The *Musa troglodytarum* L. genome provides insights into the mechanism of non-climacteric behaviour and enrichment of carotenoids

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Abstract

**Background:** Karat (*Musa troglodytarum* L.) is an autotriploid Fe’i banana of the Australimusa section. Karat was domesticated independently in the Pacific region, and karat fruit are characterized by a pink sap, a deep yellow-orange flesh colour, and an abundance of β-carotene. Karat fruit showed non-climacteric behaviour, with an approximately 215-day bunch filling time. These features make karat a valuable genetic resource for studying the mechanisms underlying fruit development and ripening and carotenoid biosynthesis.

**Results:** Here, we report the genome of *M. troglodytarum*, which has a total length of 603 Mb and contains 37,577 predicted protein-coding genes. After divergence from the most recent common ancestors, *M. troglodytarum* (T genome) has experienced fusion of ancestral chromosomes 8 and 9 and multiple translocations and inversions, unlike the high synteny with few rearrangements found among *M. schizocarpa* (S genome), *M. acuminata* (A genome) and *M. balbisiana* (B genome). Genome microsynteny analysis showed that the triplication of *MtSSUIIs* due to chromosome rearrangement may lead to the accumulation of carotenoids and ABA in the fruit. The expression of duplicated *MtCCD4s* is repressed during ripening, leading to the accumulation of α-carotene, β-carotene and phytoene. Due to a long terminal repeat (LTR)-like fragment insertion upstream of *MtERF11*, karat cannot produce large amounts of ethylene but can produce ABA during ripening. These lead to non-climacteric behaviour and prolonged shelf-life, which contributes to an enrichment of carotenoids and riboflavin.

**Conclusions:** The high-quality genome of *M. troglodytarum* revealed the genomic basis of non-climacteric behaviour and enrichment of carotenoids, riboflavin, flavonoids and free galactose and provides valuable resources for further research on banana domestication and breeding and the improvement of nutritional and bioactive qualities.

**Keywords:** Banana, Chromosome-level genome, Carotenoids, Non-climacteric behaviour

Background

Bananas (*Musa* spp.) are among the most favoured fruits worldwide and are important staple foods for people in some tropical and subtropical countries [1]. According to their distinct genetic backgrounds, four genomes are represented within *Musa* spp., including *Musa acuminata* (A genome, 2n = 2x = 22), *Musa balbisiana* (B genome, 2n = 2x = 22), *Musa schizocarpa* (S genome, 2n = 2x = 22) and the Australimusa species (T genome, 2n = 2x = 20) [2]. Bananas are typically triploids or diploids of subspecies of *Musa acuminata* or of *Musa balbisiana* and *M. acuminata*. Of the seven species of Australimusa, there is a distinct group of banana species known as Fe’i banana (*Musa troglodytarum* L.). Fe’i banana plants are...
characterized by erect bunches and produce fruit that have a deep yellow-orange flesh colour and an abundance of α-carotene and β-carotene [3]. Karat is a cultivar of Fe'i banana distributed on the island of Pohnpei. Karat used to be a traditional weaning food in Pohnpei and has regained popularity due to a campaign that promoted karat to combat vitamin A deficiency (VAD) [4]. Moreover, karat has also been found to be rich in riboflavin (vitamin B2), an essential vitamin for nervous system function and iron utilization [5]. Riboflavin also contributes to the yellow colour of karat flesh.

Carotenoids are a large group of isoprenoids that play essential roles in plants; carotenoid function as pigments in both photosynthesis and light harvesting and serve as substrates for the biosynthesis of strigolactone and abscisic acid (ABA) [6]. The carotenoid biosynthetic pathway has been described in plants [7]. 1-Deoxy-D-xylulose-5-phosphate synthase (DXS) is the first and rate-limiting enzyme of the MEP pathway, and phytoene synthase regulates the first step of carotenoid biosynthesis by condensation of geranylgeranyl diphosphate, which is rate-limiting [8, 9]. Both α- and β-carotene are produced through the cyclization of lycopene by ε-lycopene cyclase (LCYE) and β-lycopene cyclase (LCYB). The pro-vitamin A (PVA) content of Musa germplasms varied from 0 to 85.08 μg/g, with yellow-orange plantains, Papua New Guinea diploids and deep yellow-orange pulp Fe'i bananas have relatively high carotenoid contents [10]. The biosynthesis of carotenoids of the Fe'i cultivar Asupina has been examined, and MtCCD4 is thought to be the key gene that results in hyperaccumulation in Asupina [11]. MtPSY2a cloned from Asupina was successfully used to generate transgenic bananas with high PVA levels [12]. In Cavendish, MaSPL6 functions as an activator of MalCYB1.1, and MalCYB1.2 and plays essential roles in carotenoid accumulation during ripening [13]. High carbohydrate contents were also shown to contribute to the hyperaccumulation of carotenoids in the green mutant of the plantain variety Obubit Ntanga [14].

