from different species with an E-value cutoff of 1e-05. The composition of the OrthoMCL clusters was used to calculate the total number of gene families. Orthogroups that were single copy in all species analyzed were selected and aligned using MAFFT v7.310⁵⁷. Each gene tree was constructed by RAxML v8.2.4⁵⁸ with GTRGAMMA model. To construct the species phylogenetic tree, a coalescent-based method by ASTRAL v4.10.4⁵⁹, with 100 replicates of multi-locus bootstrapping⁶⁰ were used.

The divergence time between *A. carambola* and other species were estimated using MCMCTREE⁵⁸ with the default parameter. The expansion and contraction of gene family numbers were predicted using the CAFÉ⁶¹ by employing the phylogenetic tree and gene family statistics.

To further perform the phylogenetic analysis for the key enzyme GalDH in vitamin C pathway, we annotated ortholog genes from 6 other plant species using BLASTP with an E-value cutoff of 1e-05 to align coding sequences against KEGG database. In total, 55 ortholog genes were used to generate phylogenetic tree by using maximum likelihood (ML) method in RAxML v 8.2.4⁵⁸ and included 20 runs to find an optimal tree using GTRGAMMA substitution model and 100 nonparametric Bootstrap replicates.

Data availability

The data sets generated and analyzed during the current study are available in the CNGB Nucleotide Sequence Archive (CNSA: <https://db.cngb.org/cnsa>). The raw sequencing data is under ID CNR0066625, and assembly data is under ID CNA0002506. All other data generated or analyzed during this study are included in this published article and its supplementary information files.

Acknowledgments

This work was supported by funding from the National Key R&D Program of China (No. 2019YFC1711000), Shenzhen Municipal Government of China (grants JCYJ20170817145512476 and JCYJ20160510141910129), the Guangdong Provincial Key Laboratory of Genome Read and Write (grant 2017B030301011), and the NMPA Key Laboratory for Rapid Testing Technology of Drugs.

Author information

1. State Key Laboratory of Agricultural Genomics, China National GeneBank, BGI-Shenzhen, Shenzhen 518120, China
Yan-nan Fan, Sunil Kumar Sahu, Ting Yang, Wei-xue Mu, Jinpu Wei, Xun Xu, Xin Liu & Huan Liu

2. BGI-Yunnan, BGI-Shenzhen, Kunming, 650106, China

Le Cheng & Jin-long Yang

3. Forestry Bureau of Ruili, Yunnan Dehong, Ruili 678600, China

Ran-chang Mu, Jie Liu & Jian-ming Zhao

4. Department of Biology, University of Copenhagen, Copenhagen, Denmark.

Huan Liu

5. Chinese academy of forestry, Beijing, China

Yu-xian Zhao

6. Guangdong Provincial Key Laboratory of Genome Read and Write

Xun Xu

Contributions

R.-c.M., J.L., J.-m.Z., T.Y., Y.-x.Z. and W.-x.M. collected the samples; W.-x.M and S.K.S. conceived and conducted the experiments; Y.-n.F. and T.Y. analyzed the results; Y.-n.F. and S.K.S wrote the manuscript.

Corresponding authors

Correspondence to Huan Liu liuhuan@genomics.cn

Conflict of interest

The authors declare that they have no conflict of interest.

References

- 1 Muthu, N., Lee, S. Y., Phua, K. K. & Bhore, S. J. Nutritional, Medicinal and Toxicological Attributes of Star-Fruits (*Averrhoa carambola* L.): A Review. *Bioinformation* **12**, 420-424, doi:10.6026/97320630012420 (2016).
- 2 Khoo, H. *et al.* A review on underutilized tropical fruits in Malaysia. *Guangxi Agricultural Sciences* **41**, 698-702 (2010).
- 3 Ray, P. K. *Breeding Tropical and Subtropical Fruits*. XVI, 338 (Springer-Verlag Berlin Heidelberg, 2002).
- 4 Wu, S.-C., Wu, S.-H. & Chau, C.-F. Improvement of the Hypocholesterolemic Activities of Two Common Fruit Fibers by Micronization Processing. *Journal of Agricultural and Food Chemistry* **57**, 5610-5614, doi:10.1021/jf9010388 (2009).
- 5 Cabrini, D. A. *et al.* Analysis of the Potential Topical Anti-Inflammatory Activity of *Averrhoa carambola* L. in Mice. *Evid Based Complement Alternat Med* **2011**, 908059-908059, doi:10.1093/ecam/neq026 (2011).

