Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2019, 47(1):1-9. DOI:10.15835/nbha47111343

Review

Advances of the Flowering Genes of Gymnosperms

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Abstract

Flowering is an important stage in the life cycle of plants and also a turning point from vegetative growth to reproductive growth. This process is affected by many exogenous and endogenous factors. Some examples of the latter are endogenous hormones, plant growth status, nutrient composition, and flowering regulatory genes. Many gymnosperms have a long juvenile period. Previous studies attempted to shorten this period using traditional asexual propagation methods, but significant results have not been achieved. In recent years, molecular biology is used to study the flowering regulatory gene to obtain transgenic plants with early flowering trait. Thus, the production of gymnosperms is hastened, and economic efficiency is improved. Studies have shown that the flowering genes of plants act synergistically to form a complex network. In this paper, we reviewed the recent development in the study of the regulation of the flowering genes of gymnosperms, that is, from the floral meristem-specific gene, floral organ-specific gene, genes that inhibit plant flowering, and microRNA regulation of flowering. We provide a reference for the in-depth study on the genetic improvement of the flowering gene.

Keywords: floral development; flowering gene; *Gymnosperms*; juvenile period; reproductive growth

Introduction

Gymnospermae is an advanced plant between the fern and angiosperm, which is a more primitive seed plant. This species has been widely used in greening, medicinal, and dietary consumption. Some of these rare species are remains from the quaternary glaciers and are considered as rare endangered plants. Many gymnosperms have a long juvenile period. For example, the juvenile period of gingko is as long as 20-30 years (Singh *et al*., 2008). This plant takes 8-10 years to blossom and yield after being grafted. Many studies have long performed asexual reproduction technologies, for instance, cutting and grafting, for gymnosperms. Although these methods can shorten the juvenile period to a certain extent, the flowering and bearing of fruit still requires a long time. These characteristics are not conducive to the breeding of superior varieties and the application for high economic value.

The flower is the important reproductive organ of a plant. Flowering plays a central role in plant growth and species evolution. The flowering transformation is the process of transforming the plant from the vegetative growth to the reproductive growth. Flowering is affected by exogenous factors, such as light, temperature, exogenous hormones, moisture, and soil fertility, and endogenous factors (the genes involved in plant flowering regulation) (Levy and Dean, 1998; Khodorova and Michèle, 2013; Cho *et al*., 2016). The isolation of *FLORICAULA* (*FLO*) and *DEFICIENS* (*DEF*) genes from the model plant *A. majus* and the *AGAMOUS* (*AG*) gene cloned from *A. thaliana* indicate that studies on floral development have reached molecular level (Coen *et al*., 1990; Sommer *et al*., 1990; Yanofsky *et al*., 1990). The development of molecular biology has led to in-depth studies on the regulation of plant flowering, and considerable progress has been achieved. At present, the flowering regulatory genes have been isolated from *A. majus*, *A. thaliana*, *O. sativa*, *P. radiata*, *P. mariana*, and *G. gnemon* (Tröbner *et al*., 1992; Weigel *et al*., 1993; Yamaguchi *et al*., 2006; Mouradov *et al*., 1998; Rutledge *et al*., 1998; Shindo *et al*., 2001). Studies of these flowering regulatory genes and mutants have shown that these molecules can effectively promote the flowering period and shorten the juvenile period. Moreover, the widely existing microRNAs in plants are also involved in the regulation of floral organ development and the flowering time (Spanudakis and Jackson, 2014; Aukerman and Sakai, 2003). These flowering regulators constitute a complex network that collectively regulates the flowering (Jack, 2004).

