The chromosome-level genome of Gypsophila paniculata reveals the molecular mechanism of floral development and ethylene insensitivity

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Abstract

Gypsophila paniculata, belonging to the Caryophyllaceae of the Caryophyllales, is one of the most famous worldwide cut flowers. It is commonly used as dried flowers, whereas the underlying mechanism of flower senescence has not yet been addressed. Here, we present a chromosome-scale genome assembly for G. paniculata with a total size of 749.58 Mb. Whole-genome duplication signatures unveil two major duplication events in its evolutionary history: an ancient one occurring before the divergence of Caryophyllaceae and a more recent one shared with Dianthus caryophyllus. The integrative analyses combining genomic and transcriptomic data reveal the mechanisms regulating floral development and ethylene response of G. paniculata. The reduction of AGAMOUS expression probably caused by sequence polymorphism and the mutation in miR172 binding site of PETALOSA are associated with the double flower formation in G. paniculata. The low expression of ETHYLENE RESPONSE SENSOR (ERS) and the reduction of downstream ETHYLENE RESPONSE FACTOR (ERF) gene copy number collectively lead to the ethylene insensitivity of G. paniculata, affecting flower senescence and making it capable of making dried flowers. This study provides a cornerstone for understanding the underlying principles governing floral development and flower senescence, which could accelerate the molecular breeding of the Caryophyllaceae species.

Introduction

The genus Gypsophila belongs to the Caryophyllaceae family, and comprises ~150 species of the annual, biennial, and perennial plants which mainly originated from temperate Asia and Europe [1]. Among them, G. paniculata (2n = 34) is a perennial herbaceous shrub, the only species used as cut flowers in the genus Gypsophila [2]. With the clouds of tiny white or pink flowers covering the bunches of branching stems after blooming, G. paniculata is commonly used as fresh or dried filler in flower arrangements and bouquets. Due to its ornamental value, G. paniculata is listed in the top ten best-selling cut flowers in the global floricultural market [3]. In addition, the plants of G. paniculata contain various bioactive compounds with potential medicinal values, such as flavonoids, triterpene saponins, sterols, and volatiles, which increase the utilization and economic value of this ornamental plant [4].

Flower type is one of the most critical ornamental traits and the petal of many floricultural species (e.g. rose, carnation, and lisianthus). Among these diverse flower types, the double flower is considered to be the key and precious one. Thereby, breeding efforts have been invested in the creation and improvement of desirable double flower varieties [5]. The same holds for the breeding of G. paniculata, and several commercial varieties were released, such as ‘Million Stars’ and ‘Huixing 1’ [6]. However, the main breeding methods of this species are confined within conventional hybridization and subsequent phenotypic selection for specific traits, which become inoperable in the breeding of the double flower varieties due to their almost infertile reproductivesystem [7]. As a consequence, the variations existing among the current commercial cultivars are limited [8].

Received: 30 March 2022; Accepted: 2 August 2022; Published: 24 August 2022; Corrected and Typeset: 1 October 2022
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In the past decades, the genetic and molecular networks regulating flower development have been investigated in roses, carnations, and gerberas [9–11]. An ABCE model has been raised in the model plant Arabidopsis which also fits other species [12, 13]. The model indicates that the sepal formation is controlled by the A-class genes, which also function in the petal formation, together with the B-class genes. C-class gene specifies the stamen development with B-class genes and determines the carpel fate while alone. And the E-class genes function throughout the whole flower identity. In addition, the A- and C-class genes are antagonistic in expression patterns. The loss-of-function of AGAMOUS (AG) results in the elevated expression of A-class genes in the stamens, turning them into petals [14]. Similarly, the over-accumulation of APETALA2/PETALOSA (AP2/PET) due to the defects of miR172 binding reduces the AG expression in the stamens, leading to the double flower phenotype likewise [9, 10]. Although this model has been verified in many ornamentals, it remains unclear whether it is true in G. paniculata [15].

Flowers that remain open and colored long after senescence initiation are normally used as dried flowers, such as species in Limonium and Gypsophila [16]. This phenomenon may relate to ethylene regulation, as ethylene functions critically in flower senescence and petal abscission [17, 18]. In addition to ethylene, phytohormones such as abscisic acid (ABA), cytokinin (CTK), gibberellins (GAs), auxin, and jasmonic acid (JA) also influence petal senescence in a synergistic or antagonistic way. The crosstalk between different phytohormones in petal senescence and the genes involved in relative biological processes are well reviewed [19]. Moreover, transcription factors like RhHB1, RhMYB108, RhWRKY33, and DcWRKY75 affect petal senescence through positive or negative regulation of phytohormone synthesis and signal transduction [20–23]. Previous studies showed that the flowers of G. paniculata were highly sensitive to ethylene, and the endogenous ethylene content instantaneously increased in flower initiating senescence process [24, 25]. However, the flower decay or petal abscission occurred rarely during flower senescence, even though the petals became translucent, rolled, wilted, and desiccated. Abscission of petals exists in many species, and this process is highly sensitive to ethylene but not associated with petal senescence [26, 27]. To date, the knowledge of the molecular mechanisms underlying the relationship between ethylene response and petal senescence in G. paniculata flowers is limited.

In this study, a chromosome-level genome assembly of G. paniculata was presented. The genome was sequenced by a combination of long-read sequencing and Hi-C scaffolding technologies. In total, a 749.58 Mb genome was assembled and anchored to 17 pseudo-chromosomes. The molecular basis of double flower formation and regulation in G. paniculata was revealed through gene family and transcriptome analyses generated from diverse floral developmental stages, together with a forward genetic ethyl methane sulfonate (EMS) mutant screen. Meanwhile, we provide new insights into the influence of ethylene on flower senescence with a plausible explanation of the formation of dried flowers in G. paniculata. This chromosome-scale reference genome and the genetic mechanisms governing floral development in G. paniculata offer valuable resources for developing consumer-oriented selective breeding of Gypsophila.