Fe'i banana fruit are a parthenocarpic edible type [15]. The domestication of Fe'i banana occurred independently of that of plantains and other banana species through parthenocarpy and sterility processes [15]. To date, the genomes of A, B, S and Musa itinerans of Musa spp. have been published [16–19]. In addition to the latest updated genome of Musa acuminata DH PaHang, the genome data of Musa acuminata Banksii, Zebrina, and Calcutta 4 were deposited in the banana genome hub [20]. A cross-genus pangenome of banana contains representatives of the Musa and Ensete genera was presented, including genomic short reads of Musa troglodytarum L. ‘Pisang Tongkat Langit’ (tongkat), a cultivar from Eastern Indonesia [21, 22]. However, limited information is available for the Musa troglodytarum L. (T) genome, which has restricted the mining and utilization of valuable germplasm and gene resources. In this study, we de novo sequenced the genome of Musa troglodytarum L. for the first time, by integrating Oxford Nanopore, PacBio, Illumina and Hi-C sequencing techniques. Karat fruit showed non-climacteric behaviour, with an approximately 215-day bunch filling time. Metabolomic analyses and transcriptome sequencing were carried out to determine candidate genes involved in non-climacteric behaviour and the enrichment of carotenoids and riboflavin. The data from this study will be valuable for further research on improving the nutritional and bioactive qualities of banana fruit, prolonging shelf-life and reducing postharvest crop losses.

**Results**

**Assembly of the T genome**

The chromosomes of karat were fluorescently stained, and the result showed that karat is an autotriploid cultivar with 30 chromosomes (Additional file 1: Fig. S1). According to the genome survey, the T genome is 606–655 Mb in size and has a heterozygosity rate ranging from 1.25% (tongkat, TT) to 1.55% (karat, TTT) (Additional file 1: Fig. S2 and Additional file 1: Table S1-2). For genome sequencing, we generated 42 Gb of Nanopore reads, 6.9 Gb of PacBio reads and 42 Gb of Illumina reads (Additional file 1: Table S3-5). Using NextDenovo and NextPolish, we obtained an assembly with a total length of 918 Mb and contig N50 of 4.9 Mb (Additional file 1: Table S3-5). After purging haplotigs (Additional file 1: Fig. S3), we obtained 603 Mb contigs (Additional file 1: Table S6). After purging haplotigs, the contigs were arranged into 10 chromosomes (Fig. 1a and Additional file 1: Fig. S4). BUSCO analysis showed that 97.7% of the BUSCO genes were assembled (Additional file 1: Table S8). The
transcriptomes of leaves, roots, stems and fruits were sequenced for gene annotation. Using Maker2 [23], we predicted 37,577 protein-coding genes (Additional file 1: Table S9). BUSCO analysis showed that 92.5% of the BUSCO genes were predicted (Additional file 1: Table S10). Using eggNOG-mapper [24], we predicted 30,377 protein-coding genes with orthologues, 16,687 genes with GO annotation and 13,105 genes with KEGG annotation. Using RepeatMasker [25], we found that 59.62% of the T genome contained repeat elements (Additional file 1: Table S11). LTR/Gypsy and LTR/Copia accounted for 15.1% and 36.4% of the genome, respectively. As specific marker of the centromeric regions in M. acuminata genome [16, 26], Nanica LINE clusters also presented in all chromosomes (Additional file 1: Fig. S5). Using LTR FINDER [27], we identified 3,128 intact LTRs, and analysis of the insertion time showed that there was an LTR insertion burst at 1.47 MYA (Additional file 1: Fig. S6), which occurred before the burst of M. balbisiana (0.32 MYA) and after the burst of M. acuminata (1.77 MYA). Then, Illumina reads of karat and tongkat were mapped to the T genome. There were 516,884 and 459,137 indels, and 7,716,375 and 7,125,857 single-nucleotide polymorphism (SNP) sites identified in karat and tongkat, respectively (Additional file 1: Table S12 and Additional file 1: Fig. S7).

Using OrthoFinder [28], we identified 8924 single-copy genes and 27,100 orthologous gene sets in the A, B, S, T and M. itinerans genomes. There were 7791 genes specific in the T genome. According to the phylogenetic tree generated by OrthoFinder and the divergence time of M. acuminata and M. balbisiana reported in a previous study [17], we constructed an ultrametric tree showing that M. troglodytarum diverged from the ancestor of M. acuminata, M. schizocarpa and M. balbisiana 20.8 MYA (Fig. 1d). According to the 4DTV distances, the peak Ks values were approximately 0.06, 0.06, and 0.07 for M. troglodytarum–M. acuminata, M. troglodytarum–M. schizocarpa and M. troglodytarum–M. balbisiana, respectively, where a peak Ks value of approximately 0.46 indicated whole-genome duplication (Fig. 1e). Using Café [29], we identified 771, 460, 1820 and 608 expanded gene families and 3325, 3049, 562 and 6980 contracted gene families in the A, S, T and B genomes, respectively. There are 11 gene families with rapid expansions in T genome. GO functional enrichment analysis was conducted to explore the functions T genome-specific genes and rapidly evolving families (Additional file 1: Fig. S8-9 and supplementary Additional file 2: Data 1-2). GO enrichment analysis show that those genes of rapidly evolving gene families were enriched in cell morphogenesis, cell growth, defense response to insect immune system process, defense response to bacterium, defense response to fungus, response to virus etc.

