Metacaspase gene family in Rosaceae genomes: Comparative genomic analysis and their expression during pear pollen tube and fruit development

Yunpeng Cao, Dandan Meng, Tianzhe Chen, Yu Chen, Wei Zeng, Lei Zhang, Qi Wang, Wei Hen, Muhammad Abdullah, Qing Jin, Yi Lin, Yongping Cai

1 School of Life Sciences, Anhui Agricultural University, Hefei, China, 2 College of Horticulture, Anhui Agricultural University, Hefei, China

☯ These authors contributed equally to this work.
* swkx12@ahau.edu.cn

Abstract

Metacaspase (MC), which is discovered gene family with distant caspase homologs in plants, fungi, and protozoa, may be involved in programmed cell death (PCD) processes during plant development and respond abiotic and biotic stresses. To reveal the evolutionary relationship of MC gene family in Rosaceae genomes, we identified 8, 7, 8, 12, 12, and 23 MC genes in the genomes of Fragaria vesca, Prunus mume, Prunus persica, Pyrus communis, Pyrus bretschneideri and Malus domestica, respectively. Phylogenetic analysis suggested that the MC genes could be grouped into three clades: Type I*, Type I and Type II, which was supported by gene structure and conserved motif analysis. Microsynteny analysis revealed that MC genes present in the corresponding syntenic blocks of P. communis, P. bretschneideri and M. domestica, and further suggested that large-scale duplication events play an important role in the expansion of MC gene family members in these three genomes than other Rosaceae plants (F. vesca, P. mume and P. persica). RNA-seq data showed the specific expression patterns of PbMC genes in response to drought stress. The expression analysis of MC genes demonstrated that PbMC01 and PbMC03 were able to be detected in all four pear pollen tubes and seven fruit development stages. The current study highlighted the evolutionary relationship and duplication of the MC gene family in these six Rosaceae genomes and provided appropriate candidate genes for further studies in P. bretschneideri.

Introduction

Programmed cell death (PCD) is a developmental and genetically controlled cell death process, which is divided into two broad categories: environmentally induced PCD and developmentally regulated PCD in plants [1–4]. Environmentally induced PCD is primarily caused by external abiotic or biotic signals, such as drought, hormone, heat shock and pathogens stresses.
stage 2 (30DAB), Accession: SRX1595646; P. bretschneideri fruit developmental stage 3 (55DAB), Accession: SRX1595647; P. bretschneideri fruit developmental stage 4 (85 DAB), Accession: SRX1595648; P. bretschneideri fruit developmental stage 5 (115 DAB), Accession: SRX1595652; P. bretschneideri fruit developmental stage 6 (mature stage), Accession: SRX1595651; P. bretschneideri fruit developmental stage 7 (fruit senescence stage), Accession: SRX1595650; P. bretschneideri drought-tolerant for 0 h, Accession: SRX4110141; P. bretschneideri drought-tolerant for 1 h, Accession: SRX4110140; P. bretschneideri drought-tolerant for 3 h, Accession: SRX4110143; P. bretschneideri drought-tolerant for 6 h, Accession: SRX4110142; P. bretschneideri for 24 h, Accession: SRX4110139). All other relevant data are within the paper and its Supporting Information files.

Funding: This study was supported by The National Natural Science Foundation of China (grant 31640068; Yongping Can). The Funding bodies were not involved in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Competing interests: The authors have declared that no competing interests exist.

[5–8]. In contrast, developmentally regulated PCD covers most of the organs and tissues of plants, such as fruit, root, stem, leave and xylem, which caused by internal factors and occurs at predictable locations and times [9–11].