Results
G. paniculata genome assembly and annotation
The genome sequencing of G. paniculata was conducted from a wild-type plant with pink single flowers. The genome was sequenced using Illumina HiSeq, Nanopore sequencing, and Hi-C technique. Approximately 82.85 Gb of Nanopore long reads were generated, covering 102.92-fold of the 805.00 Mb genome sequence (k-mer analysis data, Figure S1, see online supplementary material). The genome size of G. paniculata was 794.66 Mb determined by flow cytometry (Figure S1, see online supplementary material), which was in line with the k-mer estimate (k = 17). After the pipeline of the MECAT assembling program and PILON, the long-read reads were assembled into a contig level with error sites corrected. A total of 749.58 Mb error-corrected contig-level assembly was obtained (281 contigs, N50 = 15.07 Mb, Table 1). In the end, the assembly results at the chromosome level, which is combined by 17 pseudo chromosome scaffolds and 82 contigs that are not anchored to any chromosome, were obtained after adjusting the assembly results produced by ALLHiC (Figs 1 and S2, see online supplementary material). According to the alignment result with eudicotyledons_odb10 using BUSCO, 99.8% universal single-copy orthologous gene groups in eudicot were obtained in the genome assembly, indicating that the result of the assembling process is relatively integrated. We adopted bwa-mem2 for aligning NGS (Next generation sequencing) reads to the assembly. It turned out that 99.84% of the NGS reads are mapped to the reference genome, and 96.56% are properly paired. By aligning the genome assembly with several assemblies of the mitochondrion and the chloroplast using BLASTN (version = 2.12.0+, P-value ≤0.001), we confirmed that the genome assembly covered a part of the mitochondrion and most of the chloroplast (Table S1, see online supplementary material).

After the pipeline of MAKER, 24459 genes and 40273 transcriptions were annotated. By the alignment with eudicotyledons_odb10 using BUSCO, 90.07% universal single-copy orthologous gene groups in eudicot were found in the annotation. After the adoption of a process combined by RepeatModeler and RepeatMasker, a total of 895344 repeat sequences covering ~77.23% of the genome were obtained (571.74 Mb), consisting of retroelement (71.33%), DNA Transposon (23.14%), and tandem repeats (9.13%). Interestingly, we found a remarkable feature in the distribution of genes in the assembly of G. paniculata genome: the gene density is much higher at the ends of chromosomes than central (Fig. 1), which is far more different from the vast majority of dicotyledonous plants’ genomes [28]. A significant negative correlation was shown between chromosome gene distribution density and distribution of average length of introns (Figure S3, see online supplementary material). Therefore, a hypothesis is raised that the distribution of extra-long introns might reduce the distribution density of genes, which was also reflected in the human genome [29].

Whole-genome duplication and genome evolution analysis
To investigate whole-genome duplication events during the evolutionary process of Gypsophila, we first clustered the orthologous gene groups derived from the intergenicomic and intragenomic analysis of G. paniculata, Dianthus caryophyllus, Spinacia oleracea, Beta vulgaris, Fagopyrum tataricum, Arabidopsis thaliana, Vitis vinifera, Oryza sativa, Ananas comosus, and Nymphea colorata. Then single-copy orthologs were adopted for the construction of the phylogenetic tree. Synonymous substitution is thought to occur at a relatively stable rate during the evolution process of orthologous genes, allowing them to be used as molecular clocks due to their absence from selection [30]. Ks and 4Dt distribution of synteny gene pairs within collinearity blocks were used for speculating the whole-genome duplication events. Long collinearity blocks
Table 1. The statistics for genome sequencing of *G. paniculata*.

| Items                          | Number   | Size       | Coverage  |
|-------------------------------|----------|------------|-----------|
| Nanopore reads                | 3 574 137| 82.85Gb    |           |
| Total contigs                 | 281      | 749.58 Mb  |           |
| Contig N50                    | 18       | 15.07 Mb   |           |
| Total protein-coding genes    | 24 459   |            |           |
| Total repetitive sequences    | 895 344  | 571.71 Mb  | 77.23%    |
| LTR retrotransposon           | 266 136  | 352.90 Mb  | 47.67%    |
| Non-LTR retrotransposon       | 99 578   | 36.22 Mb   | 4.89%     |
| DNA transposon                | 265 560  | 132.31 Mb  | 17.87%    |
| Tandem repeats                | 200 870  | 52.22 Mb   | 7.05%     |

Figure 1. Overview of the *G. paniculata* genome assembly. **a**, The 17 pseudochromosomes illustrated in different colors, **b**, gene density (sliding window size = 500 kb), **c**, transposon elements (sliding window size = 500 kb), **d**, tandem repeats (sliding window size = 500 kb), and **e**, connections of collinear blocks (with the same colors as pseudochromosomes) of the *G. paniculata* genome.

detected within *G. paniculata* (Fig. 2a) and between *G. paniculata* and *B. vulgaris* (Fig. 2b) showed the sign of a recent whole-genome triplication (WGT) event. A significant expansion of orthologs of the MRCA (most recent common ancestor) of *G. paniculata* and *D. caryophyllus* was spotted (Fig. 2b). Both distributions of 4Dtv and Ks revealed that a polyploid event had occurred before the divergence of *G. paniculata* and *D. caryophyllus* (Fig. 2c, d, e). The results indicated that *G. paniculata* shared a most recent polyploid event with *D. caryophyllus*, which may play an essential role in the evolution of the Caryophyllaceae family. Last, the evolution analysis was investigated between the species in Caryophyllidae, Rosidae and Asteridae, with the additional genome data of *Coffea arabica*, *Corymbia citriodora*, *Cuscuta australis*, *Malus domestica*, *Pyrus communis*, and *Salvia splendens*. In order to reduce the instability during phylogenetic analysis, the longest transcription of each gene was chosen with other sequences being removed. The filtered sequences were subsequently analyzed by OrthoFinder, and the phylogenetic tree was reconstructed to reveal the evolutionary relationship among the selected species. The phylogenetic tree supported that Caryophyllidae is an outgroup to both Rosidae and Asteridae, and Caryophyllidae is closer to Rosidae phylogenetically compared to Asteridae (Fig. S4, see online supplementary material).

Genetic basis of floral development in *G. paniculata*

Through the blast of identified MADS-box genes from *Arabidopsis* in *G. paniculata* genome, 95 MADS-box genes were identified, including 33 Type I MADS-box genes and 62 Type II MADS-box genes (Fig. S5 and Table S2, see online supplementary material). In the phylogenetic tree constructed together with several other angiosperm species, the Type II MADS-box genes of *G. paniculata*
are divided into 14 groups, namely SEPALATA (SEP), AGAMOUS-LIKE 16 (AGL16), APETALA 1 (AP1), FLOWERING LOCUS C (FLC), AGAMOUS/SEEDSTICK (AG/STK), SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1), SHORT VEGETATIVE PHASE (SVP), AGAMOUS-LIKE 15 (AGL15), ARABIDOPSIS NITRATE REGULATED 1 (ANR1), AGAMOUS-LIKE 12 (AGL12), OsMADS32, APETALA 3/PISTILLATA (AP3/PI), ARABIDOPSIS BSISTER (BS), and MIKC+. TM8 and AGL32 were found neither in the G. paniculata genome nor the transcriptome. In addition, no significant expansion of gene number in the 11 groups of Type II MADS-box genes was detected in G. paniculata, but in B. vulgaris, no significant expansion of gene number in the 11 groups of Type II MADS-box genes was detected in G.paniculata (except for OsMADS32, FLC, and AGL12).