Using MGRA2 [30], we constructed the ancestral genome of the A, B, S and T genomes, which resulted in 11 contiguous ancestral regions (CARs) and 20,056 ordered ancestral genes. For chromosome rearrangement, we constructed bar plots of the A, B, S and T genomes compared to CARs using MCSCAN (Fig. 1c). The ancestor of the A, B and T genomes experienced multiple chromosome rearrangements before and after their divergence. Chromosome 1 of the A, B and T genomes experienced translocation after divergence, and chromosomes 8 and 9 in the ancestry fused into chromosome 9 of the T genome. A dot plot of the synteny gene blocks between M. troglodytarum and M. acuminata also indicated the fusion of chromosomes 8 and 9 in the T genome (Additional file 1: Fig. S10-11). M. troglodytarum was domesticated independently and diverged from an ancestor of M. acuminata, M. schizocarpa and M. balbisiana 20.8 MYA. Thus, M. troglodytarum has experienced multiple translocations and inversions, unlike the high synteny with few rearrangements found among M. schizocarpa, M. acuminata and M. balbisiana.

Transcriptome and metabolome of the fruit
To determine the basis of the enrichment of carotenoids and flavonoids and the non-climacteric behaviour of karat, we integrated widely targeted metabolomics and targeted metabolomics data from karat pulp at 25, 45, 65, 115, 145, 173, 200 and 215 days after flowering (DAF) and RNA sequencing (RNA-seq) data from karat pulp at 25, 45, 65, 115, 100, 130, 145, 152, 159, 173, 200 and 208 DAF (Fig. 2a). According to widely targeted metabolomic data, we identified 877 metabolites, including flavonoids, lipids, phenolic acids, amino acids and their derivatives, organic acids, nucleotides and their derivatives, alkaloids, lignin, coumarins, tannins, terpenoids, quinones and others, 768 of them were divided into 5 clusters (Fig. 2f, Additional file 1: Fig. S12 and Additional file 2: Data 3-4). Cluster 2 represents the metabolites that increased during ripening, including alkaloids, amino acids and their derivatives, coumarins, free fatty acids, organic acids, phenolic acids, saccharides and alcohols, vitamins and others.

Carotenoids were enriched throughout fruit development
According to the quantification of the karat pulp metabolites, lutein accumulated throughout all the fruit developmental stages, with a content of 10.62–32.25 μg/g (Fig. 2b, c). The contents of α-carotene, β-carotene and phytocene increased rapidly at 200 DAF and 215 DAF, with values of 53.42, 27.90, and 10.57 μg/g, respectively, at 215 DAF. In addition, β-cryptoxanthin-laurate, rubixanthin-laurate β-cryptoxanthin, γ-carotene and others, were also increased at 215 DAF (Additional file 2: Data 5).
Microsynteny analysis of carotenoid biosynthesis pathway genes showed that *MtSSUIIs* were triplicated and *MtC-CD4s* were duplicated in the T genome (Figs. 1c and 3b). According to the RNA-seq data, the key genes of carotenoid biosynthesis, including *MtGGPPS1, MtSSUIIs, MtPSY2s, MtLCYBs, MtLCYEbs, MtZDSs, Mtβ-OH* and *Mtε-OH*, were all highly expressed across all the fruit developmental stages (Fig. 3a, c). At 200 DAF and 215 DAF, hyperaccumulation of α-carotene, β-carotene and phytene coincided with a decrease in *CCD4* expression. The triplication of *MtSSUIII* may explain the enrichment of carotenoids in karat and other Fe'i banana fruits. The lutein contents of these fruits were high throughout the fruit development process. *SSUIII* enhances the accumulation of carotenoids by interacting with *GGPPS1 and PSY*, promoting their enzymatic activity [31, 32].

**Fig. 2** Targeted metabolomics and widely targeted metabolomics analysis of karat pulp at different developmental stages. a Transverse section of karat fruit at different developmental stages. Quantitation of carotenoids (b and c, μg/g) and relative quantification of flavonoids (c) and vitamin B (c). F1, (E/Z)-phytoene; F2, Lutein; F3, α-Carotene; F4, β-Carotene; B2, riboflavin; B5, D-pantothenic acid; B6, pyridoxine; F1, 4′-hydroxy-5,7-dimethylxoyflavanone; F2, epicatechin; F3, myricetin-3-O-rutinoside; and F4, delphinidin-3-O-rutinoside. d Pink sap of pseudostem and fruits at 25 DAF. e Quantitation of flavonoids in pink sap. f Heatmap of metabolites in fruit pulp.
addition to MtSSUIls enhancing the hyperaccumulation of carotenoids, MtCCD4, a key gene that regulates various branches of carotenoid biosynthesis, regulates the accumulation of α-carotene and β-carotene during ripening and is downregulated at the end of ripening [11]. Downregulation of CCD4 is fruit-specific and may be the key reason for the enrichment of only α-carotene and β-carotene in the fruit. According to coexpression network analysis, MtCCD4 was cocoregulated with MtETO1 and MtJAZ1. MtJAZ1 is the key regulator of the JA signalling pathway and is induced by JA [33]. Multiple JA response element G-box and TGACG-box motifs were identified in the promoters of MtCCD4a and MtCCD4b (Additional file 1: Fig. S13), similar to CCD4 in Brassica napus, indicating an extensive role of JA in the regulation of CCD4 [34]. In Osmanthus fragrans, OfCCD4 were also induced by JA treatment [35]. In the full-green (FG) stage, the decreased expression level of CCD4 coincides
with the increase in the JA content, but in the full-ripening (FR) stage, the decrease in the JA content also coincides with the downregulation of MtCCD4s, implying the complex regulation of MtCCD4s by JA. For α-carotene and β-carotene rapidly accumulation in FR stage, JA may repress the accumulation of α-carotene and β-carotene by activating the expression of MtCCD4s in fruit. Therefore, further research is needed to elucidate the mechanism governing the regulation of MtCCD4s by JA, which may be spatiotemporally dependent and dose dependent.