Metacaspases (MCs) are multifunctional proteins that are involved in PCD regulation, cell cycle and senescence, and oxidative stress. According to the sequence similarities and domain structure, plant MCs are divided into two types: Type-I and Type-II [1, 12]. Additionally, a subgroup appeared from the Type-I and named as Type-I*, which exclude N-terminal zinc-finger motif [1]. These different types MC proteins contained a putative conserved caspase-like domain (PF00656), which consists of p10 and p20 subunits [1, 12]. Additionally, Type-I MC proteins have a short linker between the p20 and p10 subunits and an N-terminal pro-domain upstream of the p20 subunit. Type-II MC proteins did not contain N-terminal pro-domain upstream of the p20 subunit, but it has a longer linker between the p20 and p10 subunits than Type-I MC proteins [1, 12, 13].

MC genes play an important role in stress-induced and developmentally regulated PCD. Tsiatsiani et al. (2011) have been identified nine MCs (AtMC1–AtMC9) in Arabidopsis thaliana [2]. Under the stress of UV-C and H2O2, the expression of AtMC8 was up-regulated in A. thaliana, which accelerated the process of the PCD in protoplasts [14]. AtMC1 and AtMC2 have been reported as positive and negative regulators, respectively, to antagonize pathogen-triggered PCD [15]. AtMC2p26 (i.e. AtMC5) can activate apoptotic-like cell death during early senescence process and oxidative stress [16]. AtMC9 plays an important role in the process of autolysis during vessel cell death [9]. Additionally, the Nicotiana benthamiana NbMCA1, Capsicum annuum CaMC9, Triticum aestivum TaMC4 have been reported to function in stress response and PCD [4, 6, 17].

Systematic analysis and genome-wide identification of MC gene family have been reported in Hordeum vulgare, Solanum lycopersicum, Hevea brasiliensis, Vitis vinifera, Arabidopsis thaliana and Oryza sativa [18–23]. However, investigations on the MC gene family in Rosaceae genomes are limited. F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica are important worldwide cultivated fruit trees, and they belong to Rosaceae family [24–29]. In the present study, we identified MC gene family members in these six genomes by screening these genome sequences using bioinformatics approaches. Then we characterized their phylogenetic relationship, gene structures, chromosomal distribution, microsynteny and expression patterns. The current study explored the evolutionary relationship of MC gene family members in the Rosaceae plants and provided insights into the functions of PbMCs during P. bretschneideri pollen tube and fruit development.

Materials and methods
Identification and characterization of the MC gene family in Rosaceae genomes

Genome resources and predicted proteins of P. bretschneideri (version 1.0) was downloaded from the GigaDB (http://gigadb.org/), P. persica (version 1.0) from Ensembl Plants (http://plants.ensembl.org/index.html), P. mume (version 1.0) from PGGD database (http://chibba.agtec.uga.edu/duplication/), F. vesca (version 2.0) from PLAZA v2.5 (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v2_5/), P. communis (version 1.0) from Pear Genome Project (http://peargenome.njau.edu.cn), M. domestica (version 3.0) from GDR database (https://www.rosaceae.org/). Three strategies were used to identify gene-encoding MC from six Rosaceae plants at the whole-genome level. First, the published Arabidopsis thaliana MC protein sequences were used to query putative MC homologous proteins of F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica using BLASTp software with E-value
cutoff $1 \times 10^{-5}$. Subsequently, HMM (Hidden Markov Model) profiles of the Caspase-like domain (PF00656) in the Pfam database was searched against the local database using HMMER 3.0 software with E-values cutoff $1 \times 10^{-3}$ [30, 31]. Finally, the Pfam and Smart databases were used to check these candidate sequences that contained Caspase-like domain (PF00656) [32, 33]. Additionally, we used the MEROPS online tool (https://www.ebi.ac.uk/merops/) [2] to predict the distribution of Type-I, Type-I and Type-II MC genes in these Rosaceae genomes.

Sequence alignment and phylogenetic analysis of MC proteins in Rosaceae genomes

To further insight into the phylogenetic relationship of MC proteins in *F. vesca, P. mume, P. persica, P. communis, P. bretschneideri* and *M. domestica*, we aligned multiple sequence alignments including FvMCs, PmMCs, PpMCs, PcMCs, PbMCs and MdMCs using MAFFT software [34, 35]. For all MC proteins, we determined the best substitution model using modeltest software [36]. Subsequently, the IQ-TREE software was used to generate the Maximum Likelihood (ML) tree with 1000 bootstrap replications and VT+G4 model [37].