- 463 6 Shui, G. & Leong, L. P. Analysis of polyphenolic antioxidants in star fruit using liquid
464 chromatography and mass spectrometry. *Journal of Chromatography A* **1022**, 67-75 (2004).
- 465 7 Annegowda, H. V., Bhat, R., Min-Tze, L., Karim, A. A. & Mansor, S. M. Influence of
466 sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. *J*
467 *Food Sci Technol* **49**, 510-514, doi:10.1007/s13197-011-0435-8 (2012).
- 468 8 Cazarolli, L. H. *et al.* Anti-hyperglycemic action of apigenin-6-C- β -fucopyranoside from
469 Averrhoa carambola. *Fitoterapia* **83**, 1176-1183 (2012).
- 470 9 Suluvoy, J. K., Sakthivel, K. M., Guruvayoorappan, C. & Berlin Grace, V. M. Protective effect
471 of Averrhoa bilimbi L. fruit extract on ulcerative colitis in wistar rats via regulation of
472 inflammatory mediators and cytokines. *Biomedicine & Pharmacotherapy* **91**, 1113-1121,
473 doi:<https://doi.org/10.1016/j.biopha.2017.05.057> (2017).
- 474 10 Wei, X. *et al.* Protective Effects of 2-Dodecyl-6-Methoxycyclohexa-2,5 -Diene-1,4-Dione
475 Isolated from Averrhoa Carambola L. (Oxalidaceae) Roots on Neuron Apoptosis and Memory
476 Deficits in Alzheimer's Disease. *Cellular Physiology and Biochemistry* **49**, 1105-1114,
477 doi:10.1159/000493289 (2018).
- 478 11 Cheng, S. *et al.* 10KP: A phylodiverse genome sequencing plan. *Gigascience* **7**, giy013
479 (2018).
- 480 12 Liu, H. *et al.* Molecular digitization of a botanical garden: high-depth whole genome
481 sequencing of 689 vascular plant species from the Ruili Botanical Garden. *GigaScience*
482 (2019).
- 483 13 Weisenfeld, N. I., Kumar, V., Shah, P., Church, D. M. & Jaffe, D. B. Direct determination of
484 diploid genome sequences. *Genome research* **27**, 757-767 (2017).
- 485 14 Waterhouse, R. M. *et al.* BUSCO applications from quality assessments to gene prediction and
486 phylogenomics. *Molecular biology and evolution* **35**, 543-548 (2017).
- 487 15 Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.
488 *arXiv preprint arXiv:1303.3997* (2013).
- 489 16 Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. The COG database: a tool for
490 genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* **28**, 33-36 (2000).
- 491 17 Bairoch, A. & Apweiler, R. The SWISS-PROT protein sequence database and its supplement
492 TrEMBL in 2000. *Nucleic acids research* **28**, 45-48 (2000).
- 493 18 Quevillon, E. *et al.* InterProScan: protein domains identifier. *Nucleic acids research* **33**,
494 W116-W120 (2005).
- 495 19 Argout, X. *et al.* The genome of Theobroma cacao. *Nature genetics* **43**, 101,
496 doi:10.1038/ng.736 (2011).
- 497 20 Swarbreck, D. *et al.* The Arabidopsis Information Resource (TAIR): gene structure and
498 function annotation. *Nucleic Acids Research* **36**, D1009-D1014, doi:10.1093/nar/gkm965
499 (2007).
- 500 21 Tuskan, G. A. *et al.* The genome of black cottonwood, Populus trichocarpa (Torr. & Gray).
501 *science* **313**, 1596-1604, doi:10.1126/science.1128691 (2006).
- 502 22 Jaillon, O. *et al.* The grapevine genome sequence suggests ancestral hexaploidization in major
503 angiosperm phyla. *nature* **449**, 463 (2007).
- 504 23 Li, L., Stoeckert, C. J. & Roos, D. S. OrthoMCL: identification of ortholog groups for