**Flavonoids are enriched early during fruit development, which may be due to the expansion of MtF3’5’Hs**

The T genome has 17 F3’5’H loci, while there are eight, eight and five loci in the A, B and S genomes respectively. Microsynteny analysis showed that the flavonoid biosynthesis gene MtF3’5’H was tandemly duplicated on both chromosomes 2 and 10, resulting in nine more loci than were present in the A genome (Fig. 4b). In particular, F3’5’H on chromosome 9, which is a single locus in the A, B and S genomes, is duplicated into eight loci in the T genome. Moreover, seven of the eight loci distributed on chromosome 9 of the T genome showed highly similar expression patterns in karat. MtF3’H, which competes with MtF3’5’Hs for substrates, was largely decreased in karat. Microsynteny analysis also showed that there are three M. troglodytarum-specific regions in the upstream sequences of MtF3’H. No similar sequences were identified by BLAST in A, B, S or other genomes. The specific regions may contribute to the low expression level of MtF3’H in karat. According to the quantification of flavonoids in pink stem sap, the delphinidin-3-rutinoside chloride content was enriched. Moreover, multiple flavonoids in the pulp were found to be enriched, including 4’-hydroxy-5,7-dimethoxyflavanone, epicatechin, myricetin-3-O-rutinoside, and delphinidin-3-O-rutinoside. In particular, only 4’-hydroxy-5,7-dimethoxyflavanone was enriched at 215 DAF, while epicatechin, myricetin-3-O-rutinoside and delphinidin-3-O-rutinoside degraded largely at the end of the ripening process, coinciding with the fading of pink sap in the fruit (Fig. 2a, c–e). The duplication of MtF3’5’H and suppression of MtF3’H led to the enrichment of delphinidin-3-O-rutinoside, which differs from other types of bananas. The heatmap shows that the key genes involved in the synthesis of flavonoids were downregulated at the end of the ripening process, except for MtUFGT3s, which were highly expressed throughout the ripening process (Fig. 4a, c).

**Riboflavin is enriched in karat pulp**

According to the widely targeted metabolome analysis, riboflavin (B2), pantothenic acid (B5) and pyridoxine (B6) were enriched in karat pulp (Fig. 2c). In particular, riboflavin (B2) was enriched, especially in Fe’i banana fruit. Transcriptome analysis also showed that the riboflavin de novo synthesis genes MtRIBAI and MtFMNse showed higher expression levels in karat fruit pulp than in BXJ (BaXi Jiao, Musa acuminata L. AAA group cv. Cavendish) fruit pulp. Moreover, microsynteny analysis showed that there was a 2399-bp deletion in the 5’ UTR of MtRIBAI (Fig. 5a). As a consequence, MtRIBAI was highly expressed throughout all developmental stages, and the increased expression of MtFMNse across the ripening process, may be the reason for the enrichment of riboflavin.

**Non-climacteric behaviour of karat fruit**

Fruit ripening is distinctively different between climacteric and non-climacteric fruit. ABA and ethylene play key roles in the ripening of climacteric fruit, while non-climacteric ripening is linked to only ABA [36]. Banana is usually a climacteric fruit, while karat shows non-climacteric behaviour with a long bunch-filling time (215 days) and shelf life (harvested full yellow fruits can be stored for approximately 8 days under ambient conditions). By integrating metabolomic and comparative transcriptomic analyses, we found that the non-climacteric behaviour of karat is due to the transformation of ethylene-induced ripening into ABA-induced ripening.

Using a widely targeted metabolome, we quantified the plant hormones involved in the regulation of fruit development, including ABA, 1-aminocyclopropane-1-carboxylic acid (ACC), jasmonic acid (JA), indoleacetic acid (IAA), and salicylic acid (SA). ACC increased at 65 DAF and decreased at 145 DAF; ABA increased at 65 DAF and decreased at 215 DAF; JA increased at 65 DAF and decreased at 200 DAF; IAA decreased at 173 DAF; and SA decreased at 145 DAF but increased at 215 DAF (Fig. 5b). Karat fruit ripened at 215 DAF and was harvested at that time in June. At 65 DAF, homologues
Fig. 4 (See legend on previous page.)
of MaNAC1, MaBMY, MaAMY3, MaBMY3 and MaB-GAL6, were upregulated with the onset of fruit ripening, and the ABA and JA contents increased as the fruits reached the mature-green or full-green (FG) stages. ABA may play key roles in both FG and full-ripening (FR) stage. While ethylene, IAA, JA and SA may involve in fruit ripening during the FG stage. Moreover, there was another ABA peak at 200 DAF, when the fruit reached the FR stage, coinciding with fruit softening and the rapid accumulation of α-carotene and β-carotene (Fig. 2a and Additional file 2: Data 6-8). Though ABA, ethylene, IAA, JA and SA were all reported to activate the biosynthesis of carotenoids in many plants [13, 35, 37, 38], ABA may be one of the key factors regulating the accumulation of α-carotene and β-carotene in FR stage.