In recent years, molecular biology technology has been employed to study the flowering regulatory gene. Some early flowering-improved plants with effectively shortened juvenile period have been obtained by genetically transforming the flowering gene. For example, the wild *P.*

Received: 20 Aug 2018. Received in revised form: 22 Oct 2018. Accepted: 19 Nov 2018. Published online: 23 Nov 2018.

davidiana Dode needs 8-12 years to bloom, but the *LFY* mutant - *P. davidiana* can bloom in 5 months (Blázquez *et al*., 1997). Previous studies have shown that after transferring the *LFY* and *APETALA1* (*AP1*) genes into citrus, both transgenic plants could bloom within a year, which is 3-5 years earlier than the wild plants, with normal fruit development and stable genetic trait (Peña *et al*., 2001). Therefore, studying the regulation of flowering genes in the gymnosperm can promote the early flowering of this species and is also conducive to breeding fine varieties and improving the economic benefit of gymnosperms. We reviewed the floral meristem-specific gene, floral organ-specific gene, flowering suppression gene, and flowering microRNA. The expression patterns or functions of different flowering genes of gymnosperms are listed in Table 1.

Floral meristem-specific gene

In angiosperms, the *LEAFY* gene has an important role in determining the flowering time of plants. The *LFY* gene is currently the most studied and investigated flowering regulatory gene of the floral meristem. To date, *LEAFY* homologous genes, as an example *LFY*, *FLO*, *NFL*, and *RFL*, have been isolated from many angiosperms (Coen *et al*., 1990; Kelly *et al*., 1995; Weigel *et al*., 1995； Kyozuka *et al*., 1998). The *LFY* gene is the earliest expression of floral meristem-specific gene, and *AP1*/*CAL* and other genes are their downstream target genes (William *et al*., 2004). As a plant ages, the expression of the *LFY* gene gradually increases, reaching the highest level during reproductive growth. This process activates a series of downstream target genes to enable the plant to successfully complete flowering. The *LFY* gene can synergize with the *AP1* gene to inhibit the activity of the *EMF* gene. This process promotes the conversion of the inflorescence meristem to the floral meristem and enables the plant to complete the flowering transition (Chen *et al*., 1997). Dornelas *et al*. (2005) transformed the *PcLFY* gene of *P. caribaea* to the *A. thaliana lfy-26* mutant. The transgenic plants had the same phenotype as the wild plants. This result suggested that the *LFY* homologous genes of gymnosperms and angiosperms may have similar biological functions.

Table 1. Expression patterns or functions of different flowering genes of gymnosperms

Gene category	Gene name	Expression patterns/Function	References
Floral meristem-specific gene	*PcLFY*	female spherules	Dornelas *et al*.,2005
	GpLFY	female spherules	Shindo *et al*., 2001
	PRFLL	vegetative buds and male bulbs	Mellerowicz *et al*., 1998
	NEEDLY	female spherules	Mouradov *et al*., 1998
	Ginlfy	flower buds, saplings, and leaves of mature plant	Zhang *et al*., 2002a
	GinNdly	roots, leaves, female and male flower buds, and young fruits	Zhang *et al*., 2002b
	PaLFY	female scapes	Carlsbecker *et al*., 2004
	PaNLY	surrounding tissues	Sundström *et al*., 2002
	PoLFY	female flower buds	Vázquez *et al*., 2007
	PodNLY	ovule primordium and epimatium	Vázquez *et al*., 2007
	PmLFY	female cones and strobile	Chen *et al*., 2015
Floral organ-specific gene	*PrDGL*	pollen strobili (male cones)	Mouradov *et al*., 1999
	GpMADS1,3,4	early stage of ovule development	Shindo *et al*., 1999
	GGM7, 9, 11, 15	early stage of reproductive organ development	Becker *et al*.,2003
	CjMADS14, 15	male and female strobili	Katahata *et al*., 2014
	GBM5	stamens, ovules, gametophytes and young leaves	Jager *et al*., 2003
	GbCO	bud tip	Yan *et al*., 2017
	GbCOL16	female sporophyll and young fruit	Wang *et al*., 2017
	GbMADS9	spherules and ovules	Yang *et al*., 2016
	GbSEP	female flowers	Cheng *et al*., 2016
	GbMADS2	male flowers	Wang *et al*., 2015
	GbAGL66	flower and fruit growth	Dou *et al*., 2017
	GbAP2	leaves and female spores	Zhang *et al*., 2017
Genes that inhibit plant flowering	*PaFTL1*，*PaFTL2*	inhibit the development of male spherules and meristems inhibit the growth of needles and vegetative buds	Karlgren *et al*., 2011