The phylogenetic profiles of the genes related to flower development in G. paniculata were further investigated, and the homologous genes for the ABCE model were identified (Fig. S6, see online supplementary material). There are three AP1 homologs of A-class genes and three B-class genes consisting of two AP3 genes and one PI gene. Both duplicates of the C-class genes, AGa and AGb, are retained on the collinear blocks, which likely results from the genome duplication event of G. paniculata, as the STK group only consists of one member. In addition, four SEP homologs belonging to the E-class are found. To conclude, the distribution and number of ABCE genes in G. paniculata are similar to those in Arabidopsis, indicating that there is no significant expansion or loss of genes during the whole genome duplication of G. paniculata.

Double flower regulation and the relative genes in G. paniculata

To investigate the organ determination and development of double flowers, the floral development process was divided into three stages in wild type and double flower cultivars (‘YX1’ and ‘YX4’), namely flower bud stage, flower semi-open stage, and flower fully open stage (Fig. 3a). ‘YX1’ and ‘YX4’ are two representative commercial varieties of G. paniculata with white petals, and the difference is that the flower type of ‘YX1’ is larger than that of ‘YX4’. We analysed the RNA-seq data of type II MADS-box genes and generated its expression patterns in the whole flower and different floral developmental stages (Fig. 3b). The results showed that the expression level of AG genes (C-class) is largely decreased in two double flower cultivars compared with wild type, while the expression levels of A-, B-, and E-class genes increased to a first approximation. We then further investigated the expression pattern of ABCE model genes in different floral organs (sepal, petal, stamen, and carpel) at the fully open stage (Fig. S7, see online supplementary material). We did not detect the gene expression in stamen as it was not found in two double flower cultivars. In total, 15 genes (AP1a/b/c, AP2a/b, FETa/b, AP3a/b, PI, STK, AGa/b, and SEPa/b) were examined in different floral organs. All the expression levels of the C-class genes were reduced significantly in petal of double flower cultivars, while the expression levels of B-class genes PI and AP3b increased.
dramatically in the same organs, compared with those in wild type. The relative expression levels of A-class genes were quite low in floral organs except for AP1a and FEtA. Although slight differences were displayed between double flower cultivars and wild type, no clear pattern was summarized. The E-class genes SEPAs and SEFb showed different expression patterns as well. SEPAs expressed more or less in all the samples, whereas the expression levels were significantly high in floral organs of double flowers rather than wild type.

Previously, three double flower mutants of G. paniculata were isolated, namely dfm1, dfm2, and dfm3, through EMS induced mutation using single flower wild type seeds. The three double flower mutants displayed unequal variation in flower type and color compared with wild-type flowers (Fig. S8b, see online supplementary material). The AG genes of the three mutants were then cloned, and SNP switches and deletions were identified in the AG sequences (Fig. 3d and Table S3, see online supplementary material). In addition, the AG genes in ‘YX1’ and ‘YX4’ also showed several non-synonymous mutations, which might lead to the dysfunction or expression disorder of AG and cause the double flower phenotype. Besides, the miR172 binding site (GCCGACATCATGAGATTG) of AP2/PET is also responsible for the double flower formation in flower plants [9]. An SNP in PETs miR172 binding site (G to C) was detected in ‘YX1’ and ‘YX4’, but not in dfm lines. Lastly, random crosses between wild type and commercial double flower cultivars were performed, and several double flower progenies were isolated in the seeds harvested from wild type, indicating the dominance of the double flower phenotype (Fig. S8c, see online supplementary material). Similar to the known theory [9], our data suggest that AG and PET genes are the key genes in the regulation of double flower formation in G. paniculata in this research (Fig. 3e).

**Flower senescence and ethylene response in G. paniculata**

RNA-seq data of different samples were generated from G. paniculata wild type, ‘YX1’ and ‘YX4’ in the exploration of mechanisms underlying the trait of G. paniculata befitting for dried flowers. A total of 23449 expressed genes were clustered into 10 different color modules using WGCNA package on the basis of pairwise correlations between genes in their common expression trends among all samples. The 10 different color modules are shown by the dendrogram in Fig. 4a, in which each tree branch constitutes a module and each leaf in the branch is one gene. Notably, the magenta and turquoise modules are associated with the floral development stages in the sample of two cultivars, suggesting the genes in those modules might be involved in the flower development and senescence (Fig. 4b).

In the magenta module, 406 genes are highly expressed at the flower fully open stage (S3) in two cultivars. Based on the gene expression pattern and annotation, we obtained four genes that might be associated with flower development and senescence (Gpan01g00371, Gpan01g01321, Gpan01g01319, and Gpan01g01389, Figure S9 and Table S4, see online supplementary material). Gpan01g00371 and Gpan01g01321, the SENESCENCE-RELATED GENE 1 (SRG1) like genes, are highly expressed at the S3 stage in ‘YX1’ compared with other two plants, suggesting that the senescent process is accelerated in cultivar ‘YX1’ compared with wild type and ‘YX4’. Likely, Gpan01g01319 encodes a purple acid phosphatase, which regulates phosphate during leaf senescence and is upregulated during leaf senescence [31]. The expression of Gpan01g01319 is all upregulated during flower development in wild type and two cultivars, indicating that it is possibly involved in petal senescence as well. Moreover, Gpan01g00371, Gpan01g01321, and Gpan01g01319 also show hub roles in the gene networks in the magenta module, together with the unknown gene Gpan01g01389 (Fig. 4f). Different to the senescence marker genes, two genes that potentially delay plant senescence, Gpan07g00598 (gibberellin receptor) and Gpan05g00038 (peroxidase 5, an antioxidant enzyme), are highly expressed at stage S3 in wild type rather than that in the two cultivars. The accumulation of peroxidase helps to eliminate reactive oxygen species in plants and thus delays senescence [32]. Our data collectively imply that the above genes possibly regulated the flower senescence. Thus, it is possible that the G. paniculata naturally harboured the ability of anti-senescence and this ability was partially lost during the process of artificial breeding.

There are also 5560 co-expressed genes clustered into the turquoise module, associated with different floral development stages (S1 to S3) in the two cultivars. A lot of genes related to ethylene signal transduction and response were discovered and clustered in the turquoise module (Fig. 5a and Table S5, see online supplementary material), including ETHYLENE RESPONSE SENSOR (ERS, Gpan17g000803, Gpan15g00618, and Gpan13g00217), ETHYLENE INSENSITIVE 3-LIKE (EIL, Gpan04g00597), and ETHYLENE-RESPONsIVE TRANsCRIPtION FACtor (Gpan02g00593 and Gpan08g01162). Most of the ethylene-related genes show lower expression levels in ‘YX1’ and ‘YX4’, suggesting that the two cultivars might be insensitive to the ethylene, resulting in the delayed flower senescence. Three unknown genes, Gpan02g00074, Gpan01g00708, and Gpan01g00461, show the hub regulation roles in the gene network of the turquoise module, indicating that they may have functions in the regulation of flower senescence in the two cultivars (Figure 4f). The RNA-seq data provides some candidate genes that might be involved in the flower senescence, and also suggests that the delay of flower senescence might be determined by the ethylene insensitivity of G. paniculata.

