A genetic approach to comprehend the complex and dynamic event of floral development: a review

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The concepts of phylogeny and floral genetics play a crucial role in understanding the origin and diversification of flowers in angiosperms. Angiosperms evolved a great diversity of ways to display their flowers for reproductive success with variations in floral color, size, shape, scent, arrangements, and flowering time. The various innovations in floral forms and the aggregation of flowers into different kinds of inflorescences have driven new ecological adaptations, speciation, and angiosperm diversification. Evolutionary developmental biology seeks to uncover the developmental and genetic basis underlying morphological diversification. Advances in the developmental genetics of floral display have provided a foundation for insights into the genetic basis of floral and inflorescence evolution. A number of regulatory genes controlling floral and inflorescence development have been identified in model plants such as *Arabidopsis thaliana* and *Antirrhinum majus* using forward genetics, and conserved functions of many of these genes across diverse non-model species have been revealed by reverse genetics. Transcription factors are vital elements in systems that play crucial roles in linked gene expression in the evolution and development of flowers. Therefore, we review the sex-linked genes, mostly transcription factors, associated with the complex and dynamic event of floral development and briefly discuss the sex-linked genes that have been characterized through next-generation sequencing.

Keywords: ABCDE model, floral genetics, next-generation sequencing, sex-linked gene, transcription factor

Introduction

Understanding the dynamics of sexual manifestations has enormous significance both in classical and theoretical research. Hermaphroditism is the major sexual system in the plant kingdom, with male and female sexual structures coexisting in the same flower. The XY and WZ sex determination systems commonly found in animals have hardly developed in plants, and are recognized in only a few genera of flowering plants [1]. Additionally, dioecy or isolated sexes have developed in around 7% of all angiosperms [2], typically from complete-flowered or monoecious descendants [3]. Variation in the outcomes of sexual reproduction of plants establishes outcrosses, which are essential for promoting inherent disparities and improving the adaptability of plant types. It remains unclear whether all dioecious organisms have a characteristic sexual morphology. However, theoretical

findings indicate that sex-linked genes specifically accumulated in the recombination-suppressed regions of one of the gonosomes. This accumulation of gonosome-specific genes resulted in the corporeal differentiation of the masculine and feminine sex chromosomes [4]. More precisely, the evolution of sexual patterns was initiated with the expression of sex-linked genes within a recombination-suppressed region of a chromosome.

The concepts of phylogeny and floral genetics play a crucial role in understanding the origin and diversification of flowers in angiosperms [5]. Out of 25,500 (Approx) genes in Arabidopsis, more than 20,000 genes are responsible for pollen development at some point in time [6]. Research on the genetics and molecular biology of floral development and sex differentiation has resulted in the discovery of various floral identity genes. The majority of these genes belong to a smaller set of regulators called transcription factors (TFs), which govern the complexity of the transition from the floral meristem to the mature flower, as well as deciphering of the sexes. Floral organ speciation (sepals, petals, stamens, and carpels) and sexual variability are combined activities of floral homeotic genes, according to the ABC model [7]. Our aim here is to present a detailed review of sex-linked genes, including TFs and others that have been found to be involved in sex differentiation or floral development.

Sex–Linked Genes in Flowering Plants

Phenotyping of floral mutants and their associated genetic interaction studies have shown that A function genes alone specify sepals, while the combined activity of A and B class genes leads to petals. Stamen development reflects the combined contributions of the B and C genes, while C functioning alone leads to carpels [7,8]. A comprehensive re-evaluation of the ABC model then led to the inclusion of the E function genes (the ABCE model), which are associated with the speciation of all organ types [9,10]. Subsequently, the D class genes were added, which determine the feature of ovule development in female flowers. These floral-specific genes

code for the MADS-box family of TFs and are highly diversified in plants [11]. Based on their characterization in *A. thaliana*, *APETALA1 (AP1)* and *APETALA2 (AP2)* are A function genes, the B function is contributed by *APETALA3 (AP3)* and *PISTILLATA (PI)*, the C function is encoded by *AGAMOUS (AG)*, and the E function is carried out by multiple *SEPALLATA (SEP)* genes (i.e., *SEP1* to *SEP4*) [12].

