Horticulture Research

Horticulture Research, 2024, **11**: uhae258
https://doi.org/10.1093/hr/uhae258

Letter to the Editor

A glimpse of light on the mystery of regulating temperate fruit tree blooming time

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Dear Editor,

Spring frost damage poses a major threat to fruit production in temperate climates, which has become more severe in some regions due to climate change. Management techniques to prevent or mitigate such damage are expensive and their effectiveness often variable, increasing the demand for breeding solutions, such as the exploitation of late-flowering traits to avoid frost injury in spring. Due to the relative scarcity of late-flowering traits in germplasm, gene editing technology represents a feasible option to engineer temperate fruit crops, which requires an in-depth understanding of how target genes regulate flower development and blooming time. Such information is currently unavailable for temperate fruit trees due to lack of proper tools (e.g. effective regeneration–transformation and mutagenesis manipulation methods) to probe the intricate mechanisms complicated by a slow developmental pace, seasonal change, a dormancy cycle, and cold and warm requirements. Thus, for both gene editing and conventional breeding of late-flowering cultivars, a comprehensive understanding of flower regulatory mechanisms is essential.

The development and regulation of floral buds is much more complex in temperate fruit trees than in annual plants. Instead of flower bud establishment and subsequent bloom in one growing season for annual plants, flower development in temperate fruit trees occurs throughout late summer, fall, winter, and the following spring. The flower bud undergoes distinct developmental changes during this period, marked by the formation of rudimentary flower organs (e.g. sepal, petal, stamen, and carpel) before entering dormancy in the fall, forming internal sporogenous tissues (e.g. tapetum, pollen mother cells, ovules, etc.) within dormant floral buds during the winter chilling period, followed by rapid growth and blooming in the following spring (Fig. 1). Despite the popular belief that dormant floral buds remain in an arrested state during winter, recent studies show that they undergo morphological changes (reviewed in [1]). These developmental events are dependent on chilling exposure [1, 2]. Likewise, flower buds that have fulfilled their chilling requirement do not bloom immediately in the following spring, as they require exposure to warm temperatures for a sufficient period of time. It is thus clear that both winter chilling and spring warmth are contributing to flower development and bloom regulation in temperate fruit trees, but the mechanisms underlying this thermal regulation remain largely unknown.

Recent studies by Cirilli et al. [3] and Liu et al. [4] have shed light on this mystery. The first study at the University of Milan evaluated the late blooming traits in 133 peach accessions, identifying four that flower significantly later than the others [3]. Combining QTL mapping with genome sequencing, the team successfully mapped a late-blooming trait to a 994-bp deletion at the *Di2* locus that also confers a dominant *DOUBLE-FLOWERING* or *PETALOSA* phenotype characterized by excessive petal production [5]. At the *Di2* locus, the 994-bp deletion occurred at the 3′ end of gene model *Prupe.6G242400* predicted in the peach genome [3, 5]. The results of these studies reveal for the first time that a genetic mutation causes delayed blooming in temperate fruit trees. Parallel to these studies, the USDA-ARS team has also been working on a particularly Late-Flowering Peach (*LFP*) selection [4], which was obtained by crossing with some of the same or similar germplasm as used by Cirilli [3]. *LFP* floral buds require a longer chilling period and bloom significantly later than all other available germplasm (10–14 days) under both laboratory and field conditions [4]. Furthermore, they have increased numbers of petals, sepals, stamens, and occasionally pistils, similar to altered flower phenotypes caused by mutations in the *Arabidopsis PLURIPETALA* (*PLP*) and *ENHANCED RESPONSE TO ABSCISIC ACID1* (*ERA1*) genes. The team studied peach orthologs of *Arabidopsis* genes as well as a few peach genes, including *Prupe.6G242400*, and discovered aberrant expression, alternative splicing, and sequence variations in 4 of 11 candidate genes in the *LFP* flower buds [4]. Using the genome sequences of *LFP* and 60 other accessions, researchers found a strong link between *LFP*'s late-flowering phenotype and the same deletion at 3′ of the *Prupe.6G242400* sequence [4]. Thus, the same mutation identified by two groups, which likely originated from the same source, results in similar dominant floral phenotypes and late-flowering traits in two peach accessions.