However, unlike angiosperms, gymnosperms have double-copy *LEAFY* homologous genes (Frohlich and Parker, 2000; Himi *et al*., 2001). Frohlich and Parke (2000) proposed the "mostly male theory" based on the differences in the spatiotemporal expression of double-copy *LEAFY* homologous genes in gymnosperms and the deletion of *NLY* genes in angiosperms. However, in the study of *P. caribaea* and *G. parvifolium*, the *LEAFY* homologous genes *PcLFY* and *GpLFY* were expressed in the female spherules (Dornelas *et al*., 2005; Shindo *et al*., 2001). This result weakened the basis of the "mostly male theory".

The *LFY* homologous genes of *P. radiata*, namely, *PRFLL* and *NEEDLY*, were found to be expressed in the vegetative and reproductive organs and constitutively expressed. However, the *PRFLL* genes were expressed in vegetative buds and male bulbs. The *NEEDLY* gene was mainly expressed in the female spherules (Mellerowicz *et al*., 1998; Mouradov *et al*., 1998). In *G. biloba*, the female plant *LEAFY* homologue gene *Ginlfy* and the male plant *LEAFY* homologue gene *GinNdly* have also been cloned (Zhang *et al*., 2002a; Zhang *et al*., 2002b). The result of the expression profile showed that *Ginlfy* was tissue-specifically expressed in the leaves of the flower buds, saplings, and mature female and male plants. The *GinNdly* gene was expressed in the roots, leaves, female and male flower buds, and young fruits of *G. biloba* as constitutive expression (Guo *et al*., 2005). In *P. abies*, the *PaLFY* gene was expressed in female scapes, and *PaNLY* was expressed in the surrounding tissues (Carlsbecker *et al*., 2004; Sundström *et al*., 2002). The *PoLFY* of *P. macrophyllus* was expressed in the female flower buds, and *PodNLY* was expressed in ovule primordium and epimatium, and *PoNLY* was expressed in the cones, quills, ovule primordia, and epimatium (Vázquez *et al*., 2007). In *P. massoniana*, the *PmLFY* and *PmNLY* genes main involved in the development process of the female cones and *PmLFY* also involved in strobile development (Chen *et al*., 2015). The difference in the spatial-temporal expression of the double-copy *LFY* homologous gene of gymnosperm may be the reason for the prolonged juvenile period of gymnosperms. The *LFY* gene may undergo functional differentiation in the long-term evolution and has different functions for inhibiting the development of vegetative organs and genital organs.

Floral organ-specific gene

The MADS-box gene is found widely in plants. This gene regulates all stages of the floral development and plays a decisive role in the development of a floral organ. In addition to the *AP2* gene, most floral organ-specific genes contain the MADS-box DNA transcription factor region. Thus, this type of gene is called the MADS-box gene family. According to the ABC model of floral development (Bowman *et al*., 1991; Coen and Meyerowitz, 1991), the class A gene, *AP1*, is involved in the formation of sepal. The A and B genes determine the formation of the petal. *APETALA3* (*AP3*), *PISTILLATA* (*PI*), *DEFICIENS* (*DEF*), and *GLOBOSA* (*GLO*) are Class B genes (Falkowski and Dubinsky, 1981; Sommer *et al*., 1990; Tröbner *et al*., 1992). The class B and C genes jointly inhibit the development of the pistil and stamen, while the class C gene determines the development of the carpel. The *AG* gene of *A. thaliana* belongs to class C gene (Yanofsky *et al*., 1990). However, the ABCDE model was deduced from the continuous study of flower development (Theissen and Saedler, 2001). The class D gene, like *FLORAL BIDING PROTEIN 7* (*FBP7*) and *FBP11* of *P. hybrida*, and *AGL11* of *A. thaliana*, is the major gene that inhibits the development of ovules (Colombo *et al*., 1995; Rounsley *et al*., 1995; Angenent and Colombo, 1996). The class E gene is involved in the development of the petal, stamen, and carpel. *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3*, and *SEP4* are typical examples of this class of gene (Pelaz *et al*., 2000; Ditta *et al*., 2004). By using the characteristics of MADS-box gene in different flower organs, some genes can be applied to plant genetic manipulation in order to reconstruct the horticultural traits of plants, and also shorten the juvenile period of long gymnosperms to reduce production time and increase yield. With the deepening of these studies, the application value of MADS-box gene will be gradually revealed.