In *Silene latifolia*, the Y chromosome-linked *SlAP3* gene encodes the *Apetala 3* MADS box protein in male flowering buds [13]. *SlAP3* exhibited high similarity with the *Arabidopsis* floral identity gene *AP3*, which is responsible for floral morphogenesis and organ identity [14]. Another Y-linked sequence named CCLS96 from *S. latifolia* encoding multiple copy numbers of non-coding RNAs has been found to be responsible for male bud expression [15]. X-linked genes in *S. latifolia*, such as *MROS3X* and *SlMF1*, demonstrated significantly high expression in female floral buds [13,16]. A few XY-linked genes, such as SlssX/SlssY, SlX1/SlY1, SlX3/SlY3, SlX4/SlY4, and DD44X/Y, have also been characterized in *S. latifolia* and are believed to have housekeeping functions [17]. A list of the sex-linked genes identified in different plant species is presented in Table 1.

TFs and the Regulation of Flower Development

TFs are a group of regulatory proteins that play critical roles in altering the expression of genes associated with cellular pathways and biological processes, including sex differentiation, floral development, and the floral transition [18]. A wide range of TFs are known as major determinants of sex speciation in angiosperms. The most prominent are the MADS-box family TFs, which play important roles in many aspects of plant growth and are crucially involved in floral organ speciation and reproductive development [19]. These proteins are characterized by the presence of a 58–60 amino acid-long conserved MADS-box DNA binding domain at the N-terminus that dimerizes to specific DNA sequences called

Table 1. Major sex–linked genes characterized in different plant species

Gene	Symbol	Species	Function
AGAMOUS–LIKE 65, 66, 104	AGL65, 66,104	Arabidopsis thaliana	Pollen maturation and tube growth
AGAMOUS	AG	Arabidopsis thaliana	Homeotic C–class gene; carpel and stamen specification, lineage–specific sub functionalization of the homeotic C function; fruit development (e.g., tomato versus Arabidopsis)
SHATTERPROOF 1, 2	SHP1, 2	Arabidopsis thaliana	Carpel, ovule, and fruit development; dehiscence; periodic lateral root formation
APATELA 3, PISTILATA	AP3, PI	Arabidopsis thaliana	Floral homeotic B function, specification of petaloid organs

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Table 1. Continued

Gene	Symbol	Species	Function
FLORAL LOCUS C	FLC	Arabidopsis thaliana	Potential role in floral bud dormancy; perennial life history in Arabis alpina
SQUAMOUS	SQUA	Antirrhinum majus	Floral meristem and organ identity specification; floral transition; fruit development
	STMADS11	Arabidopsis thaliana	Control of floral transition; repression of precocious homeotic gene expression
AGAMOUS-LIKE 2	AGL2	Arabidopsis thaliana	Floral homeotic E function
A BSISTER	ABS	Arabidopsis thaliana	Endothelium development in seeds
SEEDSTICK	STK	Arabidopsis thaliana	Carpel and ovule development; periodic lateral root formation
XAANTAL1	XAL1	Arabidopsis thaliana	Transition to flowering
MADS AFFECTING FLOWERING 5	MAF5	Arabidopsis thaliana	Transition to flowering (activator)
CAULIFLOWER	CAL	Arabidopsis thaliana	Meristem identity specification
FRUITFULL	FUL	Arabidopsis thaliana	Meristem identity specification; annual life cycle regulator, with SOC1; fruit development; cauline leaf growth
AGAMOUS-LIKE 24	AGL24	Arabidopsis thaliana	Transition to flowering (activator)
SHORT VEGETATIVEPHASE	SVP	Arabidopsis thaliana	Transition to flowering (repressor)
APETALA1	AP	Arabidopsis thaliana	Meristem identity specification; homeotic A-class gene
DIANA (AGAMOUS-LIKE 61)	DIA	Arabidopsis thaliana	Central cell and endosperm development
AGAMOUS-LIKE 23	AGL23	Arabidopsis thaliana	Embryo sac development
SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1	SOC1	Arabidopsis thaliana	Transition to flowering (activator); periodic lateral root formation
AP3 MADS box gene	SLAP3	Silene latifolia	Male floral bud development
DUF538	MROS3X	Silene latifolia	Male floral bud development
PLENA	PLE	Antirrhinum majus	Specify stamen and carpel identity
Floral binding protein gene	FBP7, FBP11	Petunia hybrida	Ovule identity
FARINELLI	FAR	Antirrhinum majus	Male fertility
PrMADS1, PrMADS1, PrMADS2	PRMADS1,2,3	Pinus radiata	Petal, stamen, and carpel development, and preventing the indeterminate growth of the flower meristem.
DEFICIENS/GLOBASA	DEF/GLO	Antirrhinum majus	Petal and stamen identity
Gerbera MADS box gene	GRCD1	Gerbera hybrida	Stamen development and identity
Zea Agamous 3	ZAG3	Zea mays	Carpel development
ZmMADS1	ZMM5	Zea mays	Expressed during flower development: in egg cells and embryos
CmWIP1	CmWIP1	Cucumis melo	Stamen development in male flowers and suppression of carpel development
CmACS7	CmACS7	Cucumis melo	Stamen suppression in female flower development
OGI	OGI	Diospyros lotus	Suppression of anther development in female flowers
Domain of unknown function 247	DUF 247	Asparagus officinalis	Suppressor of pistil development
RADIALIS	RAD1 and RAD2	Rumex acetosa	Stamen whorl development
Ras-proximate-1 or Ras-related protein 1	RAP1	Rumex acetosa	Carpel and stamen whorl specification/ development
TASSELSEED2	ts2	Zea mays	Male sex determination and stamen development
STABILIZED1	STA1	Silene latifolia	Tapetum development in male flowers
Silene latifolia MADS1	SLM1	Silene latifolia	Specify stamen and carpel identity
Silene latifolia MADS2	SLM 2	Silene latifolia	Developing stamens of smut-infected female flowers
Silene latifolia MADS3	SLM 3	Silene latifolia	Repression of gynoecium development in male flowers
AGAMOUS-LIKE 80	AGL80	Arabidopsis thaliana	Central cell and endosperm development
Silene latifolia MADS4,5	SLM 4,5	Silene latifolia	Floral meristem and organ identity specification; floral transition; fruit development
Silene latifolia sepallata 1 and 3	SlSEP1 and SlSEP3	Silene latifolia	Expressed in young flower meristems, developing petals, male anthers, and female ovules