Gene structures and chromosomal locations of MC genes in Rosaceae genomes

To determine the location of MC genes on chromosomes, the published *Arabidopsis thaliana* MC genes were used as query sequences against local Rosaceae genome database using BLAST software [38]. To display gene structure of each MC gene, the GSDS website was used to parse GFF3 files and visualize them [39]. The MEME program Version 4.11.1 was used to identify the conserved motifs according to previously published manuscripts [40–42].

Microsynteny analysis of MC genes in Rosaceae genomes

The microsynteny analysis of each MC gene was carried out using Microsyn software and MCScanX pipeline [43, 44], based on the previous description [39, 45, 46]. Firstly, three files (i.e. the gene identifier file, the CDS file and the gene list file) were generated. Subsequently, we used local BLAST software to compare whole proteins of each species with E-value less than $1 \times 10^{-10}$. The position and blast output files of all protein-coding genes were imported into MCScanX software to scan the collinearity gene pairs and the Circos software was used to display the results of collinearity gene pairs [47]. Nonsynonymous ($K_a$), synonymous ($K_s$) and $K_a/K_s$ ratios were estimated using DnaSP v5 software [48].

Expression analysis of MC genes

In the present study, the RNA-Seq data were downloaded from the public NCBI database, and then these data was used to survey the expression of MC genes. The accession numbers and sample details for above the RNA-Seq data have mentioned in the availability of data and materials section. The low-quality base-calls ($Q < 20$) of raw reads were deleted by FASTX-toolkit. The clean reads were mapped to the reference genome using TopHat2 software with default parameters [49, 50]. Finally, we used the Cufflinks software to assemble and estimate the expression FPKM values [50, 51]. The R software was used to visualize the gene expression profiles of MC genes.

Quantitative real-time PCR (qRT-PCR)

The methods for collection and drying, and in *vitro* culture of pear pollen grains were based on the procedures according to the previously published manuscripts [52, 53]. The pollen
samples were collected from 40-year-old pear trees (Pyrus bretschneideri. Rehd), and mature pollen was immediately frozen in liquid nitrogen and stored at −80°C until use. The pollen grains were cultured in this medium for hydration, germination and growth. The medium components were 450 mM sucrose, 25 mM 2-(N-morpholino) ethanesulfonic acid hydrate, 15% (w/v) PEG4000, 1.5 mM H$_2$BO$_3$ and 0.5 mM Ca(NO$_3$)$_2$, with pH 6.0–6.5 (pH was adjusted with KOH). The guanidine thiocyanate extraction method was used to isolate the total RNAs by RNA Plus (Takara) [54, 55]. According to the manufacturer’s instructions, first-strand cDNAs were synthesized from DNaseI-treated total RNA using Oligo(dT) primers and reverse transcriptase (TIANGEN, China). The qRT-PCR was conducted on a CFX96 Touch Real-Time PCR Detection System (BIO-RAD), based on the manufacturer’s protocol. Primer Express 3.0 software (Applied Biosystems) was used to design the primers of PbMC genes for qRT-PCR (S1 Table). Relative expression levels of PbMC genes were normalized against the P. bretschneideri Actin gene (NCBI ID AF386514). The relative expression level was calculated as $2^{-\Delta\Delta C_t}$ as described previously [41, 56]. In the present study, three biological replicates were conducted for each sample.