Autocatalytic ethylene synthesis was disrupted in karat, but ABA synthesis was enhanced. MaERF11 is a key ethylene-related gene that negatively regulates ethylene biosynthesis by suppressing MaACS1 and MaACO1 in banana [39]. MaACS1 and MaACO1 are the key genes that regulate ethylene biosynthesis. Homologues of MaERF11, MaACS1 and MaACO1 presented expression patterns that differed from those in FJ (Fen Jiao, Musa ABB PisangAwak) and BXJ (Fig. 5c). Both MaERF11 and MtERF11 are repressor with ERA repression motif ‘DLNNPP’. Microsynteny analysis showed that there was a DNA fragment of 4,708 bp and an LTR-like fragment of 2116 bp inserted 780 bp upstream of MtERF11 and that there was a DNA fragment of 39,766 bp and an LTR-like fragment of 14,924 bp inserted 2720 bp upstream of

Fig. 5 Modulation of fruit ripening by ABA and ethylene. a Characteristics of the ERF11, ACO1 and RIBA1 gene sequences. b Relative quantitation of plant hormones involved in fruit ripening. c Expression patterns of NCED6, ERF11 and ACS1. d Coexpression network of genes involved in the modulation of fruit ripening. DAF, days after flowering; DPH, days post-harvest
**MtACO1** (Fig. 5a). Due to sequence variations, unlike that of *MaERF11* in BXJ, the expression of *MtERF11* was not suppressed during ripening as that of *MaERF11* in BXJ. The expression of *MtERF11* showed a similar expression pattern as that of *MtACS1* during ripening, which is unlike the sharp upregulation of *ACS1* in FX and BXJ. This result indicated that *MtERF11* suppressed the expression of *MtACS1* throughout the fruit development process. The promoter sequence of *MtACS1* harbour GCC-boxes, while that of *MtACO1* lacks GCC-boxes, which may explain the upregulation of *MtACO1* during ripening (Additional file 1: Fig. S14). Analysis of the cloned promoter sequences of *MtACO1* also validated the missing of GCC-boxes. As a downstream gene of the carotenoid synthesis pathway and a gene that catalyses the first step of ABA biosynthesis, *MtNCED6* may play key roles in the regulation of ABA synthesis in karat fruit, as this gene is specifically expressed during ripening, unlike in FX and BXJ [40]. Moreover, unlike in FX and BXJ, the metabolism-related gene *MtCYP707A1* was not upregulated during ripening. It may be the key gene involved in the decrease in ABA content in BXJ during ripening. *MtCYP707A2* was upregulated at 208 DAF, which may explain the decrease in ABA levels (Additional file 1: Fig. S15-17). ABA-stress-ripening (ASR) transcription factors play key roles in sucrose- and ABA-induced fruit ripening and softening via crosstalk between ABA and sucrose [41]. In BXJ, *MaASR1* and *MaASR2* increased before ripening but sharply decreased, while they were highly expressed during karat fruit ripening. *MtASR1* expression was maintained at a high level throughout the ripening period, indicating that this gene may play a role in karat fruit ripening and softening.

As starch was degraded and converted to soluble sugars during ripening, karat had lower glucose and fructose contents but higher sucrose and free galactose contents than BXJ, due to non-climacteric behaviour. Quantification of sugars in the pulp of ripening karat showed that sucrose was highly enriched, coinciding with the degradation of starch. In the pulp of dry ripened fruit, the sucrose content reached 233.96 mg/g and was the predominant sugar (Fig. 6d and Additional file 2: Data 9). Given that the pulp of the ripening fruit is 67% water [4], karat pulp has approximately 27.13 and 24.95 mg/g glucose and fructose, respectively, which is less than the approximately 60 mg/g glucose and fructose found in BXJ [42]. During ripening, the starch-degradation-related genes *MtBMYS* and *MtAMYs* were upregulated, coinciding with the accumulation of sucrose, fructose and glucose. However, in contrast to that of their homologues in BXJ and FX, the expression of these genes did not sharply increase in karat (Fig. 6e and Additional file 1: Fig. S18). Sucrose synthase genes were highly expressed in karat during ripening, which differed from the decrease in BXJ and FX. Moreover, the level of free galactose in the pulp at ripening was 0.27 mg/g. The galactose synthesis-related genes *Mtα-GALs* and *Mtβ-GALs* had higher expression levels than those in FX and BXJ, which may be due to the non-climacteric behaviour. *GALK* is involved in galactose metabolism [43]. Compared to *MaGALK*, *MtGALK* is a 5’ prime untranslated region (UTR) premature start codon-gain variant that has two premature ORFs in the 5’ UTR (Fig. 6a–c). Premature ORFs may suppress the translational activity of transcripts [44]. *MtGALK* also has an alternative transcript in which the sixth exon is missing, which may be due to a DNA sequence insertion in the sixth intron (Fig. 6c). The cloned *MtGALK* cDNA sequences validated the variation of in 5’ UTR and alternative splice. Moreover, the karat shoot buds presented better growth vigour than BXJ under exogenous application free galactose (Additional file 1: Fig. S19). Therefore, the free galactose content in the pulp was higher than that in the pulp of other banana fruits and may be due to sequence variations in the 5’ UTR and sixth intron of *MtGALK*. Non-climacteric ripening behaviour may be another reason for the accumulation of free galactose; similar to the fruit of non-climacteric plum cultivars, which shows increased expression levels of α-GALs and β-GALs and increased accumulation of free galactose [45].

