Molecular Phylogenetics and Evolution 114 (2017) 111–121

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Evolution of the *miR5200-FLOWERING LOCUS T* flowering time regulon in the temperate grass subfamily Pooideae

Meghan McKeown [a,1], Marian Schubert [b,1], Jill C. Preston [a,*], Siri Fjellheim [b]

[a] Department of Plant Biology, The University of Vermont, 63 Carrigan Drive, Burlington, VT 05405, USA
[b] Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, PO Box 5003, 1432 Ås, Norway

ARTICLE INFO

Article history:
Received 24 October 2016
Revised 4 June 2017
Accepted 7 June 2017
Available online 8 June 2017

Keywords:
Flowering time
FT
miR5200
Photoperiod
Pooideae

ABSTRACT

Flowering time is a carefully regulated trait controlled primarily through the action of the central genetic regulator, *FLOWERING LOCUS T* (*FT*). Recently it was demonstrated that a microRNA, miR5200, targets the end of the second exon of *FT* under short-day photoperiods in the grass subfamily Pooideae, thus preventing *FT* transcripts from reaching threshold levels under non-inductive conditions. Pooideae are an interesting group in that they rapidly diversified from the tropics into the northern temperate region during a major global cooling event spanning the Eocene-Oligocene transition. We hypothesize that miR5200 photoperiod-sensitive regulation of Pooideae flowering time networks assisted their transition into northern seasonal environments. Here, we test predictions derived from this hypothesis that miR5200, originally found in bread wheat and later identified in *Brachypodium distachyon*, (1) was present in the genome of the Pooideae common ancestor, (2) is transcriptionally regulated by photoperiod, and (3) is negatively correlated with *FT* transcript abundance, indicative of miR5200 regulating FT. Our results demonstrate that miR5200 did evolve at or around the base of Pooideae, but only acquired photoperiod-regulated transcription within the *Brachypodium* lineage. Based on expression profiles and previous data, we posit that the progenitor of miR5200 was co-regulated with *FT* by an unknown mechanism.

1. Introduction

Flowering time is critical to plant survival and reproductive success, and is tightly regulated by the integration of several exogenous and endogenous signals, including photoperiod, temperature, and developmental age (Andrés and Coupland, 2012; Bernier and Périlleux, 2005; Lacey, 1986; Lang, 1952; Song et al., 2015). One of the most important promoters of the floral transition is *FLOWERING LOCUS T* (*FT*), whose small protein product acts as a 'florigen', moving from leaves through the phloem to the shoot apex (Corbesier et al., 2007; Turck et al., 2008). Although the role of FT as the central florigen is highly conserved across many flowering plant groups (Turck et al., 2008), recent work suggests that many points of regulation have evolved in response to abiotic signals such as photoperiod (Greenup et al., 2009; Wu et al., 2013) and vernalization (Fjellheim et al., 2014; Yan et al., 2006). Studies examining the evolutionary history of *FT* regulation in response to the environment are critical to inform our understanding of how plant genetic architecture may be modified in response to current and future climatic changes.

Recently it was demonstrated that transcription of *FT*-like genes *FTL1* and *FTL2/VERNALIZATION 3* (*VRN3*) (hereafter *FTL2*) in the long-day (LD) temperate grass *Brachypodium distachyon* (tribe Brachypodieae) (Schwartz et al., 2010) are negatively regulated by the microRNA miR5200 (previously known as miR2032) in a photoperiod-sensitive manner (Wei et al., 2009; Wu et al., 2013). On chromosome one in *B. distachyon,* two potential genes encode miR5200 transcripts, and are thus named *MIR5200a* and *MIR5200b* (Wu et al., 2013). Another miRNA, with a one-nucleotide difference to miR5200a, has also been identified, and named miR5200c (Wu et al., 2013; Zhang et al., 2009). Suggesting evolutionary conservation, *MIR5200a* and *MIR5200b* are syntenic in *Triticum monococcum* (tribe Triticeae, einkorn wheat) and *B. distachyon* (Abrouk et al., 2012; Lucas and Budak, 2012; Wu et al., 2013).