Results

Identification of MC genes in Rosaceae genomes

To identify the MC gene family members in Rosaceae genomes, HMM model and BlastP were used to search against the whole-genome sequence, with the procedures described previously [45, 57]. We identified 70 genes as members of MC gene family in these six Rosaceae genomes, including 8 FvMC genes in Fragaria vesca, 7 PmMC genes in Prunus mume, 8 PpMC genes in Prunus persica, 12 PcMC genes in Pyrus communis, 12 PbMC genes in Pyrus bretschneideri and 23 MdMC genes in Malus domestica. The identified MC genes were designated as FvMC, PmMC, PpMC, PcMC, PbMC, and MdMC followed by number. The theoretical pI values of 24 MC proteins, such as MdMC08 (9.13), FvMC06 (8.98) and FvMC06 (8.53) were above 7, suggesting that these proteins were alkaline, however, the remaining sequences encoded by the other MC genes were acidic (<7). The detailed parameters of MC genes were listed in S2 Table, including theoretical pI, molecular weight, protein length, and chromosome location.

Phylogenetic and structural analysis of MC genes in Rosaceae genomes

MCs could be grouped into three classes (Type I’, Type I and Type II) according their conserved domains [2]. In the present study, to obtain insight into the phylogenetic relationships and evolutionary history in the MC family, MC proteins from F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica were compared for comprehensive phylogenetic analysis. As shown in Fig 1 and S1 Fig, the Maximum-likelihood tree of these MC genes was classified into three classes, which was supported the above discrimination based on their conserved domains. Subsequently, we found that the class I contained 42 type I MC genes, and it was further divided into four subclasses: A, B, C and D with each subclass having 14, 10, 8 and 10 members, respectively. The class II had 20 type II MC genes, and it was categorized into two subclasses: F and G, with each class containing 5 and 15 members, respectively. The class III contained 8 type I’ MC genes, which is named E. MC genes in P. mume, P. persica, P. communis and M. domestica were distributed in all the seven classes, but there are no members from F. vesca in class C and no members from P. bretschneideri in class E and F (Fig 1).

To gain understanding in the structural diversity of MC gene family members, we built exon-intron organization maps of each MC gene, as shown in Fig 2. The 70 MC gene family members have different numbers of introns, ranging from 1 to 17, with the exception of MdMC06, MdMC23, PbMC06, PcMC06 and PcMC04 having no introns. While PcMC02
containing the highest introns (17) among these MC genes. Additionally, we found that the most MC gene family members clustered in the same subfamily contained similar exon-intron (i.e. intron numbers and exon length) distribution patterns. For example, four genes (PmMC01, PpMC06, PcMC12 and MdMC04) locating in the subfamily F shared one intron, ...
and four genes (FvMC06, MdMC08, PmMC05 and PpMC08) belonging to subfamily D had four introns. Likewise, most MC gene family members in the subfamily A had no or one intron except PbMC03, MdMC07 and PbMC07, which contained 2, 2 and 4 introns, respectively. Subsequently, we also investigated the conserved motifs of MC proteins from Rosaceae genomes to understand the diversified functions of these proteins. Twenty conserved motifs were identified and designated as motif 1 to motif 20 in MC proteins, as shown in S3 Table and Fig 2. Additionally, we used the Pfam and SMART database to annotate each of the putative motifs from MEME software. Motif 1, motif 2, motif 3, motif 4, motif 6 and motif 10 were found to encode the Caspase-like domain (PF00656) and were scanned one or more times among all the seven subfamily members. For example, motif 1 and motif 3 were scanned in subfamily F, and motif 1, motif 2, motif 3 and motif 10 were scanned in subfamily C. Remarkably, most MC members within the same subfamily, especially the most closely related members (e.g. PmMC05/PpMC08, PbMC01/MdMC16, PcMC05/PcMC09 and PbMC04/PcMC08), generally contained highly similar motif compositions and distributions, indicating function may be similarities among these MC proteins. We also noted that different subfamilies had great difference within motif compositions and distributions, such as proteins in subfamily C contained motif 8, motif 12, motif 13, motif 14, motif 16 and motif 17, while subfamily E members possessed motif 1, motif 7 and motif 18. At the same time, several motifs were exclusively identified in a particular subfamily, indicating that these motifs may play an important role in the subfamily, for example, motif 7 was unique to the proteins in subfamily E.