"CArG boxes" [20]. Based on protein domain structures, the MADS-box genes have been divided into two lineages: type I and type II. The type I or M-type gene forms a heterogeneous group with short DNA sequences ($\approx$ 180 bp) encoding only the MADS domain and are classified as Mα, Mβ, and Mγ based on phylogeny. Although they constitute the major component of MADS-box genes in many plants, their functional attributes have only been characterized recently [21]. The type II or MIKC genes are characterized by the presence of additional domains, including an intervening (I) domain, a keratin-like (K) domain, and a C-terminal (C) domain. They are classified as canonical (MIKCC) or star type (MIKC*) depending on the alteration of their motif structure. Additionally, MIKCC genes are further divided into 14 clades based on phylogenetic relationships and distinct sequence motifs in their C-terminal domains [22]. An alteration in the C-terminal motif results in the transcriptional activation of specific DNA sequences through the formation of multimeric MADS-box protein complexes [19]. The MIKC subfamilies are often conserved and exhibit similar functions in the growth of different plants, as well as in reproductive and vegetative speciation, such as in the differentiation of the floral meristem (*APETALA 1* [*AP1*], *FRUITFUL* [*FUL*], and *CAULIFLOWER*), the development of floral organs (*AP1, APETALA3* [*AP3*], *PISTILLATA* [*PI*], *AGAMOUS* [*AG*], and *SEPALLATA 1-3* [*SEP1-3*]), the regulation of flowering time (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANT 1* [*SOC1*], *FLOWERING LOCUS C* [*FLC*], *SHORT VEGETATIVE PHAGE* [*SVP*], *AGAMOUS-LIKE 24* [*AGL24*]), fruit maturation (*SHATTERPROOF 1-2* [*SHP1-2*]), embryonic development (*TRANSPARENT TESTA 16* [*TT16*]), and root growth (*AGAMOUS-LIKE 17* [*AGL17*]) (reviewed in Smaczniak et al. 2012; Theiben et al. 2016) [19,20]. The key function of MIKCC genes is classified into five classes: A, B, C, D, and E, each with multiple MADS-box TFs directly involved in the development of the floral quartet model [19,20]. The ABCDE model accounts for the regulation of flowering plants. Several reports have shown that MIKCC genes are fundamental to organogenesis, such that the combination of A + E genes identifies sepals, A + B + E specifies petals, B + C + E yields stamens, C + E gives carpels, and C + D + E specifies ovules. In contrast, MIKC* genes have been implicated in floral transition and gametophytic development [19]. Moreover, the *FLC* subfamily of genes also plays a role in controlling flowering through vernalization in *A. thaliana* [23]. *AGL12* has been implicated in pigment accumulation and root development in the floral transition in rice and proliferation of the root meristem in *Arabidopsis* [24]. Similarly, *TM8* genes control flower development in tomatoes and grapevines [25]. Most of the SOC family proteins