According to reports, many MADS-box genes have been isolated from *P. abies*, *P. mariana*, *G. gnemon* (Tandre *et al*., 1995; Rutledge *et al*., 1998; Sundström *et al*., 1999; Winter *et al*., 1999). In *P. radiata*, *PrDGL* was expressed in emergent male cone primordia and persisted through the early stages of pollen cone bud differentiation (Mouradov *et al*., 1999). The *GpMADS1, 3, 4* gene of *G. Prvifolium* were expressed during the early stage of ovule development in the differentiating nucellus and envelopes (Shindo *et al*., 1999). The studies of *G. gnemon* have shown that the expression of all four MADS-box genes (*GGM* 7, 9, 11, 15) is limited to reproductive units (especially in the early stage of reproductive organ development), the *GGM15* transcript is even restricted to male reproductive organs (Becker *et al*., 2003). These genes were specifically expressed in the reproductive organs but not in vegetative organs. The role of MADS box gene in the floral development of gymnosperm and angiosperm has been perceived to be very conservative (Theißen, 2001). However, new ideas have gradually emerged in subsequent studies. The MADS-box genes played a role in the growth of floral organs and were expressed during flowering initiation, fruit growth, differentiation, and formation of meristems, embryos, roots, and vascular tissues (Van der Linden *et al*., 2002). This concept was confirmed by the expression of some gymnosperm. For instance, the homologue gene *GBM5* of *G. biloba* in the stamens, ovules, and gametophytes of this species as well as in the young leaves of both male and female plants (Jager *et al*., 2003). In the study of *C. japonica*, the *CjMADS15* gene was expressed in all organs except pollen, and was especially expressed in needles, *CjMADS14* gene was expressed mainly in male and female strobili. These two genes play important roles during the development of male and female strobili in *C. japonica* (Katahata *et al*., 2014). The study of these MADS-box gene functions is of great theoretical significance for explaining various physiological phenomena in plants.

Our research group has reported some floral organ-specific genes of *G. biloba*. The *CONSTANT* gene regulates the expression of the downstream *FT* gene in the leaves by responding to photoperiodic signals. The *FT* protein accumulates in the leaves and is transferred to the shoot tips to induce the flowering of the plant. The *CONSTANT* homologous gene *GbCO* has the highest expression level in the *G. biloba* bud tip and is regulated by a photoperiod. It promotes the early flowering by activating the downstream *FT* gene (Yan *et al*., 2017). The *GbCOL16* gene of *G. biloba* is then cloned, with the highest expression in the leaves. The expression in the male spores is higher than that in the female sporophyll and young fruit. This phenomenon indicates that *GbCO* and *GbCOL16* have similar flowering regulatory mechanisms with the *CO* gene (Wang *et al*., 2017). *GbMADS9* of *G. biloba* belongs to the B_{sister}-class MADS-box gene and is expressed in the spherules and ovules. Overexpression of *GbMADS9* leads to the early flowering of transgenic *A. thaliana* and enhances the ability of *A. thaliana* to tolerate permeation. Thus, *GbMADS9* may be involved in the regulation of the flowering time of *G. biloba* and enhance the ability of this species to withstand abiotic stresses (Yang *et al*., 2016). The repression levels of *GbSEP* and *GbMADS2* cloned from the female and male flowers of *G. biloba* are significantly higher than those in the roots, stems, and leaves. The expression of *GbSEP* in the female flowers is significantly higher than those in the male flowers, however *GbMADS2* is opposite to *GbSEP*. The expression of the two genes increases as the flowers grow. This phenomenon indicates that these genes may be involved in the growth of *G. biloba* (Cheng *et al*., 2016; Wang *et al*., 2015). *GbAGL66* is strongly expressed in the roots and flowers, with the highest level in the roots. This gene is also detected in the fruit. Thus, the *GbAGL66* gene may be involved in the regulation of flower and fruit growth (Dou *et al*., 2017). *GbAP2* gene is expressed in the roots, stems, leaves, male spores, female spores, and fruits and strongly expressed in the leaves and female spores. These characteristics indicate that *GbAP2* may be involved in the growth and development of *G. biloba* (Zhang *et al*., 2017). These studies indicate that the MADS-box gene plays an important role in regulating the flowering time and floral organ development of *G. biloba*, and provides a reference for the flower-specific gene regulation of other gymnosperms.