Next, the key genes involved in the pathways of ethylene biosynthesis and signaling transduction were investigated through genomic data of eight species, including early angiosperm (Nyctophila colorata), monocots (Ananas comosus, O. sativa, and Musa acuminata), and eudicots (A. thaliana, Rosa chinesis, Chimonanthus salicifolius and G. paniculata). Eleven core gene families were identified via comparative genome analysis, namely S-ADENOSYL METHIONINE (SAM), 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE (ACS), 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE (ACO), ERS, RESPONSIVE TO ANTAGONIST 1 (RAN1), REVERSION TO ETHYLENE SENSITIVITY 1 (RTE1), CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), ETHYLENE INSENSITIVE (EIN), EIN3-BINDING F-BOX PROTEIN (EBF), EIL, and ETHYLENE RESPONSE FACTOR (ERF) (Fig. 5a). Among these gene families, only one member is detected in RAN1, CTR1, and EIN gene families in G. paniculata, and the gene numbers of other families in G. paniculata genome are close to that of other monocots and eudicots, like SAM, ACS, ACO, and RTE1 genes. Interestingly, the ERS genes, located on the membrane and transporting ethylene signal from extracellular to the cytoplasm [33], have the largest gene number (eight) in G. paniculata, similar to in banana (M. acuminata). A huge gene number shrinkage is identified in ERF family, in which only 34 genes are detected in G. paniculata, while there are 205 and 118 members identified in banana and Arabidopsis, respectively, implying that there is a dramatical reduction of the ERF gene number in G. paniculata.

A comparative synteny map was further constructed using C. salicifolius, A. thaliana, R. chinesis, and G. paniculata to infer the narrowed mechanism of the ERF gene family in G. paniculata.
Figure 3. The MADS-box gene expression profile and proposed regulation network of double flower formation in G. paniculata. a. The morphological characteristics of the single-flowered (wild type) and double-flowered ('YX1' and 'YX4') G. paniculata were displayed here. Three distinguishable flower development stages were shown for each variation, namely flower bud stage (S1), semi-open stage (S2), and fully open stage (S3). b. The transcriptome analysis of G. paniculata MADS-box genes from three flower development stages in various flower types. Four groups (ABCE) were classified according to the expression of MADS-box genes. Expression values were scaled by log2(FPKM +1), in which FPKM is fragments per kilobase of exon per million mapped reads. c, qPCR-based gene expression level of PETa, AGa, and STK in different floral organs of the fully open stage (sepal, petal, and carpel). Because no stamen was found in two double flower cultivars, we did not detect gene expression in that organ. The expression of more ABCE mutants and cultivars (detailed mutation information is shown in Table S3, see online supplementary material). d. The representative detected mutations in AG and PET genes from EMS mutants and cultivars (detailed mutation information is shown in Table S3, see online supplementary material). e. The regulation network of double flower formation in G. paniculata.

In the genome of G. paniculata, all of 34 ERF genes are mapped onto 14 chromosomes, except the chromosomes 4, 5, and 12. In detail, four ERF genes are located on chromosome 2 and 13, while only one member is found on chromosome 15 and 17, respectively. Moreover, six duplicated syntenic blocks composed of 22 ERF genes are identified on ten chromosomes of G. paniculata genome, all generated from segmental duplication. Besides, six tandem duplication ERF gene pairs are also found on chromosomes 1, 2, 7, 11, 13, and 14. However, a total of 71 interspecific syntenic blocks are identified between C. salicifolius and A. thaliana, and 76 interspecific syntenic orthologous gene pairs are found in A. thaliana and R. chinensis, whereas only 25 syntenic blocks exist between R. chinensis and G. paniculata.

The expression profiles of the above gene families in different flower development stages and tissues were conducted using G. paniculata wild type and two cultivars (Fig 6). The ethylene synthesis and signal transduction genes are highly expressed in all tissues and development stages, such as SAM, ACO, RAN1, EIN2, EBF, and EIL. However, the expression level of ERS is relatively low in all detected tissues, indicating the ERS genes were not highly active during the floral development of G. paniculata. As expected, ERFs show much lower expression during plant development, except for one gene (Gpan039g00752).

To support the hypothesis that the insensitivity of G. paniculata to ethylene is due to the abnormal function of ethylene receptors, we investigated the expression level of ERS under short exposure (0 h, 4 h, and 12 h) to exogenous ethylene using the double flowers of two cultivars ('YX1' and 'YX4'). Eight ETR genes identified in the genome of G. paniculata were detected, which could be divided into two groups based on the phylogenetic analysis, namely ERS1 and ERS2 (Fig 6b and c). The expression of the ERS1 and ERS2 genes decreased significantly after ethylene treatment in cultivar ‘YX1’, while two ERS1 and ERS2 genes (Gpan089g01336 and Gpan10g00832) showed decreasing after 4 h ethylene treatment in cultivar ‘YX4’. This is also consistent with the observed senescent process that ‘YX1’ ages faster than ‘YX4’. These results prove that ethylene
receptors of *G. paniculata* do not respond to exogenous ethylene in commercial cultivars and the ethylene sensitivity varies in different varieties, which may be caused by artificial selection during plant breeding. Our data collectively suggest that the low expression level of *ERS* genes and the reduced number of downstream *ERF* genes might be related to ethylene insensitivity in *G. paniculata* due to non-ethylene response, resulting in the petal reservation during the process of flower senescence.

**Discussion**

*G. paniculata*, the only species used as cut flowers in the genus *Gypsophila*, is an important cut flower for global floricultural markets. In this study, we have sequenced the genome of *G. paniculata* wild type, which is the first genome of the *Gypsophila* genus. On the premise of a pseudochromosome genome assembly and a relatively complete annotation, the WGT events are well illustrated in different ways, such as genome collinearity and orthologs analysis, which are shared by *G. paniculata* and *D. caryophyllus* and may play an essential role in the evolution of Caryophyllaceae.