Using weighted gene coexpression network analysis (WGCNA), we constructed a weighted coexpression network. To identify candidate genes involved in fruit development and ripening, flavonoid and carotenoid biosynthesis, we selected *MtXB3*, *MtNAC1*, *MtCCD4s*, *MtERFs*, *MtNCED6*, and *MtDFR* to extract subnetwork from coexpression network (Fig. 5d and Additional file 2: Data 10). *MtXB3*, a homologue of *MaXB3* that negatively regulates fruit ripening, was downregulated at 130 DAF. *MtXB3* was coexpressed with *MtTT8*, *MtLC*, *MtMYB114*, *MtDFR*, and *MtANR*, which may play key roles in flavonoid biosynthesis. *MtERF11* and *MtACS1* were coexpressed with *MtSAP1*, *MtSAP4*, *MtERF4*, *MtERF105*, *MtERF107* and *MtERF109*. *MtNCED6* was coregulated with *MtRIBA1*, *MtACO1*, *MtSAUR1*, *MtCCD4a* and *MtCCD4b*. And *MtCCD4a* and *MtCCD4b* negatively regulate the accumulation of α-carotene and β-carotene and were coregulated with *MtJAZ1* and *MtETO1*.

**Discussion**

Karat is an edible parthenocarpic cultivar of the Australimusa section known as Fe’i banana. Different from the known karyotype of Pisang tongkat langit, in which TT and Asupina were combined with ATT, in karat, these genomes were combined with TTT. In this study, we constructed a high-quality T genome with a size of...
603 Mb, representing 92% of the estimated genome size of 655 Mb. Transposable element (TE) contents comprise 59.62%, 45.16%, 49.55% and 56.69% of the T, A, B and S genomes, respectively [16–18]. The T genome was larger than the A, B and S genomes (523, 521 and 587 Mb, respectively), which may be due to the presence of more TE contents. This is especially true for LTR/Copia (36.41%) and LTR/Gypsy (15.07%), which account for 51.48% of the T genome. Through chromosome rearrangement analysis of the T genome, we found that ancestral chromosomes 8 and 9 fused into modern chromosome 9. Phylogenetic analysis based on orthologous genes shows that the divergence time between *M. troglodytarum* and the ancestor of *M. acuminata* and *M. schizocarpa* was about 20.8 MYA, indicating a much earlier divergence time between Callimusa and Musa than previous reported 37.9-50.7 MYA [46–48]. This may be because of utilizing more informative characters to construct phylogenetic trees in genome-wide studies. After divergence from the ancestor of *M. acuminata* and *M. schizocarpa*, the *M. troglodytarum* genome experienced multiple chromosome rearrangements and sequence variations, which resulted in Fe’i banana fruit having many specific features, such as a red or purple sap rich in delphinidin-3-rutinoside chloride, a deep yellow-orange-coloured flesh that is rich in β-carotene and riboflavin, high contents of galactose, and a non-climacteric ripening pattern. By integrating metabolome, comparative transcriptome and microsynteny analyses, we revealed the genomic basis of these features, which provides insights into the improvement of nutritional and bioactive qualities, the prolonging of fruit shelf-life and reduction in postharvest crop losses.

Some fruit, such as those of melon, pepper, plum, and pear, show both climacteric and non-climacteric behaviour. In the non-climacteric varieties, endogenous
ethylene synthesis is suppressed [49–53]. Banana is usually a climacteric fruit, while karat shows non-climacteric behaviour with the transformation of ethylene-induced ripening into ABA-induced ripening. The triplication of MtSSU1IIs resulted in the enhancement of ABA biosynthesis, and the suppression of ethylene biosynthesis by a mutation in MtERF11 may be the primary reason for the non-climacteric behaviour of karat. In banana, a complex network regulating fruit ripening has been reported, and a dual-loop circuit, involving NAC and MADS transcription factors and ethylene signalling, was shown to control banana fruit ripening [54]. MaMADS1, MaMADS2 and MaMADS36 are also necessary for banana fruit ripening [55–57]. However, the expression patterns of MtMADS1, MtMDA2a and MtMDA2b, which are homologues MaMADS1 and MaMADS2, were different from those in BXJ; thus, these genes may function in an ethylene-dependent mode. A multilayered regulatory cascade comprising MaNAC1, MaNAC2, MaXB3, MaERF11, MaACS1 and MaACO1 is involved in ripening, of which MaERF11 functions as a repressor of ethylene biosynthesis by repressing MaACS1 and MaACO1 [39, 58, 59]. MaERF11 was repressed by MaNAC2 and MaNAC1; both genes were repressed by MaXB3. During ripening, the repression of MaACS1 was removed with the downregulation of MaXB3. However, in karat, the regulatory cascade was disrupted by the insertion of LTRs, and MtERF11 repressed the expression of MtACS1 throughout the fruit development process. Additionally, the comparative transcriptome analysis indicated that the regulatory network comprising NAC1, NAC2, XB3, ERF11, ACS1 and ACO1 was conserved in Fj, BXJ and karat. Moreover, ABA-related transactors such as ABI5, ASR1, and ASR2 were also involved in the regulation of fruit ripening in Fj, BXJ and karat. These data will provide important resources for further research on the fruit ripening process of banana.