Received: 17 July 2024; **Accepted:** 5 September 2024; **Published:** 30 August 2024; **Corrected and Typeset:** 1 November 2024

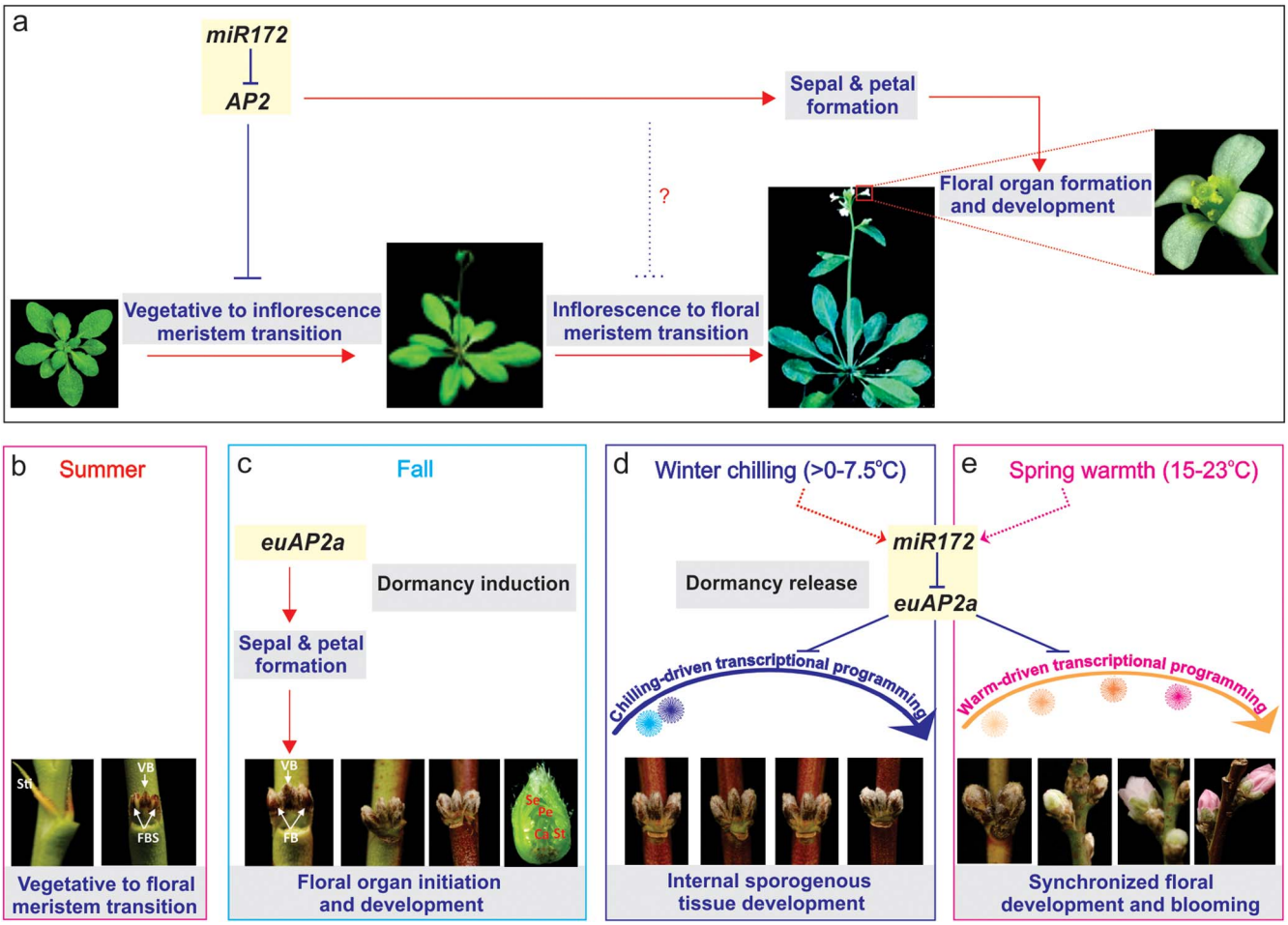

Figure 1. The illustration of mechanistic similarities and differences between *Arabidopsis AP2* and peach *euAP2a*. (a). *Arabidopsis*' reproductive process begins with the transition from vegetative to inflorescence meristem, which subsequently gives rise to the floral meristem, where petal, sepal, stamen, and carpel primordia arise and further developed before flowering. *AP2* specifies sepal and petal identity while inhibiting the vegetative-to-inflorescence transition to delay flowering. However, miR172 negatively regulates it, and the miR172-*AP2* regulatory module fine-tunes flower developmental pace. (b–e). Peach grown in a northern atmosphere converts its vegetative meristem to floral meristem at the end of summer (b), as indicated by the abscission of stipules (Sti) and the formation of flower bud scales (FBS). During the early fall, the floral meristem gives rise to sepal (Se), petal (Pe), stamen (St), and carpel (Ca) primordia, which develop into rudimentary flower organs in the late fall before the floral buds enter dormancy (c). While in a dormant state, the floral buds undergo numerous but delicate developmental events, including the formation of sporogenous tissue (d), which is solely dependent on chilling temperatures. Floral buds resume synchronized, warm-driven development programming after winter chilling and bloom once heat requirements are met. As with *Arabidopsis AP2*, peach *euAP2a* confers sepals and petals during early fall stages (c), but represses flower development by inhibiting stage-specific activation of chilling (FX1)- and warm (FX2)-responsive co-expression modules or transcriptional programming during the chilling and following warm treatments (d, e) rather than the transition from vegetative to inflorescence (a). Since *miR172* is a negative regulator, mutations in *miR172* binding sites within the *euAP2* transcript result in gain-of-function mutations in *euAP2a*, further reinforcing *euAP2a*'s repression of transcriptional programming and delaying bloom. The *miR172-euAP2* module-mediated regulation therefore plays a key role in delicately gauging flower development pace to ensure blooming at the right time. Sti – Stipules. FBS – floral bud scales. VB - Vegetative buds. FB – floral buds in a triple bud. Se – Sepal. Pe – Petal. St – Stamen, Ca – Carpel.

The *Prupe.6G242400* gene encodes an orthologue (coined euAP2a) of the *Arabidopsis* APETALA2 (AP2) protein, a founding member of the AP2-like transcription factor family. Both *Arabidopsis* AP2 and Prupe.6G242400/euAP2a belong to the euAP2 lineage within the family, which have miR172 binding sites within their transcripts [6]. This binding site enables negative regulation of *AP2* by miR172, either through translational repression or posttranscriptional cleavage [7, 8]. AP2 functions as both a transcriptional activator and repressor to regulate an array of genes promoting sepal and petal identity and repressing the transition from vegetative to