The genome of *G. paniculata* provides a better understanding of floral development, which is the key ornamental trait in its breeding process [34]. The application of databases about the floral development regulation networks in several flowering plants serves to identify the homologs of floral development genes in *G. paniculata*, together with the comparative transcriptome data analyses. In the expression profile of MADS-box genes in *G. paniculata*, the expression of *AG* genes showed a significant decrease in double flower cultivars, whereas the expression of *AP2/PET* genes showed a sharp increase in the whole flower at different stages. In EMS mutants and double flower cultivars, variety mutations were identified in *AG* genes, which is a core gene playing an essential role in double flower formation in many flowering plants [35–38]. For instance, *AGa* and *AGb*, the *AG* subfamily, are widely expressed in stamens and carpels in most flower plants, such as *Nymphaea colorata*, *D. caryophyllus*, and *Eustoma grandiflorum*, and the mutation or reduced expression in petal of *AG* will cause the formation of double flower [39–41]. Moreover, it has been reported that mutations in the miR172 target site of *AP2/PET* were responsible for the double flower formation in *Arabidopsis*, petunia, rose, peach, and...
Figure 5. The key genes of G. paniculata involved in ethylene biosynthesis and signaling transduction pathways. a, The number of key genes regulating ethylene biosynthesis and signaling transduction. The species were selected from basal angiosperm N. colorata, monocots (A. comosus, O. sativa, and M. acuminata), and eudicots (A. thaliana, R. chinensis, C. salicifolius, and G. paniculata). b, Synteny analysis of ERF genes between G. paniculata, C. salicifolius, A. thaliana, and R. chinensis. Gray lines in the background indicate the collinear blocks within G. paniculata and other plant genomes, while the red lines highlight the syntenic ERF blocks. c, Schematic representation for the chromosomal distribution and interchromosomal relationships of G. paniculata ERF genes.

Dianthus [42]. In present study, we detected an SNP substitution in PETa from two double flower cultivars, but no mutations in EMS mutants, which had fewer petals compared to the cultivar. This could be that the disrupted AG have a cumulative effect with overaccumulation of PET on double flower formation. In all, the revealing of the floral development regulation in G. paniculata provides the genetic basis for molecular breeding using genetic engineering technology, such as genome editing.

Dried flowers and dyed flowers are important forms of cut G. paniculata flowers. Our results suggested that low expression of ethylene receptors and the massive loss of ethylene response factors were related to ethylene insensitivity during flower senescence. This phenomenon has also been observed in carnation flowers, which are highly sensitive to ethylene but petals do not fall naturally [42, 43]. Six putative ethylene receptors were identified in carnation genomic sequences, and ETR1, ERS1, and ERS2 genes were classified in one subfamily via phylogenetic analysis. These three genes were expressed constitutively or at undetectable levels in different floral tissues during flower senescence in carnation reported by previous independent research [44]. We found the ethylene response factors, ERF genes, were largely lost in G. paniculata genome (34 members in G. paniculata, while there were 205 and 118 members identified in banana and Arabidopsis), as well as in carnation genome (41 ERFs of carnation vs 106 ERFs of rose) [45]. These results were in line with our data in G. paniculata during flower senescence. Moreover, similar results have also been achieved in geranium and Delphinium, demonstrating that the effect of ethylene on flower senescence may not be carried out by regulating the expression of ethylene receptor genes [46–48]. Thus, we speculate that the partial gene loss and low ethylene response of ethylene receptors during the evolution of Caryophyllaceae species changed the ethylene response network, which basically abandoned the gene function of ethylene response factors, resulting in the low expression level and massive gene loss of ERF genes.

Conclusions

In summary, we have presented a high-quality genome of G. paniculata, the first reference genome of the Gypsophila genus. This G. paniculata genome will be a significant contribution to this ornamental plant research community. The whole-genome duplication signatures demonstrate two major duplication events in the evolutionary history of G. paniculata. Through this high-quality chromosome-level genome, we identified and analysed the MADS-box genes to illustrate the floral development regulation of G. paniculata. Notably, with the sequence polymorphism analyses of EMS double-flowered mutants and commercial cultivars, the disruption of AG and/or the misregulation of miR172-mediated PET expression are found to be associated with the double flower formation. We also found that the transcriptional regulation and the reduction in the number of ethylene-related genes lead to the insensitivity of G. paniculata to ethylene, satisfying the characteristics of dried flowers. This G. paniculata genome will also serve as a great resource to improve ornamental traits through molecular breeding in the future due to the fact that G. paniculata is increasingly consumed and demanded for new varieties in the global floricultural market.

Materials and methods

Genome and transcriptome sequencing

Total DNA for genome sequencing was extracted from fresh young leaves of a single wild type plant of G. paniculata. RNA for transcriptome sequencing was extracted from several organs and
Figure 6. The ethylene biosynthesis and signaling transduction pathways of G. paniculata. Heat maps of the expression of ethylene biosynthesis and signaling transduction pathway genes during flower development in wild type and two cultivars ('YX1' and 'YX4'). The R, S, and L refer to root, stem, and leaf. The S1, S2, and S3 are flower bud stage (S1), semi-open stage (S2), and fully open stage (S3), respectively. b and c, Expression of ERS genes in petals of two cut G. paniculata flowers determined by qPCR. Flowers were treated with 10 ppm ethylene for 0 h, 4 h, and 12 h. The air treatment (0 h) was used as a control. 'YX1' and 'YX4' were the name of two cultivars used in the experiment.
was used to extract high-quality DNA, and a Nanopore library with insertion fragments larger than 20 KB was constructed. The Oxford Nanopore Technology (San Diego, California, USA) sequencer was used to conduct single-molecule real-time sequencing of DNA, after which the original reads were filtered to remove low-quality data and adapter sequences.

**Estimation of genome size**

The Jellyfish ([https://github.com/gmarcais/Jellyfish](https://github.com/gmarcais/Jellyfish)) program was used for K-mer (k = 17) distribution analysis of G. paniculata to estimate the genome size. For the low error rate of next-generation sequencing, it is commonly used in K-mer distribution analysis, whose correctness is closely related to the accuracy of the sequencing result. At the same time, we also performed the genome size determination by a flow cytometer (BD FACscalibur, San Diego, California, USA, BD Bioscience, USA) using leaf samples. The nuclei of the samples were stained with DNA fluorochrome PI (propidium iodide, 50 mg/mL) and were measured samples. The nuclei of the samples were stained with DNA fluorochrome PI (propidium iodide, 50 mg/mL) and were measured through flow cytometer with at least 10,000 nuclei. The coefficient of variation (CV) of both sample and reference for every measurement was controlled within 5%. The genome size was determined by comparing with a standard reference genome of *Oryza sativa* L. * japonica*. cv. Nipponbare (genome size is 420 Mb) based on the method of Arumuganathan and Earle [49].