Conclusions

In this study, we de novo sequenced the genome of Musa troglodytarum L. for the first time. Genome microsynteny analysis showed that the duplication of MtSSU1IIs due to chromosome rearrangement may lead to the accumulation of carotenoids and ABA in the fruit. And the expression of duplicated MtCCD4s is repressed during ripening, leading to the accumulation of α-carotene, β-carotene and phytolene. The duplication of MtF3′5′H and suppression of MtF3′H led to the enrichment of delphinidin-3-O-rutinoside, which is different from that in the other types of bananas. The free galactose content in the pulp was higher than that in the pulp of other banana fruit and may be due to the sequence variations in the 5′ UTR and sixth intron of MtGALK. Due to an LTR fragment insertion upstream of MtERF11, karat cannot produce large amounts of ethylene but can produce ABA during ripening, resulting in non-climacteric behaviour and prolonging bunch filling time. In conclusion, integrating metabolome, comparative transcriptome and microsynteny analysis, we revealed the genomic basis of non-climacteric behaviour and enrichment of carotenoids, riboflavin, flavonoids, free galactose of karat (Musa troglodytarum L.). These data provide insights into the improvement of the nutritional and bioactive qualities, prolongation of shelf-life and reducing postharvest crop loss.

Methods

Sampling, sequencing and assembly

Karat plants growing in a greenhouse of the National Gene Bank of Tropical Crops in Danzhou, Hainan, China, were sampled for genome sequencing. This Musa germplasm was collected from Kosrae of The Federated States of Micronesia during a plant resources investigation and cooperation. The genomic DNA of leaves was extracted for genomic library construction. The DNA high-molecular-weight genomic DNA extraction was performed using an SDS-based method [60]. For Illumina sequencing, libraries with 350 bp insertions were constructed. For PacBio sequencing, libraries with 20,000 bp insertions were constructed and sequenced on the PacBio RS II system using P6-C4 chemistry. For Nanopore single-molecule sequencing, libraries with high-molecular-weight genomic DNA were constructed on PromethION. In total, 42,304,446,276 bp reads were produced by Nanopore single-molecule sequencing, 42,215,455,800 bp Illumina short reads were produced, and 6,961,206,933 bp PacBio reads were produced.

Hi-C libraries were created from young leaves of M. troglodytarum at BioMarker Technologies Company as described previously [61]. Briefly, the leaves and formaldehyde were mixed together and then lysed, and then the cross-linked DNA was digested with DpnII overnight. Sticky ends were biotinylated and proximity-ligated to form chimaeric junctions that were enriched and then physically sheared to a size of 500–700 bp. Chimaeric fragments representing the original cross-linked long-distance physical interactions were then processed into paired-end sequencing libraries, and 110 Gb of paired-end reads were produced on the Illumina HiSeq X Ten platform.

For RNA-seq, total RNA was extracted from leaves, roots, pseudostems and fruits using TRIzol reagent according to the manufacturer’s instructions. After removing genomic DNA using DNase I (Takara), mRNAs were obtained using oligo (dT) beads and subsequently broken into short fragments, followed by cDNA
synthesis. Paired-end sequencing was conducted on a HiSeq X Ten platform (Illumina, CA, USA).

**Genome assembly and annotation**

Using GenomeScope 2.0 [62], a program that employs a polyploid-aware mixture model to assess heterozygous and polyploid genomes, we estimated the genome size using jellyfish-produced K-mer counts. Nanopore long reads were imported for assembly by NextDenovo v2.3.0 (https://github.com/Nextomics/NextDenovo), with the errors first corrected by the NextCorrect module and then assembled into 918,212,466 bp contigs by the NextGraph module. The Illumina short reads and PacBio reads, NextPolish (https://github.com/Nextomics/NextPolish) was subsequently used to polish the preliminary assembly with the default parameters. Then, the allelic haplotigs were eliminated by Purge_Haplotigs (v1.1.1) [63]. The final assembly was then assembled into a scaffold based on proximity-guided assembly by ALLHIC [64].

RepeatModeler v1.0.11 with default parameters was used to identify TEs de novo [65]; this program employs two different software programs: RECON (v1.08) and RepeatScout (v1.0.5). For consensus building and classification steps, the consensus TE libraries generated above were imported into MAKER2 for further repeat annotations by employing RepeatMasker (v4.05). TClass (v2.1.3) was subsequently used to classify unknown TEs using a support vector machine (SVM) method. Tandem Repeat Finder (v4.07) was then used to identify repeats within the genomes with the following parameters: ‘1 1 2 80 5200 2000 –d –h’.

To detect LTRs, the LTR_retriever pipeline with default parameters was used to integrate and remove false positives from the initial predictions of LTR FINDER [27, 66]. BUSCO v5.22 was used to evaluate genome completeness using the viridiplantae_odb10 dataset, which includes 425 single-copy and conserved protein-coding genes [67]. The LTR insertion time was estimated by the transcripts using the viridiplantae_odb10 dataset, which includes 425 single-copy and conserved protein-coding genes [67].

After filtering out proteins produced by MAKER2 that had low AED values, the ab initio predictors AUGUSTUS, GENEMARK and SNAP were trained again. Then, utilizing high-confidence and high-sensitivity transcripts produced by StringTie (v2.1.1) [68] as inputs, the MAKER2 pipeline was run again. Using eggNOG-mapper (v2) [24] and its default database, genes were assigned functional annotations.