inflorescent meristem [7–9]. The loss-of-function mutants of *Arabidopsis ap2*, together with mutations in other functionally redundant genes, show sepal-to-carpel and petal-to-stamen transformations, as well as early flowering. However, plants with *AP2* gain-of-function mutations, resulting from the loss of the miR172 binding sequence that usually inhibits translation or cleaves *AP2* mRNA, produce more petals and flower later [7, 8]. This phenotypic response has been observed in various plant species, including tobacco [3, 5, 8], *Dianthus*, petunia, and rose plants [10]. It is evident that *euAP2 genes* are mechanistically and functionally conserved across a wide range of plants. In line with this conservedness, the loss of *miR172* binding sites, resulting from deletion of exon 10 of *Prupe.6G242400* in two peach accessions, leads to similar floral organ proliferation and late-flowering phenotypes [3–5], suggesting that miR172-*euAP2a* 'module play similar regulatory roles in peach'.

Although *euAP2* genes suppress flowering in general, they target distinct flower development stages in *Arabidopsis* and peach. *euAP2a* in peach represses the developmental pace of floral buds, as opposed to its counterparts in annuals that inhibit the transition from vegetative to inflorescence meristem (Fig. 1), suggesting that it is mechanistically distinct, as further evidenced by transcription programming analyses of peach floral buds [4]. As part of a dynamic transcriptional program, two co-expression modules encompassing >300 genes are activated during the chilling period, followed by the sequential activation of four modules during the subsequent warm period, ultimately leading to flowering. m These modules are, however, suppressed during chilling and delayed during subsequent warm/blooming periods in the *LFP* buds. The data suggest that *euAP2a* negatively regulates these chilling- and warm-dependent transcriptional programming events (Fig. 1), and the gain-of-function mutation of *euAP2a* in *LFP* reinforces this negative regulation, resulting in a significant delay in flowering. Interestingly, the activation of these co-expression modules is accompanied by a transient downregulation of *euAP2a* levels in 'wild-type' floral buds, but this inverse responsive dynamic disappears in the *LFP* flower buds when miR172 binding sites are absent [4], suggesting the role of miR172 binding in fine-tuning thermal-responsive transcriptional programming. Thus, euAP2a likely relays miR172-mediated regulatory inputs from external thermal signals to transcriptional programming orchestrating floral developmental pace, a mechanism that appears to be absent in annual plants (Fig. 1). Yet, it remains unclear whether miR172 is regulated by or responds to external thermal conditions. Nevertheless, current studies reveal that *euAP2a* controls flower development in a miR172-dependent manner, paving the way for the identification of potential upstream regulators, downstream effectors, and parallel regulators that specifically regulate flowering time independent of floral organs.

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