**Genome assembly**

We applied MECAT process [50] to assemble the fragments in the constructed library to the contig level. High-quality read data from the next-generation sequencing were used to correct low-quality assembly results of the nanopore sequencing by pilon ([https://github.com/broadinstitute/pilon](https://github.com/broadinstitute/pilon)). The Hi-C sequencing result was adopted for the construction of pseudo chromosome level scaffolds. BWA (0.7.17-r1188) [51] was applied for the alignment of Hi-C reads against contig sequences, then low-quality alignments were removed. The optimized alignment result is used by ALLHiC (v0.8.11) process [52] for preliminary assembly. In the end, the assembly results at the chromosome level were obtained after adjusting the assembly results produced by ALLHiC.

We aligned the NGS reads to the assembly using bwa-mem2 (v2.2.1) (bwa-mem2 mem -t 40 -o Gpan.sam Gpan.WG.chromosome fasta.gz R1.fastq.gz R2.fastq.gz) [53]. Samtools (v1.15.1) was chosen to calculate the mapping rate of NGS reads in the fast file (samtools flagstat Gpan.sam) [54]. BUSCO (Benchmarking Universal Single-Copy Orthologs) [55] is a widely adopted method to evaluate the integrity of genome assembly and annotation. We used BUSCO (v3.1.0, python run_BUSCO.py -i Gpan.asm.fasta -o Gpan.genome.busco -l ~/data/busco/eudicotyledons_odb10/ -m genome -c 1) to align the assembly result with the database named eudicotyledons_odb10 to find that 93.8% of universal single-copy orthologous gene groups in eudicot are aligned with it.

**Genome annotation**

The protein sequences of *S. oleracea*, *B. vulgaris*, *F. tataricum*, *A. thaliana*, *V. vinifera*, *O. sativa*, *A. comosus*, and *Nymphaea colorata* were used as homologous reference sequences to predict the gene structure in the genome. Combined with the transcriptome data, the maker ([http://www.yandelli-lab.org/software/maker.html](http://www.yandelli-lab.org/software/maker.html)) was applied to annotate the genome. After the structural annotation, we applied BUSCO to judge the quality of the annotation (python run_BUSCO.py -i Gpan.pep -o Gpan.prot.busco -l ~/data/busco/eudicotyledons_odb10/ -m proteins -c 1). Then we used eggnoG-mapper [56] (emapper.py -i Gpan.pep -d virNOG -o Gpan_genomes.NOG.virNOG —cpu 20 -m diamond) and HMMsearch [57] (hmmscan -o out.txt —tblout out.tbl —cpu 20 —noali -E 1e-5 ~/data/pfam/Pfam-A hmm Gpan.pep) against Pfam database (http://pfam.xfam.org) to annotate the genome functionally. MCSscan, a collinearity-analysing tool contained in JCVI toolkit [58], was applied to analyse the collinearity of genomic genes (python -m jcvicompare.catalog ortholog —dbtype = prot —no_strip_names Gpan Gpan). Using the circos software, we drew a circos diagram to describe the basic information of the genome including chromosome length, gene density, transcription element density, and genome collinearity.

For repetitive sequence annotation, both de novo- and homology-based approaches were applied for more comprehensive results. RepeatMasker (RepeatMasker -gff -pa 20 -lib consensi.fa.classified Gpan.gene.fasta) and RepeatModler (RepeatModeler -pe 20 -engine wublast -database dbname) were used for the searching and detection of transposable elements and Tandem Repeat Finder for tandem repeat sequences.

**Gene family analyses and species phylogenetic tree**

To study the evolutional status of *G. paniculata*, we analysed the gene families with the genome of *G. paniculata*. The protein sequences of *G. paniculata*, *N. colorata colorata*, *Amborella trichopoda*, *V. vinifera*, *Solanum lycopersicum*, *A. thaliana*, *O. sativa*, *A. comosus*, and *Sorghum bicolor* were selected to construct a species phylogenetic tree. We clustered the datasets of proteins of chosen species into orthologs and paralogs at first, then filtered the genes of single-copy orthologs for the alignment and construction of species phylogenetic tree. We chose Orthofinder, a commonly adopted gene-family-clustering tool, as the main tool of the following analysis (orthofinder -t 20 -a 20 -f July11 -S diamond -A mafft). It is an integrated workflow combined with mainstream bioinformatic tools, such as DIAMOND and IQTREE, which can separate and cluster protein sequences from different genomes into paralogous gene groups and orthologous gene groups.

**Estimation of divergence time and gene family expansion**

Before further analysis, we get the calibrated divergence time of *B. vulgaris* and *S. oleracea* and of *O. sativa* and *A. comosus* from the TimeTree database, whose divergence records are usually supported by synthetical research. The calibration time is then used as a correction to our analysis in further construction of divergence time. To estimate the divergence time, single-copy orthologous genes are in need to analyse the fourfold degenerate site. After the single-copy orthologous genes and basic phylogenetic tree were obtained, the longest coding sequences (CDS) of each single-copy orthologous gene was extracted according to the orthofinder results. Clustalo (v1.2.4) [59] was used for multiple sequence alignment of CDSs, and the results after gap removal were set to phylip format. We adopted MCMACTree, a tool comprised in paml software package [60], to construct the ultrametric tree, which not only contains the phylogenetic relationship, but also contains a 95% confidence interval for the divergence time. CAFÉ (v4.2.1) [61] workflow was selected to analyse gene family expansion.

**Polyploidization events**

The longest protein sequences of each gene within the genome of *G. paniculata*, *B. vulgaris*, *C. arabica*, and *D. caryophyllus* were used for synteny and collinearity detection within themselves to shed light
MADS-box gene family analysis
We used the peptide sequences of *G. paniculata*, *N. colorata*, *A. trichopoda*, *V. vinifera*, *S. lycopersicum*, *A. thaliana*, *O. sativa*, *A. comosus*, and *S. bicolor* to analyse the differences of MADS-box gene blocks between them. HMMsearch [57] was applied for the annotation of gene family by selecting homologous protein sequences with E value less than 1e-8. The abnormal sequences that are too long or too short were manually deleted, and the longest transcript was chosen when multiple transcripts coexist. Different groups of MADS-box genes were separated to be aligned by MAFFT [62] and MUSCLE [63] to construct phylogenetic trees for each gene family.

WGCNA analysis
In order to search the hub genes involved in the flower senescence of *G. paniculata*, 21,737 genes (FPKM >1, and the means variation of FPKM >0.5) were selected to do weight correlation network analysis (WGCA) using R package [64]. An adjacency matrix was constructed with power = 7 by raising the co-expression measure. Then the genes were hierarchically clustered and formed an adjacency matrix. The topological overlap (TO) was calculated by using that adjacency matrix. Hierarchical clustering tree was constructed by dynamic hybrid tree cut algorithm with 0.5 merged by using that adjacency matrix. The correlation of each gene module with the different samples was also qualified. The correlation of each gene module with the different samples was also qualified. The correlation of each gene module with the different samples was also qualified.