**Genome structure and evolution**

The genome data of *M. acuminata* (GCA_000313855.2) [69], *M. balbisiana* (GCA_004837865.1) [70] and *M. itinerans* (GCA_001649415.1) [71] was downloaded from NCBI. The genome data of *M. schizocarpa* was downloaded at http://www.genoscope.cns.fr/plants [72]. The RNA-seq data of BXJ and FJ were downloaded from Bioproject accession number PRJNA3949594. The short reads genome sequencing data included in SRR15675960 for tongkat and SRR16526594 for karat were used to estimate genome size.

Orthologues were identified by OrthoFinder (v2.2.7) [28] with default parameters, using the longest transcripts of protein-coding genes from *M. tropogutyram, M. acuminata, M. balbisiana, M. schizocarpa* and *M. itinerans*. According to the orthologue set mentioned above and the results from MCScanX (v0.8) [73], putative proteotyes (pPGs) were predicted. Then, the pPGs ordered according to the gene location in each species were integrated into GRIMM format and imported into MGRA2 [30] for ancestral genome reconstruction, in which heuristic higher breakpoint reuse was used. Then, the exhaustive set of ordered proteotyes (oPGs) was imported for collinear genes reported by MCScanX [73]. The rooted tree generated by OrthoFinder was used for the construction of ultrametric trees of *M. tropogutyram, M. acuminata, M. balbisiana* and *M. itinerans*.

**Metabolomic analyses**

Carotenoid, flavonoid, monosaccharide and disaccharide contents were analysed via targeted metabolomics. Karat pulp at 215 DAF was used for monosaccharide and disaccharide analysis. The pink sap of karat was used for flavonoid and carotenoid analysis. The carotenoid, flavonoid, monosaccharide and
disaccharide contents were analysed as described in previous studies [76–78]. The sample extracts were analysed using a UPLC-ESI-MS/MS system (ExionLC™ AD https://sciex.com.cn/; MS, Applied Biosystems 6500 Triple Quadrupole, https://sciex.com.cn/). Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a QQQ-linear ion trap mass spectrometer (QTRAP), QTRAP® 6500+ LC–MS/MS system equipped with an ESI turbo ion-spray interface operating in positive ion mode and controlled by Analyst v1.6.3 software (AB-SCIEX).

For widely targeted metabolomics analyses, karat pulp at 25, 45, 65, 115, 173, 200 and 215 DAF was used. The metabolomics analyses were performed as described in a previous study [79]. Briefly, the sample extracts were analysed using a UPLC–ESI–MS/MS system (Shimadzu LC–MS/MS system equipped with an ESI turbo ion-spray interface operating in positive ion mode and controlled by Analyst 1.6.3 software (AB-SCIEX).

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### Supplementary Information

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#### Additional file 1: Figure S1. Fluorescent staining of karat chromosomes. The root tip of Musa troglodytarum L. karat plants staining with DAPI and screened under ultraviolet and fluorescence microscopy. Figure S2. GenomeScope profile of karat and tongkat. Figure S3. Bimodal histogram for Purge Haplotigs processing. The cutoff values for low, mid, and high points were 6.42 and 105, respectively. Figure S4. HI-C mapping of chromosomes of the T genome. Figure S5. Heatmap of density of Nanica LINE. Figure S6. Analysis of LTR insertion times of the A, B, S and T genomes. Ma, Musa acuminate; Mb, Musa balbisiana; Ms, Musa schizocarpa, and Mt, Musa troglodytarum L. Figure S7. Distribution of SNP and indel sites in karat and tongkat. Indel sites in tongkat (A) and karat (B) and SNP sites in tongkat (C) and karat (D). Figure S8. GO enrichment analysis of T genome specific genes. Figure S9. GO enrichment analysis rapidly evolving gene families in M. troglodytarum. Genes were annotated by eggNOG-mapper. Data 2. List of genes of rapidly evolving families in M. troglodytarum. Genes were annotated by eggNOG-mapper. Data 3. Metabolites of karat pulp identified by widely targeted metabolomics analysis. Data 4. Clustered metabolites of karat pulp identified by widely targeted metabolomics analysis. Data 5. Carotenoid contents of pulp at different developmental stages. Data 6. Expression levels of genes involved in fruit ripening in karat. Data 7. Expression levels of genes involved in fruit ripening in BXJ. Data 8. Expression levels of genes involved in fruit ripening in BXJ. Data 9. Sugar contents in the pulp of ripening karat fruit. Data 10. Expression levels of nodes of the ripening-related coexpression network.

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### Authors’ contributions

L.X. and Z.Y.L. designed the research; Z.Y.L., J.B.W., B.L.H., Y.L.F., Y.L.J., Y.C. and X.B.W. performed the experiments; L.X., Z.Y.L. and J.B.W. analysed the data; Q.Q.Y, Q.L.W. and C.Y.M. supplied the materials; J.B.W. and Z.Y.L. wrote the manuscript; and L.X. revised the manuscript. All authors critically read and approved the final version of the manuscript.

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### Availability of data and materials

The Musa troglodytarum L. genome sequences and raw sequence data from RNA-seq with accessions SRX12729179-SRX1272972 and genome assembly with assembly ASA2354706v1 have been deposited under BioProject accession number PRJNA772007 [80]. The GFF format Gene and TE annotation file of the Musa troglodytarum L. genome was available in figshare [81]. The cloned promoter sequences of MtACS1, MtACO1 and cDNA sequences of MtGAUX and MtSSUII were deposited in figshare [82].

### Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
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