Biosynthesis of mature plant microRNAs from their initial transcripts occurs through various processing steps. In general,

Abbreviations: FT, FLOWERING LOCUS T; LD, long day; SD, short day.

* Corresponding author at: 111 Jeffords Hall, 63 Carrigan Drive, Burlington, VT 05405, USA.
E-mail address: Jill.Preston@uvm.edu (J.C. Preston).

[1] Denotes shared first authorship.

http://dx.doi.org/10.1016/j.ympev.2017.06.005
1055-7903/

trimming of the 3′- and 5′-ends of the primary miRNA transcript (pri-miRNA) produces the precursor miRNA (pre-miRNA) comprising a complementary stem region and a loop. Excision of the double-stranded miRNA duplex, consisting of miRNA/miRNA∗ (where miRNA∗ is complementary to the mature miRNA), is mediated by Dicer-like1 (DCL1). The mature miRNA molecule binds to ARGONAUT (AGO), which together with other proteins forms the miRNA-induced silencing complex (miRISC) that is able to cleave mRNA transcripts complementary to the miRNA (Voinnet, 2009). It is becoming clear that the abundance of mature miRNAs can be regulated at several points in the maturation process. These points of regulation may be condition specific (Kai and Pasquinelli, 2010; Meng et al., 2011). Processes that affect the final abundance of mature miRNA range from pri- and pre-miRNA base editing to miRISC loading, and AGO protein complex binding (Meng et al., 2011; Winter et al., 2009). One recent hypothesis proposes that several different AGO proteins control the stability of mature miRNAs and thus may determine miRNA abundance in the cell (Winter et al., 2009).

FTL1 and *FTL2* from *B. distachyon* possess sequences complementary to the mature form of miR5200a and miR5200b, suggesting that both miRNAs target *FT*-like transcripts for degradation (Wu et al., 2013). Supporting this hypothesis, overexpression and knock-down of *B. distachyon* Bd21-3 miR5200 previously resulted in delayed and accelerated flowering times, respectively, with concurrent decreases or increases in *FT* expression (Wu et al., 2013). Similar to *FTL1* and *FTL2*, *B. distachyon* miR5200a and miR5200b displayed diurnal expression patterns, peaking four hours before dawn (Wu et al., 2013). MiR5200a and miR5200b transcript abundance was also relatively high under four and eight hour short day (SD) conditions, but nearly undetectable under 16- and 20-h LD photoperiods (Wu et al., 2013). When plants grown in LDs were moved to SDs, miR5200a/b expression increased after seven days, while *FTL1/2* expression steadily decreased (Wu et al., 2013). Interestingly, repressive histone marks (H3K27me3) were enriched surrounding hairpin structure regions of *MIR5200* in LDs, while promoting histone marks (H3K4me3) were present in SDs, matching expression changes seen in these conditions (Wu et al., 2013). Together, these data support a role for the regulation of miR5200a and miR5200b transcription in the SD repression of FT-regulated flowering in *B. distachyon* Bd21-3.

Triticum aestivum (tribe Triticeae, common wheat) and *B. distachyon* are members of the Pooideae subfamily of grasses that originated and quickly transitioned from the tropics into the northern temperate region about 50–35 million years ago (Bouchenak-Khelladi et al., 2010; Christin et al., 2008; Gaut, 2002; Vicentini et al., 2008), during a gradual cooling period at the middle and end of the Eocene (Mannion et al., 2014; Stickley et al., 2009; Zachos et al., 2001). The subfamily, which comprises over 4200 species (Soreng et al., 2015), is thus hypothesized to have evolved several seasonal adaptations, such as a response to vernalization that prevents precocious flowering during the seasonal low winter temperatures, while triggering flowering under the inductive LD photoperiods of summer (Fjellheim et al., 2014; McKeown et al., 2016; Preston and Sandve, 2013; Ream et al., 2013; Woods et al., 2016). LD flowering is crucial for temperate plants because it enables them to efficiently exploit the short growing season. In the core Pooideae, which is the species-rich sister clade to the Brachypodieae and includes all temperate cereals and forage grasses (e.g. *T. aestivum*, *Hordeum vulgare* [tribe Triticeae, barley], *Avena sativa* [tribe Poeae, oats], and *Lolium perenne* [tribe Poeae, perennial ryegrass]) (Saarela et al., 2015), photoperiod and vernalization networks are entwined, and are often regulated by overlapping mechanisms (Ream et al., 2014). Despite this, miR5200 expression does not appear to be influenced by vernalization in both vernalization responsive and non-responsive *B. distachyon* accessions (Wu et al., 2013), suggesting that an independent photoperiod-sensitive module has evolved to regulate *FT*.