Despite remarkable progress in the regulation of floral organ-specific gene, and numerous flower development-related transcription factors have been cloned from various plants, how to use genetic engineering to precisely control plant development and how to use these transcription factors applied to agricultural production is still an urgent problem to be solved.

Genes that inhibit plant flowering

The *EMBRYONIC FLOWER* (*EMF1* and *EMF2*) gene plays an important role in maintaining vegetative growth and inhibiting flower development (Calonje *et al*., 2008; Moon *et al*., 2003). The *emf1* and *emf2* mutants do not undergo vegetative growth but flowered directly (Sung *et al*., 1992; Yang *et al*., 1995). These phenomena indicate that the loss of *EMF* function results in the early flowering of the plant. As the plant ages and the exogenous environment changes, the inhibition of *EMF* gene is gradually decreasing (Yang *et al*., 1995). When the expression drops to a certain level, the plant enters the flowering transformation. The expression of *LFY* and *AP1* genes, begins to increase, overcoming the floral repression of *EMF* and enabling the plant to complete its flowering (Sung *et al*., 2003). Many angiosperm *EMF* genes, for instance in *A. thaliana*, *O. sativa*, and *B. oleracea*, have been reported (Sung *et al*., 1992; Li *et al*., 2006; Liu *et al*., 2012), but studies of *EMF* genes in gymnosperms have not been reported.

TFL is a member of the *FT*/*TFL* gene subfamily and plays a key role in flowering. To date, the effect of the *TFL* gene on flowering inhibition has been reported in many plants (Alvarez *et al*., 1992; Bradley *et al*., 1996; Nakagawa *et al*., 2002). The *LFY* and *AP1*/*CAL* are generally believed to suppress flowering by inhibiting the expression of the *TFL1* gene in flowers. However, some studies have indicated that the *TFL1* transcription was inhibited by *AP1* but promoted by *LFY* (Shannon and Meeks-Wagner, 1991; Liljegren *et al*., 1999; Pillitteri *et al*., 2004; Serrano-Mislata *et al*., 2017). In the mutant strains without *TFL1* function, the inflorescent meristem was rapidly transformed to the floral meristem, which significantly promoted flowering (Banfield and Brady, 2000). Studies have shown that after transferring the antisense *MdTFL1* into apples, the transgenic plants blossomed within 8-10 months after being grafted (Kotoda *et al*., 2006). This characteristic indicated that removing the *TFL* gene to inhibit flowering could shorten the juvenile period. In gymnosperms, the *PaFTL* gene of Norway spruce was functionally similar to the *TFL1-like* gene (Karlgren *et al*., 2011). *PaFTL1* could inhibit the development of male spherules and meristems, while *PaFTL2* could inhibit the growth of needles and vegetative buds.