act as activators, whereas *SVP*-related genes act repressors of floral patterning and the floral meristem in both monocots and dicots [26,27].

In addition to the MADS-box family of proteins, TFs encoding zinc fingers also function as major components of the architecture for plant development and organ differentiation [28]. The expression of *CmWIP1*, a sex-linked gene encoding C_2H_2 zinc finger protein, has been found to cause carpel abortion and the development of male flowers in *Cucumis melo* [29]. The MYB family of TFs plays a significant role in plant development and sex differentiation, as has been established in

A. thaliana. AtMYB21, AtMYB24, AtMYB57, AtMYB108/BOS1, AtMYB35/TDF1, AtMYB80, and AtMYB99 are independently and individually responsible for anther development and/or function [30,31]. AtMYB33 and AtMYB65 assist in both anther and pollen development [32]. AtMYB115 and AtMYB118 are associated with embryogenesis [33], while AtMYB125 controls male germ cell division and differentiation [34]. AtMYB105 and AtMYB117 control lateral organ separation and axillary meristem formation [35]. Recently, an MYB-like gene (male-specific expression [MSE1]) linked to early anther development has been isolated from *Asparagus officinalis* [36]. Homeodomain-leucine zippers (HD-Zips) are a specific group of plant TFs with significant role in plant development, floral differentiation, and embryogenesis [37]. MeGI, an HD-Zip gene, acts as a regulatory factor for anther fertility and as a major sex determinant in the dioecious persimmon *Diospyros lotus* [38]. Furthermore, many other TFs including WRKY, F-box, SPL, GATA, YABBY, and DELLA have been implicated in various processes of plant development and floral differentiation [19,20].

Characterization of Sex-Linked Genes through Next-Generation Sequencing

The complex and dynamic event of floral development depends on the tight regulation of gene expression and controlled environmental cues [39]. In recent years, several studies have been reported regarding floral development and whorl speciation in hermaphrodites, as well as unisexual plants of model and non-model species [40-45]. However, the majority of these studies are based on mutant analysis. For example, a sex determination gene (*TASSEL-SEED2*) in maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion [46], and a conserved mutation in the active site of 1-aminocyclopropane-1-carboxylic acid synthase leads to andromonoecy in melons [47]. However, sex differentiation is a complex phenomenon in angio-

sperms, with the involvement of several genes that are differentially expressed in diverse tissues and developmental phases [48]. Under these circumstances, the identification and characterization of a few sex-linked genes at a particular stage may not be able to provide the entire mechanism of sex determination in a given species [49]. In other words, it is essential to characterize myriads of genes from different developmental stages of dioecious species to understand the complexity of plant sex determination. Large-scale gene expression analysis methods such as mRNA differential display, suppression subtractive hybridization, reverse-transcription polymerase chain reaction, and microarrays have been previously used to assess the vital stages of sex determination in a wide range of plants [50,51]. However, these methods had limited applications for understanding sex determination due to their poor sensitivity, the inconvenience of cross-hybridization, and the non-availability of the total genome sequences. The advent of next-generation RNA-sequencing (RNA-Seq) technology has offered a powerful, economical, and highly sensitive method for the discovery of novel transcripts and the assessment of transcriptome dynamics [52]. Moreover, a *de novo* assembly of RNA-Seq reads can be efficiently used for gene discovery in non-model plant species where the total genome information is unavailable [53].