- 505 eukaryotic genomes. *Genome research* **13**, 2178-2189 (2003).
- 506 24 Chase, M. W. *et al.* An update of the Angiosperm Phylogeny Group classification for the
507 orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*
508 **181**, 1-20 (2016).
- 509 25 Jia, X., Xie, H., Jiang, Y. & Wei, X. Flavonoids isolated from the fresh sweet fruit of Averrhoa
510 carambola, commonly known as star fruit. *Phytochemistry* **153**, 156-162,
511 doi:<https://doi.org/10.1016/j.phytochem.2018.06.007> (2018).
- 512 26 Moresco, H. H., Queiroz, G. S., Pizzolatti, M. G. & Brighente, I. M. Chemical constituents
513 and evaluation of the toxic and antioxidant activities of Averrhoa carambola leaves. *Brazilian*
514 *Journal of Pharmacognosy* **22**, 319-324 (2012).
- 515 27 Wheeler, G. L., Jones, M. A. & Smirnoff, N. The biosynthetic pathway of vitamin C in higher
516 plants. *Nature* **393**, 365 (1998).
- 517 28 Laing, W. A., Wright, M. A., Cooney, J. & Bulley, S. M. The missing step of the L-galactose
518 pathway of ascorbate biosynthesis in plants, an L-galactose guanylttransferase, increases leaf
519 ascorbate content. *Proceedings of the National Academy of Sciences* **104**, 9534-9539,
520 doi:10.1073/pnas.0701625104 (2007).
- 521 29 Kurup, S. B. & Mini, S. Averrhoa bilimbi fruits attenuate hyperglycemia-mediated oxidative
522 stress in streptozotocin-induced diabetic rats. *journal of food and drug analysis* **25**, 360-368
523 (2017).
- 524 30 Liu, Y., Zhang, X. & Tian, X. Extraction and purification of flavonoids in Carambola. *Journal*
525 *of Shenyang Agricultural University* **40**, 491-493 (2009).
- 526 31 Yang, D., Xie, H., Jia, X. & Wei, X. Flavonoid C-glycosides from star fruit and their
527 antioxidant activity. *Journal of Functional Foods* **16**, 204-210 (2015).
- 528 32 Jia, X., Xie, H., Jiang, Y. & Wei, X. Phytochemistry Flavonoids isolated from the fresh sweet
529 fruit of Averrhoa carambola, commonly known as star fruit. (2018).
- 530 33 Moresco, H. H., Queiroz, G. S., Pizzolatti, M. G. & Brighente, I. Chemical constituents and
531 evaluation of the toxic and antioxidant activities of Averrhoa carambola leaves. *Revista*
532 *Brasileira de Farmacognosia* **22**, 319-324 (2012).
- 533 34 Tiwari, K., Masood, M. & Minocha, P. Chemical constituents of Gmelina philippinensis,
534 Adenocalymna nitida, Allamanda cathartica, Averrhoa carambola and Maba buxifolia. *Journal*
535 *of the Indian Chemical Society* (1979).
- 536 35 Gunasegaran, R. Flavonoids and anthocyanins of three Oxalidaceae. *Fitoterapia* **63**, 89-90
537 (1992).
- 538 36 Sahu, S. K., Thangaraj, M. & Kathiresan, K. DNA extraction protocol for plants with high
539 levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol.
540 *ISRN Molecular Biology* **2012** (2012).
- 541 37 10X Genomics. <https://www.10xgenomics.com>, (2017).
- 542 38 Marçais, G. & Kingsford, C. A fast, lock-free approach for efficient parallel counting of
543 occurrences of k-mers. *Bioinformatics* **27**, 764-770 (2011).
- 544 39 Luo, R. *et al.* SOAPdenovo2: an empirically improved memory-efficient short-read de novo
545 assembler. *Gigascience* **1**, 18 (2012).
- 546 40 Tarailo-Graovac, M. & Chen, N. Using RepeatMasker to identify repetitive elements in

547 genomic sequences. *Current protocols in bioinformatics* **25**, 4.10. 11-14.10. 14 (2009).