The *FLC* gene inhibits flowering, and the encoded MADS-box transcriptional regulatory protein is an inhibitor of flowering. The *FLC* gene regulates the flowering time by responding to vernalization that can downregulate the activity of the *FLC* gene and promote the flowering of the *A. thaliana* late flower type and late flower mutant (Sheldon *et al*., 2000). Studies have shown that *FLC* suppressed flowering by inhibiting the expression of two downstream flowering genes, namely, *SOC1* (*AGL20*) and *FT* (Lee *et al*., 2000; Michaels and Amasino, 2001; Michaels *et al*., 2005), which promote flowering. In a recent study, the *ft-1*/*flc-21* double mutant was identical to the *flc-21* phenotype, but unlike *ft-1* (Chen and Penfield, 2018). This phenomenon indicated that *FLC* was downstream of *FT*, which was inconsistent with previous findings. However, studies of *FLC* genes in gymnosperms have not been reported. Few studies have been conducted on the genes suppressing the flowering of gymnosperms. Thus, additional studies are needed to further understand the molecular mechanisms that inhibit these flowering genes.

microRNA regulation of flowering

The microRNAs are endogenous non-coding small RNAs of 21-24 nucleotides in length and found in a wide variety of plants. These molecules can regulate genes at the post-transcriptional level by completely or partially matching with the target gene mRNAs. These molecules regulate the expression of plants gene by transcriptional cleavage or inhibition of target gene mRNA translation (Reinhart *et al*., 2002; Bartel, 2004). The microRNA functions of some early identified plants are conserved, and studies on flowering microRNAs have focused on model plants (Reinhart *et al*., 2002; Chi *et al*., 2011).

A microRNA has multiple target genes or multiple microRNAs to regulate a target gene to form a complex flowering regulatory network. Acting as a post-transcriptional regulator, the microRNAs function broadly to control many aspects of plant biology and plant development, and play a key role in the regulation of plant flowering and floral organ development (Nag and Jack, 2010; Wu, 2013). The miR156 controls the transformation from vegetative growth to reproductive growth through the target regulation of SPL (SQUAMOSA Promoter-binding protein-like) transcription factors (Yang *et al*., 2011). The miR172 regulates the flowering time and floral organ development through the translation inhibition or cleavage of *AP2-like* family genes (Jung *et al*., 2007; Glazińska *et al*., 2009). According to reports, miR156 and miR172 are involved in regulating the timing of sensitivity of the vernalization response in *Cardamine flexuosa*, and modulated the expression of *CfSOC1* gene to regulate flowering (Zhou *et al*., 2013). The miR159 modulates the MYB transcription factors and maintains the normal growth of anthers (Millar *et al*., 2005). The miR164 family (miR164a, miR164b, and miR164c) regulates the transformation among petals, pistils and stamens (Aida *et al*., 1997). The target genes are the NAC family of transcription factors (e.g., CUC1 and CUC2).

In the model plant *A. thaliana*, miR156 can postpone the flowering period, while miR172 can predate this period. The expression of miR156 decreases from the young to the adult, but the expression of miR172 increase (Wu *et al*., 2006). The miR156 negatively regulates the SPL3 expression and inhibits *A. thaliana* flowering. The miR156 target genes SPL9 and SPL10 positively regulate the expression of miR172 and have an indirect effect on inducing flowering (Wu *et al*., 2009). The miR172 negatively regulates the *AP2* gene. This process reduces flowering inhibition and facilitates the successful complete flowering (Aukerman and Sakai, 2003). The miR164 negatively regulates CUP SHAPED COTYLEDON 1 (CUC1) and CUC2, and this process affects the growth of the meristem and the generation of floral organ primordia (Mallory *et al*., 2004). To date, many novel and conserved microRNAs involved in flower development in angiosperms have been discovery and profiled (Wang *et al*., 2012; Wang *et al*., 2014; Sun *et al*., 2015). In these studies, most of the conserved miRNAs in these species are highly conserved among plants (e.g., miR156, miR167 and miR172 etc.), indicating that these miRNAs have important and conserved functions in plant development. These works provide a good reference for the study of flower development of gymnosperms.