Next-generation sequencing (NGS) technologies have facilitated gene discovery and the global analysis of molecular mechanisms related to growth and development in numerous plant species, including members of the Cucurbitaceae family. Transcriptome profiling and comparison between gynoecious and hermaphrodite cucumber plants resulted in the identification of 200 differentially expressed genes (DEGs) with a significant role in plant sex determination process [54]. In another study, Solexa sequencing was performed to determine the transcript profile of apical tissues from a gynoecious mutant and a monoecious wild type of cucumber [40]. A total of 143 upregulated and 600 downregulated genes were identified in the mutant type. The study suggested that multiple genes from plant hormone signaling pathways, including *ACS, Asr1, CsIAA2, CS-AUX1, TLP*, and *EREBP*, play critical roles in sex determination and floral development in cucumbers. Similarly, RNA-Seq analysis of two near-isogenic lines of melons (male sterile line DAH3615-MS and male fertile line DAH3615) resulted in the identification of 1,259 DEGs significantly associated with male fertility [55]. The majority of these genes were linked to pathways related to pollen development, stamen development, and pollen tube elongation.

Among other plant species, a genome-wide high-throughput transcriptomic sequencing for young floral buds of sterile and fertile plants of *Brassica napus* and subsequent mapping onto the AA and BB genomes revealed a total of 3231 genes of *B. rapa* and 3,371 genes of *B. oleracea* with considerable differential expression levels [56]. That study reported 760 DEGs specific to fertile and 44 DEGs specific to sterile plants. After Gene Ontology (GO) annotation, 11 DEGs were identified as involved in pollen wall assembly (GO: 0010208), of which three DEGs were beta-1,3-glucanase genes (Bra028343, Bra037057, Bra038969) implicated in male gametophyte development and pollination. Similarly, 454 pyrosequencing and a comparative analysis during the development of male and female flowers of the monoecious species *Quercus suber* revealed DEGs in the early and late stages of development of female and male flowers, some of which were shown to be involved in pollen development, ovule formation, and flower development of other species with a monoecious, dioecious, or hermaphroditic sexual system [57]. Interestingly, a homolog for *POLYGALACTURONASE-1*, which is expressed 356 times more in female tissues, has been previously associated with pollen [58-60] and carpel development [61]. Another gene, *QsENDO-BETA-1,3-1,4 GLUCANASE*, a member of the glycoside hydrolase family, which is 199 times more expressed in female samples, has been linked to male sterility due to defects in anther dehiscence [62]. Illumina sequencing of inflorescent meristems and the flowering stages of sugar apples (*Annona squamosa* L.) resulted in 71,948 unigenes, 147 of which were represented by various TF families involved in floral transition and development [63]. Likewise, different NGS platforms and diverse sequencing chemistries have been utilized to characterize DEGs in the male and female *Salix suchowensis* [64], DEGs linked with pistil abortion in Japanese apricots [65], genes linked to sex type differentiation in *Ginkgo biloba* L. [66] and genes associated with the regulatory mechanism of floral development in olive (*Oleaeuropaea* L.) [67]. Most recently, RNA-Seq analysis was performed to study the floral bud differentiation in *Magnolia sinostellata* [68]. The study revealed 82 genes out of a total of 11,592 DEGs involved in flowering and 20 genes were found to be critically involved in bud differentiation at different stages of flower development. Overall, these studies suggest that NGS analysis and the associated bioinformatics components have laid the foundation for the genome-wide characterization and functional prediction of genes linked to floral development and sex differentiation in angiosperms.

Conclusion

To understand the complex and dynamic event of floral development, research in floral evolution and development is using a combination of approaches to elucidate the genetic basis for the enor-

mous diversity in floral morphology. The need of the hour is to understand how this variation has contributed to the radiation of angiosperms. Sex-linked genes, especially TFs, are key players in flower development, and further research needs to be done in this promising area to comprehend the event of floral development.

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Conceptualization: JNM, SS. Data curation: JNM, SS. Formal analysis: JNM, SS, PM. Writing – original draft: JNM, SS, PM. Writing – review & editing: JNM, SS, PM.

Conflicts of Interest

There is no potential conflict of interest relevant to this article.