548 41 Jurka, J. *et al.* Repbase Update, a database of eukaryotic repetitive elements. *Cytogenetic and*

549 *genome research* **110**, 462-467 (2005).

550 42 Benson, G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic acids*

551 *research* **27**, 573-580 (1999).

552 43 Price, A. L., Jones, N. C. & Pevzner, P. A. De novo identification of repeat families in large

553 genomes. *Bioinformatics* **21**, i351-i358 (2005).

554 44 Xu, Z. & Wang, H. LTR_FINDER: an efficient tool for the prediction of full-length LTR

555 retrotransposons. *Nucleic acids research* **35**, W265-W268 (2007).

556 45 Campbell, M. S., Holt, C., Moore, B. & Yandell, M. Genome annotation and curation using

557 MAKER and MAKER-P. *Current Protocols in Bioinformatics* **48**, 4.11. 11-14.11. 39 (2014).

558 46 Kent, W. J. BLAT—the BLAST-like alignment tool. *Genome research* **12**, 656-664 (2002).

559 47 Birney, E., Clamp, M. & Durbin, R. GeneWise and genomewise. *Genome research* **14**, 988-

560 995 (2004).

561 48 Stanke, M., Schöffmann, O., Morgenstern, B. & Waack, S. Gene prediction in eukaryotes with

562 a generalized hidden Markov model that uses hints from external sources. *BMC*

563 *bioinformatics* **7**, 62 (2006).

564 49 Lomsadze, A., Ter-Hovhannisyan, V., Chernoff, Y. O. & Borodovsky, M. Gene identification

565 in novel eukaryotic genomes by self-training algorithm. *Nucleic acids research* **33**, 6494-6506

566 (2005).

567 50 Korf, I. Gene finding in novel genomes. *BMC bioinformatics* **5**, 59 (2004).

568 51 Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids*

569 *research* **28**, 27-30 (2000).

570 52 Lowe, T. M. & Eddy, S. R. tRNAscan-SE: a program for improved detection of transfer RNA

571 genes in genomic sequence. *Nucleic acids research* **25**, 955-964 (1997).

572 53 Nawrocki, E. P., Kolbe, D. L. & Eddy, S. R. Infernal 1.0: inference of RNA alignments.

573 *Bioinformatics* **25**, 1335-1337 (2009).

574 54 Nawrocki, E. P. *et al.* Rfam 12.0: updates to the RNA families database. *Nucleic acids*

575 *research* **43**, D130-D137 (2014).

576 55 Zwaenepoel, A. & Van de Peer, Y. wgd—simple command line tools for the analysis of

577 ancient whole-genome duplications. *Bioinformatics* **35**, 2153-2155,

578 doi:10.1093/bioinformatics/bty915 (2018).

579 56 Proost, S. *et al.* i-ADHoRe 3.0—fast and sensitive detection of genomic homology in

580 extremely large data sets. *Nucleic acids research* **40**, e11-e11 (2011).

581 57 Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7:

582 improvements in performance and usability. *Molecular biology and evolution* **30**, 772-780

583 (2013).

584 58 Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large

585 phylogenies. *Bioinformatics* **30**, 1312-1313 (2014).

586 59 Mirarab, S. *et al.* ASTRAL: genome-scale coalescent-based species tree estimation.

587 *Bioinformatics* **30**, i541-i548 (2014).

588 60 Seo, T.-K. Calculating bootstrap probabilities of phylogeny using multilocus sequence data.

589 *Molecular biology and evolution* **25**, 960-971 (2008).
590 61 De Bie, T., Cristianini, N., Demuth, J. P. & Hahn, M. W. CAFE: a computational tool for the
591 study of gene family evolution. *Bioinformatics* **22**, 1269-1271 (2006).
592