Homologs of *B. distachyon* miR5200 have been found in several core Pooideae species, but are lacking from the fully sequenced genomes of rice (*Oryza sativa*, Ehrhartoideae), maize (*Zea mays*, Panicoideae), sorghum (*Sorghum bicolor*, Panicoideae), and *Arabidopsis thaliana* (Brassicaceae) (Wu et al., 2013). Based on this observation, and given the potential importance of the miR5200-FT regulon in repressing temperate winter flowering, we hypothesize that miR5200 appeared *de novo* early within Pooideae, and was quickly integrated into the photoperiod pathway to negatively regulate *FT* transcription during the non-inductive SD photoperiods of winter. Specifically, we hypothesize that 1) miR5200 evolved at the base of Pooideae, 2) SD-induced miR5200 expression is conserved across Pooideae, and, 3) the negative correlation between *FT* and miR5200 transcript abundance is conserved across Pooideae, suggesting a prominent role for transcriptional regulation of miR5200 in mediating *FT* expression patterns. To test these hypotheses, we isolated miR5200 transcripts from phylogenetically representative Pooideae species, and compared the expression profiles of miR5200- and *FT*-like genes under different photoperiods. Our results support an origin of miR5200 at least prior to the diversification of Pooideae minus the earliest-diverging tribe Brachyelytreae, but suggest that control of miR5200 transcription by photoperiod evolved independently in the *B. distachyon* Bd21-3 accession.

2. Materials and methods

2.1. Plant growth and experimental design

Five phylogenetically diverse species were chosen for the study: *Nardus stricta* (tribe Nardeae, collected in Romania, [46.69098, 22.58302], July 2012), *Stipa lagascae* (tribe Stipeae, PI 250751, U. S. Department of Agriculture [USDA] National Plant Germplasm System [NPGS] via Germplasm Resources Information Network [GRIN]), *Melica nutans,* (tribe Meliceae, collected in Germany, [50.70708, 11.23838], June 2012), *B. distachyon* (line Bd21, tribe Brachypodieae, W6 36678, Western Regional Plant Introduction Station), and *Hordeum vulgare* (tribe Triticeae, barley) 'Morex' (USDA NPGS via GRIN, 35761). The first three species belong to lineages outside of the Brachypodieae-core Pooideae clade, referred to as early-diverging lineages (Fig. 1A). As a control we included the specific *B. distachyon* accession Bd21-3 (W6 39233, USDA NPGS via GRIN) used in Wu et al. (2013) (personal communication Long Mao).

Seeds of *N. stricta* were germinated on 1% agar plates for two weeks, before being planted in soil and randomly assigned to one of four 17-20 °C growth rooms at the Centre for Plant Research in Controlled Climate, Norwegian University of Life Sciences NMBU, in Ås, Norway. *S. lagascae* and Bd21 seeds were placed directly in soil at the start of the experiment and divided into treatments as described for *N. stricta*. Two replicates of the experiment occurred simultaneously with two treatment chambers and two control chambers. Initially, all chambers were set to 16-h light: 8-h dark LD photoperiod conditions for three weeks. Subsequently, the two treatment chambers were set to an 8-h light: 16-h dark SD photoperiod regime for two weeks, followed by LDs until flowering. The two control chambers were set to LDs for the entire experiment, and all chamber temperatures cycled from 20 °C during the day to 17 °C at night. The longest fully expanded leaf was sampled for RNA from four independent individuals for each species at three weeks post planting (set to day 0) and after one week in treatment and control conditions (day 7), times identical to those reported in Wu et al. (2013). To assess the influence of time on gene