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References

1. Ming R, Yu Q, Moore PH. Sex determination in papaya. Semin Cell Dev Biol 2007;18:401-408.
2. Renner SS, Ricklefs RE. Dioecy and its correlates in the flowering plants. Am J Bot 1995;82:596-606.
3. Vyskot B, Hobza R. Gender in plants: sex chromosomes are emerging from the fog. Trends Genet 2004;20:432-438.
4. Charlesworth D. Plant sex determination and sex chromosomes. Heredity (Edinb) 2002;88:94-101.
5. Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, et al. Polyploidy and angiosperm diversification. Am J Bot 2009;96:336-348.
6. Zhao D, Yu Q, Chen C, Ma H. Genetic control of reproductive meristems. In: Annual Plant Reviews: Meristematic Tissues in Plant Growth and Development (McManus MT, Veit BE, eds.). Sheffield: Sheffield Academic Press, 2001. pp. 89-142.
7. Coen ES, Meyerowitz EM. The war of the whorls: genetic interactions controlling flower development. Nature 1991;353:31-37.
8. Bowman JL, Smyth DR, Meyerowitz EM. Genes directing flower development in Arabidopsis. Plant Cell 1989;1:37-52.
9. Colombo L, Franken J, Koetje E, van Went J, Dons HJ, Angenent GC, et al. The petunia MADS box gene FBP11 determines ovule identity. Plant Cell 1995;7:1859-1868.
10. Ma C, Yang J, Cheng Q, Mao A, Zhang J, Wang S, et al. Comparative analysis of miRNA and mRNA abundance in determinate cucumber by high-throughput sequencing. PLoS One 2018;13: e0190691.
11. Gramzow L, Theissen G. A hitchhiker's guide to the MADS world of plants. Genome Biol 2010;11:214.
12. Chanderbali AS, Berger BA, Howarth DG, Soltis PS, Soltis DE. Evolving ideas on the origin and evolution of flowers: new perspectives in the genomic era. Genetics 2016;202:1255-1265.
13. Matsunaga S, Isono E, Kejnovsky E, Vyskot B, Dolezel J, Kawano S, et al. Duplicative transfer of a MADS box gene to a plant Y chromosome. Mol Biol Evol 2003;20:1062-1069.
14. Jack T, Brockman LL, Meyerowitz EM. The homeotic gene APETALA3 of Arabidopsis thaliana encodes a MADS box and is expressed in petals and stamens. Cell 1992;68:683-697.
15. Sugiyama R, Kazama Y, Miyazawa Y, Matsunaga S, Kawano S. CCLS96.1, a member of a multicopy gene family, may encode a non-coding RNA preferentially transcribed in reproductive organs of Silene latifolia. DNA Res 2003;10:213-220.
16. Kejnovsky E, Hobza R, Cermak T, Kubat Z, Vyskot B. The role of repetitive DNA in structure and evolution of sex chromosomes in plants. Heredity (Edinb) 2009;102:533-541.
17. Matsunaga S. Sex chromosome-linked genes in plants. Genes Genet Syst 2006;81:219-226.
18. Stewart D, Graciet E, Wellmer F. Molecular and regulatory mechanisms controlling floral organ development. FEBS J 2016;283: 1823-1830.
19. Smaczniak C, Immink RG, Angenent GC, Kaufmann K. Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Development 2012;139:3081-3098.
20. Theissen G, Melzer R, Rumpler F. MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. Development 2016;143: 3259-3271.
21. Masiero S, Colombo L, Grini PE, Schnittger A, Kater MM. The emerging importance of type I MADS box transcription factors for plant reproduction. Plant Cell 2011;23:865-872.
22. Becker A, Theissen G. The major clades of MADS-box genes and

their role in the development and evolution of flowering plants. Mol Phylogenet Evol 2003;29:464-489.

23. De Lucia F, Crevillen P, Jones AM, Greb T, Dean C. A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. Proc Natl Acad Sci U S A 2008;105: 16831-16836.

24. Tapia-Lopez R, Garcia-Ponce B, Dubrovsky JG, Garay-Arroyo A, Perez-Ruiz RV, Kim SH, et al. An AGAMOUS-related MADS-box gene, XAL1 (AGL12), regulates root meristem cell proliferation and flowering transition in Arabidopsis. Plant Physiol 2008; 146:1182-1192.

25. Heijmans K, Morel P, Vandenbussche M. MADS-box genes and floral development: the dark side. J Exp Bot 2012;63:5397-5404.

26. Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han JH, et al. Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. Development 2008;135:1481-1491.

27. Melzer S, Lens F, Gennen J, Vanneste S, Rohde A, Beeckman T. Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. Nat Genet 2008;40:1489-1492.