Chromosomal distribution and intraspecies microsynteny of MC genes in Rosaceae genomes

According to the positions of MC genes in Rosaceae genomes, we determined the physical locations of these genes among the chromosomes. These data suggested that the distribution of 70 MC genes on the chromosomes of the six Rosaceae species was not evenly distributed (Fig 3). For example, FvMCs were distributed on four of 7 chromosomes (chr 3, chr 5, chr 6 and chr 7), PbMCs were dispersed on six of 17 chromosomes (chr 1, chr 5, chr 7, chr 9, chr 10 and chr 12), while PcMCs only were dispersed on three of 17 chromosomes (chr 5, chr 6 and chr 10). For M. domestica, chromosome 10 harbors 6 MdMCs, the highest number among all M. domestica chromosomes (S2 Table), and followed by chromosome 15 contained 4 MdMCs. For P. communis and P. bretschneideri, chromosome 10 also harbors the highest number (3) among all P. communis and P. bretschneideri chromosomes (Fig 3 and S2 Table).

Based on the whole genome analysis in these six Rosaceae genomes, P. bretschneideri possessed the most putative duplicated gene pairs, such as PbMC07/PbMC08 and PbMC03/PbMC07, M. domestica contained nine gene pairs, P. communis had five gene pairs (S4 Table), whereas other three species not contained any gene pairs (Fig 4). It might because these P. bretschneideri, P. communis and M. domestica have experienced two genome-wide duplication events [27, 46]. Remarkably, we found that no any gene pairs were generated by tandem duplication in M. domestica, P. bretschneideri and P. communis, indicating that tandem duplication may have made little or no contribution to the expansion of the MC gene family in these six Rosaceae genomes. Additionally, the relationships of the flanking sequences of these MC gene pairs were further analyzed using MCScanX and MicroSyn software (Figs 4 and 5). In P. bretschneideri, we identified three conserved genes flanking six pairs. Eight other pairs of MC...
genes had more than three pairs of conserved flanking genes, such as *PbMC07/PbMC08* and *PbMC03/PbMC07* had 17 and 16 gene pairs of conserved flanking genes, respectively. In *P. communis*, we found eleven conserved genes flanking two pairs, *PcMC01/ PcMC04* and *PcMC01/ PcMC05*. Three other pairs of *MC* genes (*PcMC02/ PcMC08, PcMC04/ PcMC05* and *PcMC06/ PcMC07*). Based on the GFF3 annotation information, the physical location of each *MC* was mapped. The scale is indicated by mega bases (Mb). The inner circle represents the species evolution tree, and the blue star indicated the whole genome duplication (WGD) event.

https://doi.org/10.1371/journal.pone.0211635.g003
PcMC06/ PcMC07) contained less than five pairs of conserved flanking genes. In *M. domestica*, all pairs of MC genes had more than four pairs of conserved flanking genes, such as MdMC06/ MdMC16 had eight gene pairs of conserved flanking genes. Therefore, we speculated that large-scale duplication events may contribute to the expansion of MC gene family members during evolution in these three Rosaceae species (Fig 5).
Interspecies microsynteny of MC genes in Rosaceae genomes

For better understand the evolutionary relationship of MC genes among F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica, interspecies microsynteny analysis was carried out to identify orthologous MC genes. The flanking MC genes were analyzed to identify involved duplication events. In the present study, 23 out of 70 intraspecies MC genes were identified to involve in the interspecies microsynteny (Fig 5D and S4 Table). Among them, there were 13 orthologous gene pairs between P. communis and P. bretschneideri, 3 orthologous gene pairs between P. persica and P. bretschneideri, 2 orthologous gene pairs between F. vesca and P. bretschneideri, 2 orthologous gene pairs between P. bretschneideri and M. domestica, 1 orthologous gene pair between P. mume and P. bretschneideri, 3 orthologous gene pairs between P. communis and F. vesca, 1 orthologous gene pair between M. domestica and P. communis, 1 gene pair between P. mume and P. persica, 1 orthologous gene pair...
between *P. mume* and *F. vesca*, 1 orthologous gene pair between *P. mume* and *M. domestica*, and 1 orthologous gene pair between *F. vesca* and *P. persica* were identified (Fig 5D and S4 Table). Among *P. bretschneideri* and other five Rosaceae plants, we identified the different number of orthologous gene pairs, which may be due to different loss rates of duplicated genes during evolution.