We reviewed some microRNAs involved in the regulation of flower development in gymnosperms, which are listed in Table 2. In the gymnosperm Norway spruce, of the 22 conserved miRNA families, 8 miRNAs conserved in embryonic plants and 13 other miRNAs conserved in angiosperms were detected, indicating that these miRNAs are present in the common ancestor of spermatophytes (Xia *et al*., 2015). It has been reported that pab-miR159a may regulate *PaGaMYB* expression in Norway spruce and may participate in seed germination and flower development (Yakovlev *et al*., 2010). Numerous microRNAs involved in the regulation of floral organ development in angiosperms have been identified from leaves and ovules of *G. biloba*. Some of these microRNAs were expressed in ovules, including the miR156, miR164, miR167, miR169, miR172, and miR390 families (but not miR164c and miR169b), indicating these microRNA plays an important role in the development of floral organ (Wang *et al*., 2016). Some conserved microRNAs (miR166a, miR166b, miR172, miR399, and miR776) were also isolated from *P. taeda*, and these molecules were expressed in the female and male gametophytes and needle tissues with almost identical levels. This result suggested that these microRNAs were involved in the development of the male and female gametophytes. Pta-miR157a and pta-miR157b were highly expressed in the needles and mature pollen, but lowly expressed in germinated pollen. This expression pattern is opposite to pta-miR161.2 and pta-miR164b, indicating that these microRNAs Participated in the development of pollen (Quinn *et al*., 2015). However, the specific mechanisms of microRNAs in the regulation of flowering of gymnosperms remain ambiguous. Further investigation on the specific regulatory functions of these microRNAs will contribute to the understanding of the floral development in gymnosperms.

Table 2. MicroRNAs involved in the regulation of flower development in gymnosperms

Species	microRNA name	Expression pattern/Function	References
Ginkgo biloba	miR156, miR164, miR167, miR169, miR172, miR390	ovules	Wang *et al*., 2016
Picea abies	pab-miR159a	seed germination and flower development	Yakovlev *et al*. ,2010
Pinus taeda	miR166a, miR167b, miR172, miR399, miR776	female and male gametophytes	Quinn *et al*., 2015
Pinus taeda	pta-miR157a, pta-miR157b	highly expressed in the needles and mature pollen, but lowly expressed in germinated pollen	Quinn *et al*., 2015
Pinus taeda	pta-miR161.2, pta-miR164b	lowly expressed in the needles and mature pollen, but highly expressed in germinated pollen	Quinn *et al*., 2015

Outlook

Flowering is a very complex physiological and biochemical process regulated by a network of genes composed of various flowering regulators (Schultz and Haughn, 1991). The *LEAFY* is lowly expressed during vegetative growth. Affected by diverse internal and external factors, a plant gradually releases the flowering inhibition. When a certain threshold value is reached, *LEAFY* activates the floral organ specificity gene *AP1,* and the synergistic effect smoothly completes the flowering conversion (Parcy *et al*., 1998). An in-depth study of the synergistic effects of flowering regulators of gymnosperms and the effects of regulatory networks on the floral development will provide a theoretical basis for reducing the juvenile period and genetically improving gymnosperms. To date, studies on the mechanism and application of the flowering regulators that regulate flowering have mainly focused on model plants, such as *A. thaliana* and *N. tabacum* (Eckardt, 2005). Studies on gymnosperms are rarely reported. Additional investigations should be performed to reveal the mechanism of flowering regulation of gymnosperms and the mechanism of their interactions.

With the development of molecular biology, the clonal identification of numerous plant flowering genes provides a theoretical basis to understand the evolutionary processes and phylogenetic relationships of plants. A series of studies have shown that the flowering genes were highly conserved among closely related species. This finding revealed the homology of plant origin. Therefore, the transcription of these flowering homologous genes into gymnosperms within a prolonged juvenile period facilitate the rapid blossoming and reproduction of transgenic plants. This process will greatly increase the economic benefits of gymnosperms. However, given the difficulties in the tissue culture, in vitro regeneration, and gene transformation techniques of some gymnosperms, these genes can only be transcribed into ectopic expressions in plants. Several limitations should be solved to transcribe the flowering genes into corresponding gymnosperms to obtain flowering transgenic plant. Given the continuous development of tissue culture technology and molecular biology technology, the genetic improvement of gymnosperms will result in greater progress and better production of gymnosperms.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 31670608).

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