28. Li C, Wang Y, Xu L, Nie S, Chen Y, Liang D, et al. Genome-wide characterization of the MADS-box gene family in radish (Raphanus sativus L.) and assessment of its roles in flowering and floral organogenesis. Front Plant Sci 2016;7:1390.

29. Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, et al. A transposon-induced epigenetic change leads to sex determination in melon. Nature 2009;461:1135-1138.

30. Cheng H, Song S, Xiao L, Soo HM, Cheng Z, Xie D, et al. Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in Arabidopsis. PLoS Genet 2009;5:e1000440.

31. Mandaokar A, Browse J. MYB108 acts together with MYB24 to regulate jasmonate-mediated stamen maturation in Arabidopsis. Plant Physiol 2009;149:851-862.

32. Millar AA, Gubler F. The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. Plant Cell 2005;17:705-721.

33. Wang X, Niu QW, Teng C, Li C, Mu J, Chua NH, et al. Overexpression of PGA37/MYB118 and MYB115 promotes vegetative-to-embryonic transition in Arabidopsis. Cell Res 2009;19: 224-235.

34. Brownfield L, Hafidh S, Borg M, Sidorova A, Mori T, Twell D. A plant germline-specific integrator of sperm specification and cell cycle progression. PLoS Genet 2009;5:e1000430.

35. Lee DK, Geisler M, Springer PS. LATERAL ORGAN FUSION1

and LATERAL ORGAN FUSION2 function in lateral organ separation and axillary meristem formation in Arabidopsis. Development 2009;136:2423-2432.

36. Murase K, Shigenobu S, Fujii S, Ueda K, Murata T, Sakamoto A, et al. MYB transcription factor gene involved in sex determination in Asparagus officinalis. Genes Cells 2017;22:115-123.

37. Samad AF, Sajad M, Nazaruddin N, Fauzi IA, Murad AM, Zainal Z, et al. MicroRNA and transcription factor: key players in plant regulatory network. Front Plant Sci 2017;8:565.

38. Akagi T, Henry IM, Tao R, Comai L. Plant genetics. A Y-chromosome-encoded small RNA acts as a sex determinant in persimmons. Science 2014;346:646-650.

39. Fornara F, de Montaigu A, Coupland G. SnapShot: control of flowering in Arabidopsis. Cell 2010;141:550.

40. Wu T, Qin Z, Zhou X, Feng Z, Du Y. Transcriptome profile analysis of floral sex determination in cucumber. J Plant Physiol 2010; 167:905-913.

41. Yoo MJ, Chanderbali AS, Altman NS, Soltis PS, Soltis DE. Evolutionary trends in the floral transcriptome: insights from one of the basalmost angiosperms, the water lily Nuphar advena (Nymphaeaceae). Plant J 2010;64:687-698.

42. Zahn LM, Ma X, Altman NS, Zhang Q, Wall PK, Tian D, et al. Comparative transcriptomics among floral organs of the basal eudicot Eschscholzia californica as reference for floral evolutionary developmental studies. Genome Biol 2010;11:R101.

43. Zhang XM, Zhao L, Larson-Rabin Z, Li DZ, Guo ZH. De novo sequencing and characterization of the floral transcriptome of Dendrocalamus latiflorus (Poaceae: Bambusoideae). PLoS One 2012;7:e42082.

44. Logacheva MD, Kasianov AS, Vinogradov DV, Samigullin TH, Gelfand MS, Makeev VJ, et al. De novo sequencing and characterization of floral transcriptome in two species of buckwheat (Fagopyrum). BMC Genomics 2011;12:30.

45. Varkonyi-Gasic E, Moss SM, Voogd C, Wu R, Lough RH, Wang YY, et al. Identification and characterization of flowering genes in kiwifruit: sequence conservation and role in kiwifruit flower development. BMC Plant Biol 2011;11:72.

46. DeLong A, Calderon-Urrea A, Dellaporta SL. Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. Cell 1993;74:757-768.

47. Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, et al. A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. Science 2008;321:836-838.

48. Charlesworth D, Mank JE. The birds and the bees and the flowers

and the trees: lessons from genetic mapping of sex determination in plants and animals. Genetics 2010;186:9-31.