**Expression patterns of *PbMCs* in response to drought stress**

Drought is a major abiotic stress that can affect plant productivity, growth and development. MCs play an important role in the process of PCD during stress responses in plants. To further understand the possible involvement of *PbMCs* in response to drought stress, the expression profiles of *PbMCs* were analyzed by RNA-seq data. As shown in Fig 6A, only four out of 12 *PbMCs* were expressed under drought stress. Among these *PbMC* genes, three genes (*PbMC03*, *PbMC04* and *PbMC05*) displayed up-regulation at least one time point after treatment. At the same time, we also found that the expression of *PbMC08* showed continuous decrease after a long period of stress treatment, and resulting in 5-fold decrease at 6 h after treatment, and then the expression level increased after 24 h. In contrast, the *PbMC04* gene was strongly and rapidly induced by drought treatment, reached the maximum level at 6 h after treatment, suggesting the existence of a possible feedback regulatory mechanism (Fig 6A).

**Expression patterns of *PbMCs* during pollen tube and fruit development stages**

For flowering plants, pollen germination and pollen tube growth are very important for sexual reproduction. Tip-growth is the most important feature of pear pollen germination and pollen tube growth. The pollen tube extends very rapidly and pollen tube death occurs within 24 h of *in vitro* culture [52, 53]. These results suggested that pollen and pollen tube is an ideal model system for studying the molecular mechanisms of a series of cellular processes, such as cell growth and death. In the present study, seven *MC* genes were found transcriptionally active in at least one stage, and five genes (*PbMC01*, *PbMC02*, *PbMC03*, *PbMC07* and *PbMC08*) out of the seven genes were identified transcriptionally active in all four stages: including mature pollen grains (MP), hydrated pollen grains (HP), pollen tube (PT) and top-growth pollen tube (SPT), indicating that these genes may play a role in reproduction (Fig 6B). Subsequently, we used the qRT-PCR experiment to verify the expression patterns, and the results were shown in Fig 7. These data suggest that these *PbMC* genes showed similar expression patterns as transcriptome data.

To gain insight into the expression patterns of *PbMCs* in *P. bretschneideri* fruit development, gene expression profile analysis was carried out in seven pear fruit developmental stages (i.e. 15 days after full blooming (DAB), 30 DAB, 55 DAB, 85 DAB, 115 DAB, mature stage and fruit senescence stage). The transcription levels of the *PbMCs* were measured using FPKM values (Fig 6C). Of the 12 *MC* genes identified in *P. bretschneideri*, nine *PbMCs* were detected to be expressed in one or more developmental stages, and three genes (*PbMC01*, *PbMC03* and *PbMC04*) out of nine *PbMCs* were identified to be expressed in all seven fruit development stages (Fig 6C). Notably, several *PbMCs* continuously reduced or increased at one or several stages, such as *PbMC01* and *PbMC12*, which were up-regulated in Fruit_stage4 (85 DAB), *PbMC02*, *PbMC06* and *PbMC10*, which were highly expressed at Fruit_stage1 (15 DAB), *PbMC07* was up-regulated in Fruit_stage6 (mature stage), indicating that these gene play an important role in the fruit-specific developmental stages.
Discussion