Gene cloning and RT-qPCR analysis
The genomic and cDNA sequence of *AG*, *AP2*, and *PET* genes were isolated from the genome dataset of *G. paniculata*. The primers for gene cloning were designed by Primer Premier 5 (Table S6, see online supplementary material). PCR cloning was performed as described previously [65].

Severn ETR genes were identified from the genome data and used for the expression analysis RT-qPCR was performed to analyse the expression profile of the *G. paniculata*, which was treated with 10 ppm ethylene in an airtight vacuum dryer for 0 h, 4 h, and 12 h. RT-qPCR was performed using LightCycler® 480 II Real-time PCR Instrument (Roche, Basel, Switzerland) with QuantiFast® SYBR® Green PCR Kit (Qiagen, Dusseldorf, Germany). RT-qPCR reactions were performed as described previously [65]. ACTIN was used as a reference gene for RT-qPCR reactions. The gene-specific primers for qPCR of the target genes were shown in Table S6, see online supplementary material.

Acknowledgements
F.L. acknowledges funding from National Natural Science Foundation of China (31960608), Yunnan Fundamental Research Projects (20211AT070147), and High-level Talent Introduction Program of Yunnan Province – Industrial Talent Special Project (YNQR-CYRC-2020-004). C.Y. thanks the Green Food Brand—Build a Special Project (Floriculture) supported by Science and Technology (53000021000000012742). J.W. is funded by China Agriculture Research System of MOF and MARA (CARS-23-G56). L.Z. thanks the Fundamental Research Funds for the Central Universities (2021QNA6008). We want to thank the support from Yuxi Yuxing Biological Technology Co., Ltd. (Yunnan, China).

Author contributions
F.L. managed the project. F.L, L.Z., and J.W. conceived and designed the study. F.L., Y.G., C.J., and X.W. wrote the manuscript. F.L., J.R., C.Y., and J.W. collected the plant materials for sequencing and did the cross breeding. F.L., Y.C., and H.G. conducted the EMS mutagenesis experiment, cloned the genes, and analysed the sequence. Y.G., H.Q., and L.Z. assembled and annotated the genome, performed gene family clustering, and comparative phylogenomics. F.L., C.J., X.W., F.Z., and L.Z. conducted transcriptome sequencing and analysis. F.L., C.J., X.W., X.L., S.F., and F.Z. conducted the ethylene analysis. All authors reviewed and approved the manuscript.

Data availability
All data are publicly available in the China National GeneBank (https://www.cngb.org/) under project number CNP0003304. The genome assembly sequences and gene annotations are available at https://ftp.cngb.org/pub/CNSA/data4/CNP0003304/CNS0579838/CNA0050895/.

Conflict of interests
The authors declare no competing interests.

Supplementary data
Supplementary data is available at Horticulture Research online.