49. Renner SS. The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. Am J Bot 2014;101:1588-1596.

50. Cho J, Koo DH, Nam YW, Han CT, Lim HT, Bang JW, et al. Isolation and characterization of cDNA clones expressed under male sex expression conditions in a monoecious cucumber plant (*Cucumis sativus* L. cv. Winter Long). Euphytica 2005;146:271-281.

51. Gao WJ, Li SF, Zhang GJ, Wang NN, Deng CL, Lu LD. Comparative analysis of gene expression by microarray analysis of male and female flowers of *Asparagus officinalis*. Biosci Biotechnol Biochem 2013;77:1193-1199.

52. Garber M, Grabherr MG, Guttman M, Trapnell C. Computational methods for transcriptome annotation and quantification using RNA-seq. Nat Methods 2011;8:469-477.

53. Xiao M, Zhang Y, Chen X, Lee EJ, Barber CJ, Chakrabarty R, et al. Transcriptome analysis based on next-generation sequencing of non-model plants producing specialized metabolites of biotechnological interest. J Biotechnol 2013;166:122-134.

54. Guo S, Zheng Y, Joung JG, Liu S, Zhang Z, Crasta OR, et al. Transcriptome sequencing and comparative analysis of cucumber flowers with different sex types. BMC Genomics 2010;11:384.

55. Rhee SJ, Seo M, Jang YJ, Cho S, Lee GP. Transcriptome profiling of differentially expressed genes in floral buds and flowers of male sterile and fertile lines in watermelon. BMC Genomics 2015;16:914.

56. Yan X, Dong C, Yu J, Liu W, Jiang C, Liu J, et al. Transcriptome profile analysis of young floral buds of fertile and sterile plants from the self-pollinated offspring of the hybrid between novel restorer line NR1 and Nsa CMS line in *Brassica napus*. BMC Genomics 2013;14:26.

57. Rocheta M, Sobral R, Magalhaes J, Amorim MI, Ribeiro T, Pinheiro M, et al. Comparative transcriptomic analysis of male and female flowers of monoecious Quercus suber. Front Plant Sci 2014;5:599.

58. Allen RL, Lonsdale DM. Molecular characterization of one of the maize polygalacturonase gene family members which are ex-

pressed during late pollen development. Plant J 1993;3:261-271.

59. Tebbutt SJ, Rogers HJ, Lonsdale DM. Characterization of a tobacco gene encoding a pollen-specific polygalacturonase. Plant Mol Biol 1994;25:283-297.

60. Rhee SY, Osborne E, Poindexter PD, Somerville CR. Microspore separation in the quartet 3 mutants of *Arabidopsis* is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. Plant Physiol 2003;133:1170-1180.

61. Ogawa M, Kay P, Wilson S, Swain SM. ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are Polygalacturonases required for cell separation during reproductive development in *Arabidopsis*. Plant Cell 2009;21:216-233.

62. Yu L, Sun J, Li L. PtrCel9A6, an endo-1,4-beta-glucanase, is required for cell wall formation during xylem differentiation in populus. Mol Plant 2013;6:1904-1917.

63. Liu K, Feng S, Pan Y, Zhong J, Chen Y, Yuan C, et al. Transcriptome analysis and identification of genes associated with floral transition and flower development in sugar apple (*Annona squamosa* L.). Front Plant Sci 2016;7:1695.

64. Liu J, Yin T, Ye N, Chen Y, Yin T, Liu M, et al. Transcriptome analysis of the differentially expressed genes in the male and female shrub willows (*Salix suchowensis*). PLoS One 2013;8:e60181.

65. Alagna F, Cirilli M, Galla G, Carbone F, Daddiego L, Facella P, et al. Transcript analysis and regulative events during flower development in olive (*Olea europaea* L.). PLoS One 2016;11:e0152943.

66. Du S, Sang Y, Liu X, Xing S, Li J, Tang H, et al. Transcriptome profile analysis from different sex types of *Ginkgo biloba* L. Front Plant Sci 2016;7:871.

67. Shi T, Gao Z, Wang L, Zhang Z, Zhuang W, Sun H, et al. Identification of differentially-expressed genes associated with pistil abortion in Japanese apricot by genome-wide transcriptional analysis. PLoS One 2012;7:e47810.

68. Fan L, Chen M, Dong B, Wang N, Yu Q, Wang X, et al. Transcriptomic analysis of flower bud differentiation in *Magnolia sinostellata*. Genes (Basel) 2018;9:212.