In recent studies, the vast majority of published articles suggested that the MC genes exist as multi-gene families in the genome [2, 18, 19]. Although the number of MC gene family have been investigated in several species [18–23], such as Arabidopsis thaliana, Vitis vinifera, Hevea brasiliensis and Oryza sativa. However, the MC genes from Rosaceae genomes have not been characterized in detail to our knowledge. In the present study, 70 MC genes were identified in six Rosaceae genomes; 12 of these sequences were from P. bretschneideri, and 8, 7, 8, 12, and 23 MC genes in F. vesca, P. mume, P. persica, P. communis and M. domestica, respectively. By using the MEROPS online tool (https://www.ebi.ac.uk/merops/) [2], we predicted the distribution of Type-I* (8), Type-I (42) and Type-II (20) MC genes in these genomes, which consistent with the predictions of phylogenetic analysis of these MC gene family numbers. Using a comparative genomic approach, Fagundes et al. (2015) identified MC genes in Viridiplantae, and
then the distribution of Type-I and -II MC genes was predicted in 42 plant species [1]. They found that the total number of Type-I MC genes was more than twice the number of Type-II MC genes [1], which is basically consistent with our results. The diversity of gene structure not only provides additional evidence for supporting phylogenetic groupings, but it also plays an important role in the evolution of gene families [58]. Exon-intron structure analysis suggested that the most of Type I MC genes contained four or five exons and Type II MC genes had two or three exons. We also noted that the lengths of the exons were more conserved than the introns in Type I MC genes (Fig 2). Our data was consistent with the previous published articles that Type I MC genes contained more exon numbers than Type II MC genes [1, 22]. Additionally, the identification and phylogenetic classification of MC genes were further supported by the exon-intron structure analysis in Rosaceae genomes.

Gene duplication plays an important role in the process of biological evolution [59]. In some plants, microsynteny has been extensively described, such as GELP, WOX and MYB gene family in Rosaceae genomes [39, 41, 46]. In the present study, based on the microsynteny analysis, we did not observe the microsynteny relationships among FvMC01, FvMC03, FvMC04, FvMC06 and FvMC07, PcMC09, PcMC10, PcMC11 and PcMC12, and 22 with other MC genes in these four species (P. mume, P. persica, P. bretschneideri and M. domestica), implying that either these genes were formed through complete transposition and loss of their primogenitors or they were ancient genes without detectable linkage to other MC genes. Remarkably, two or more MC genes from these Rosaceae genomes (F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica) were orthologous to the MC genes of the same species, indicating that these gene pairs may play important roles in the expansion of MC gene family during evolution, such as PcMC01, PcMC04 and PcMC05 are orthologous genes to PbMC03, as well as PbMC04 and PbMC09 are orthologous genes to PcMC08.

So far, there is increasing evidence that MC activity plays an important role for PCD in plant growth and development, and it is essential for plant pollen and embryonic development.
For example, Watanabe et al. (2011) observed the stronger GUS expression of AtMCP2d promoter in vascular bundles of roots, pollen and embryonic cells [61]. In the present study, we explored the expression pattern of MC genes in pear pollen tube development and fruit development. Previous studies have shown that different abiotic stresses can induce PCD, and abiotic stress-induced PCD has a significant effect on plant growth and development [18–22, 62]. MC genes have also been shown to play key roles in the abiotic stress response in plants. Oryza sativa MC genes showed differential expression patterns in response to cold, drought, and salt stresses, and the expression of most OsMC genes was down-regulated when responding to salt and drought stresses [63]. In Hevea brasiliensis, Liu et al. (2016) reported that all of the HbMCs, except HbMC5, displayed transcriptional changes when responding to salt, drought, as well as cold stresses [19]. In Hordeum vulgare, qPCR analysis suggested that the expression of HvMC4 was significantly increased upon excess-B supplementation [23]. In the present study, we found that several PbMC genes also respond to drought stresses, including PbMC03, PbMC04 and PbMC08. Interestingly, the PbMC08 shown continuous down-regulated after a long period of stress treatment. In contrast, the PbMC04 gene was strongly and rapidly induced by drought treatment. The opposite expression pattern indicates that different PbMC genes contained divergent regulatory mechanisms to respond drought stress. Our data suggested that PbMC genes might play an important role in pear reproductive development and abiotic response.