References
1. Lu D, Nicholas JT. Gypsophila linnaeaeus. In: Wu Z, Raven PH (eds.), *Flora of China*, Chapter 6. Beijing/St. Louis: Science Press/Missouri Botanical Garden, 2001, 108–13.
2. Li F, Wang G, Yu R et al. Effects of seasonal variation and gibberellic acid treatment on the growth and development of Gypsophila paniculata. *HortScience*. 2019;54:1370–4.
3. Wani MA, Nazki IT, Din A et al. Sustainable Agriculture Reviews. In: Lichtfouse E (ed.), *Floriculture Sustainability Initiative: The Dawn of New Era*, Chapter 27. Cham: Springer International Publishing, 2018, 91–127.
4. Jin C, Sun D, Wei C et al. Gas chromatography-mass spectrometry analysis of natural products in Gypsophila paniculata. *HortScience*. 2021;56:1195–8.
5. Li F, Jin C, Zhang L et al. Hyper-recombinant plants: an emerging field for plant breeding. *Crit Rev Plant Sci*. 2021;40:446–58.
6. Li F, Mo X, Wu L et al. A novel double-flowered cultivar of Gypsophila paniculata mutagenized by 60Co γ-ray. *HortScience*. 2020;55:1531–2.
7. Wang SM, Piao XC, Park SY et al. Improved micropropagation of Gypsophila paniculata with bioreactor and factors affecting ex vitro rooting in microponic system. *In Vitro Cell. Dev Biol. Plant*. 2013;49:70–8.
8. Zvi MMB, Zuker A, Ovadis M et al. Agrobacterium-mediated transformation of gypsophila (Gypsophila paniculata L.). *Mol Breed*. 2008;22:543–53.
9. Wang Q, Zhang X, Lin S et al. Mapping a double flower phenotype-associated gene DcAP2L in Dianthus chinensis. *J Exp Bot*. 2020;71:1915–27.
10. François L, Verdenaud M, Fu X et al. A miR172 target-deficient AP2-like gene correlates with the double flower phenotype in roses. *Sci Rep*. 2018;8:12912.
11. Li S, Cheng Y, Sun D et al. Identification and expression of TOP3α in Gerbera hybrida. Hortic Plant J. 2021;7:167–73.
12. Krizek BA, Fletcher JC. Molecular mechanisms of flower development: an armchair guide. Nat Rev Genet. 2005;6:688–98.
13. Zhang L, Chen F, Zhang X et al. The water lily genome and the early evolution of flowering plants. Nature. 2020;577:79–84.
14. Dubois A, Raymond O, Maene M et al. Tinkering with the C-function: a molecular frame for the selection of double flowers in cultivated roses. PLoS One. 2010;5:e9288.
15. Shibuya T, Murakawa Y, Nishidate K et al. Characterization of flowering-related genes and flowering response in relation to blue light in Gypsophila paniculata. J Hortic. 2017;86:94–104.
16. Morgan E, Funnell K. Ornamental Crops. In: Van Huylenbroeck J (ed.), Limonium, Chapter 21. Cham: Springer International Publishing, 2018,513–27.
17. Aalifar M, Aliniaeifard S, Arab M et al. hierarchical, 2018,513–27.
18. Liu Y, Tang M, Liu M et al. The molecular regulation of ethylene in fruit ripening. Small Methods. 2020;4:1900485.
19. Ma N, Ma C, Liu Y et al. Petal senescence: a hormone view. J Exp Bot. 2018;69:719–32.
20. Lu P, Zhang C, Liu J et al. RhHB1 mediates the antagonism of gibberellins to ABA and ethylene during rose (Rosa hybrida) petal senescence. Plant J. 2014;78:578–90.
21. Zhang S, Zhao Q, Zeng D et al. RhMYB108, an R2R3-MYB transcription factor, is involved in ethylene-and JA-induced petal senescence in rose plants. Hortic. Res. 2019;6:131–1.
22. Jing W, Zhao Q, Zhang S et al. RhWRKY33 positively regulates onset of floral senescence by responding to wounding- and ethylene-signaling in rose petals. Front Plant Sci. 2021;12:726797–7.
23. Xu H, Luo D, Zhang F. DcWRKY75 promotes ethylene induced petal senescence in carnation (Dianthus caryophyllus L.). Plant J. 2021;108:1473–92.
24. Van Doorn WG, Reid MS. Role of ethylene in flower senescence of Gypsophila paniculata L. Postharvest Biol Technol. 1992;1:265–72.
25. Hooberichts FA, de Jong AJ, Woltering EJ. Apoptotic-like cell death marks the early stages of gypsophila (Gypsophila paniculata) petal senescence. Postharvest Biol Technol. 2005;35:229–36.
26. Doorn WGV. Effect of ethylene on flower abscission: a survey. Ann Bot. 2002;89:689–93.
27. Doorn WW, Stead A. Abscission of flowers and floral parts. J Exp Bot. 1997;48:821–37.
28. Chen F, Su L, Hu S et al. A chromosome-level genome assembly of rugger rose (Rosa rugosa) provides insights into its evolution, ecology, and floral characteristics. Horticulture Research. 2021;8:141.
29. Verstreeg R, van Schaar BDC, van Batenburg MF et al. The human transcriptome map reveals extremes in gene density, intron length, GC content, and repeat pattern for domains of highly and weakly expressed genes. Genome Res. 2003;13:1998–2004.
30. Yang Z, Rannala B. Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. Mol Biol Evol. 2006;23:212–26.
31. Gao W, Lu L, Qiu W et al. OsPAP26 encodes a major purple acid phosphatase and regulates phosphate remobilization in rice. Plant Cell Physiol. 2017;58:885–92.
32. Kan C, Zhang Y, Wang HL et al. Transcription factor NAC075 delays leaf senescence by deterring reactive oxygen species accumulation in Arabidopsis. Front Plant Sci. 2021;12:164.
33. Lin Z, Ho CW, Grierson D. AtTRP1 encodes a novel TPR protein that interacts with the ethylene receptor ERS1 and modulates development in Arabidopsis. J Exp Bot. 2009;60:3697–714.
34. Chen F, Song Y, Li X et al. Genome sequences of horticultural plants: past, present, and future. Hortic Res. 2019;6:1–23.
35. Parcy F, Bomblies K, Weigel D. Interaction of LEAFY, AGAMOUS and TERMINAL FLOWER1 in maintaining floral meristem identity in Arabidopsis. Development. 2002;129:2519–27.
36. Aida R, Komano M, Saito M et al. Chrysanthemum flower shape modification by suppression of chrysanthemum-AGAMOUS gene. Plant Biotechnol. 2008;25:55–9.
37. Liu Z, Zhang D, Liu D et al. Exon skipping of AGAMOUS homolog PtseAG in developing double flowers of Prunus lannesiana (Rosaceae). Plant Cell Rep. 2013;32:227–37.
38. Dreni L, Kater MM. MADS reloaded: evolution of the MADS-box superfamily in angiosperms. New Phytol. 2014;201:717–32.
39. Chunlian J, Huaining G, Suping Q et al. AGAMOUS correlates with the semi-double flower trait in carnation. Ornam Plant Res. 2022;1:1–6.
40. Liang Y, Li F, Gao Q et al. The genome of Eustoma grandiflorum reveals the whole-genome triplication event contributing to ornamental traits in cultivated lisanthus. Plant Biotechnol J. 2022.
41. Gattolin S, Cirilli M, Chessa S et al. Mutations in orthologous PEROXISOME-ETE-type genes cause a dominant double-flower phenotype in phylogenetically distant eudicots. J Exp Bot. 2020;71:2585–95.
42. Yu Y, Wang H, Liu J et al. Transcriptional regulation of two MADS-box-like genes of carnation during flower senescence and upon ethylene exposure, wounding treatment and sucrose supply. Plant Biol. 2011;13:719–24.
43. Naing AH, Soe MT, Kyu SY et al. Nano-silver controls transcriptional regulation of ethylene-and senescence-associated genes during senescence in cut carnations. Sci Hort. 2021;287:110280.
44. Shibuya K, Nagata M, Tanikawa N et al. Comparison of mRNA levels of three ethylene receptors in senescing flowers of carnation (Dianthus caryophyllus L.). J Exp Bot. 2002;53:399–406.
45. Yagi M, Kosugi S, Hirakawa H et al. Sequence analysis of the genome of carnation (Dianthus caryophyllus L.). DNA Res. 2014;21:231–41.
46. Dervinis C, Clark DG, Barrett JE et al. Effect of pollination and exogenous ethylene on accumulation of ETR1 homologue transcripts during flower petal abscission in geranium (pelargonium x hortorum L.H. bailey). Plant Mol Biol. 2000;42:847–56.
47. Kuroda S, Hakata M, Hirose Y et al. Ethylene production and enhanced transcription of an ethylene receptor gene, ERS1, in delphinium during abscission of florets. Plant Physiol Biochem. 2003;41:812–20.
48. Tanase K, Ichimura K. Expression of ethylene receptors dl-ERS1-3 and dl-ERS2, and ethylene response during flower senescence in delphinium. J Plant Physiol. 2006;163:1159–66.
49. Arumuganathan K, Earle ED. Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol Biol Report. 1991;9:229–41.
50. Xiao C-L, Chen Y, Xie SQ et al. Assembly of allele-aware, chromosome-scale autoploidy genomes based on hi-C data. Nature Plants. 2019;5:833–45.
54. Li H, Handsaker B, Wysoker A et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25:2078–9.
55. Simão FA, Waterhouse RM, Ioannidis P et al. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31:3210–2.
56. Huerta-Cepas J, Forslund K, Coelho LP et al. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol. 2017;34:2115–22.
57. Finn RD, Clements J, Eddy SR. HMMER web server: interactive sequence similarity searching. Nucleic Acids Res. 2011;39:W29–37.
58. Tang H, Bowers JE, Wang X et al. Synteny and collinearity in plant genomes. Science. 2008;320:486–8.
59. Sievers F, Higgins DG. Clustal omega for making accurate alignments of many protein sequences. Protein Sci. 2018;27:135–45.
60. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol. 2007;24:1586–91.
61. Han MV, Thomas GWC, Lugo-Martinez J et al. Estimating gene gain and loss rates in the presence of error in genome assembly and annotation using CAFE 3. Mol Biol Evol. 2013;30:1987–97.
62. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.
63. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.
64. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559–9.
65. Li F, Cheng Y, Ma L et al. Identification of reference genes provides functional insights into meiotic recombination suppressors in Gerbera hybrida. Hortic Plant J. 2022;8:123–32.