Conclusion

In the present study, 70 MC genes were identified in Rosaceae genomes, which including 8, 7, 8, 12, 12, and 23 MC genes in the genomes of F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica, respectively. Subsequently, we carried out comparative genomic and systematic analysis, such as phylogenetic relationships, exon-intron structures, microsynteny, conserved motifs, and expression patterns. Our results suggested the vast majority of MC gene of P. communis, P. bretschneideri and M. domestica was expanded by large-scale gene duplication. Expression profiling revealed that PbMC01 and PbMC03 were able to be detected in all four pear pollen tube and seven fruit development stages. This understanding of MC expression provides a new avenue for functional analyses of pear during pollen tube and fruit development.

Supporting information

S1 Fig. Phylogenetic tree of MC genes from six Rosaceae species (F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica) and Arabidopsis thaliana. The Maximum-likelihood tree was generated using IQ-TREE software, and MsMC (CBN76943.1) from Ectocarpus siliculosus was used as the out-group.

(PDF)

S1 Table. Primers in this study.

(XLSX)

S2 Table. Information of MC genes in six Rosaceae species, including F. vesca, P. mume, P. persica, P. communis, P. bretschneideri and M. domestica.

(XLSX)

S3 Table. Twenty MEME motif sequences of MC proteins in six Rosaceae species.

(XLSX)
S4 Table. Homologous gene pairs of MC among between *F. vesca*, *P. mume*, *P. persica*, *P. communis*, *P. bretschneideri* and *M. domestica*.

(XLSX)

**Acknowledgments**

We extend our thanks to the reviewers and editors for their careful reading and helpful comments on this manuscript. This study was supported by The National Natural Science Foundation of China (grant 31640068). The Funding bodies were not involved in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Availability of data and materials**

*P. bretschneideri* stop-growth pollen tube, Accession: SRX1356346; *P. bretschneideri* pollen tube, Accession: SRX1356343; *P. bretschneideri* hydrated pollen grains, Accession: SRX1356152; *P. bretschneideri* mature pollen grains of pear, Accession: SRX1356151; *P. bretschneideri* fruit developmental stage 1 (15DAB), Accession: SRX1595645; *P. bretschneideri* fruit developmental stage 2 (30DAB), Accession: SRX1595646; *P. bretschneideri* fruit developmental stage 3 (55DAB), Accession: SRX1595647; *P. bretschneideri* fruit developmental stage 4 (85 DAB), Accession: SRX1595648; *P. bretschneideri* fruit developmental stage 5 (115 DAB), Accession: SRX1595650; *P. bretschneideri* fruit developmental stage 6 (mature stage), Accession: SRX1595651; *P. bretschneideri* fruit developmental stage 7 (fruit senescence stage), Accession: SRX1595652; *P. bretschneideri* drought-tolerant for 0h, Accession: SRX4110141; *P. bretschneideri* drought-tolerant for 1h, Accession: SRX4110140; *P. bretschneideri* drought-tolerant for 3h, Accession: SRX4110143; *P. bretschneideri* drought-tolerant for 6h, Accession: SRX4110142; *P. bretschneideri* for 24 h, Accession: SRX4110139

**Author Contributions**

**Formal analysis:** Yu Chen.

**Resources:** Wei Zeng, Lei Zhang, Qi Wang, Wei Hen, Muhammad Abdullah, Yi Lin.

**Software:** Dandan Meng, Muhammad Abdullah, Qing Jin.

**Validation:** Yunpeng Cao.

**Visualization:** Tianzhe Chen.

**Writing – original draft:** Yunpeng Cao.

**Writing – review & editing:** Yunpeng Cao, Yongping Cai.

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