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VIEWPOINT

Molecular control of masting: an introduction to an epigenetic summer memory

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• **Background** Mast flowering ('masting') is characterized by mass synchronized flowering at irregular intervals in populations of perennial plants over a wide geographical area, resulting in irregular high seed production. While masting is a global phenomenon, it is particularly prevalent in the alpine flora of New Zealand. Increases in global temperature may alter the masting pattern, affecting wider communities with a potential impact on plant–pollinator interactions, seed set and food availability for seed-consuming species.
• **Scope** This review summarizes an ecological temperature model (ΔT) that is being used to predict the intensity of a masting season. We introduce current molecular studies on flowering and the concept of an 'epigenetic summer memory' as a driver of mast flowering. We propose a hypothetical model based on temperature-associated epigenetic modifications of the floral integrator genes *FLOWERING LOCUS T*, *FLOWERING LOCUS C* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*.
• **Conclusions** Genome-wide transcriptomic and targeted gene expression analyses are needed to establish the developmental and physiological processes associated with masting. Such analyses may identify changes in gene expression that can be used to predict the intensity of a forthcoming masting season, as well as to determine the extent to which climate change will influence the mass synchronized flowering of masting species, with downstream impacts on their associated communities.

Key words: Epigenetic, floral integrator genes, ambient temperature pathway, masting, mast flowering, perennial plant, ΔT model of mast flowering.

INTRODUCTION

Mast flowering, or masting, which is synchronized highly variable flowering and seed production by perennial plants, is a well-researched phenomenon at the ecological level (Kelly, 1994; Pearse *et al.*, 2016). How this is regulated at the molecular level is not understood. In this review, we introduce masting and the predictive temperature model (Kelly *et al.*, 2013). We briefly introduce the molecular pathways to flowering, particularly focusing on epigenetic control of flowering. We also highlight the limited knowledge of these pathways in mast flowering plants. Finally, we introduce the hypothesis of an 'epigenetic summer memory' as the mechanism underpinning mast flowering and present a model based on temperature-associated epigenetic modifications of *FLOWERING LOCUS T* (*FT*), encoding the flowering hormone.

MAST FLOWERING AND SEED PRODUCTION

Masting is synchronized irregular seed production by a perennial plant population spread across a wide geographical area (Fig. 1). In a high seed year (mast year), a species undergoes heavy flowering and sets a large number of seeds; in other years (non-mast years), plants have moderate, low or zero flowering (Kelly, 1994; Kelly *et al.*, 2008; Pearse *et al.*, 2016). Masting allows a plant population to synchronize its flowering to achieve greater reproductive efficiency (Kelly and Sork, 2002). Synchronous flowering over a large area extending to hundreds or thousands of kilometres prevents the aggregation of seed consumers over a local area. Furthermore, the consumers are starved during a non-mast year and satiated with food during a mast year, leading to fluctuations in the population of the consumers over a period of time (Kelly *et al.*, 2000). Additionally, if seed consumers firmly favour a specific fruit or seeds of a species, then less favoured species may escape seed predation by masting in synchrony with the favoured groups (Kelly and Sork, 2002).

The phenomenon of masting is particularly observed in long-lived plant populations, predominantly in woody and wind-pollinated species (Herrera *et al.*, 1998). Plants belonging to 37 different families have been shown to exhibit masting patterns, with the Pinaceae being the most studied plant family (Kelly and Sork, 2002). Much of the alpine flora of New Zealand exhibits strong masting behaviour (Kelly and Sork, 2002), providing a pulse of produce for native invertebrates and birds (Norton and Kelly, 1988).

The evolution of mast flowering in a long-lived plant species is a result of the interaction between various functional constraints at the population level and evolutionary selective forces

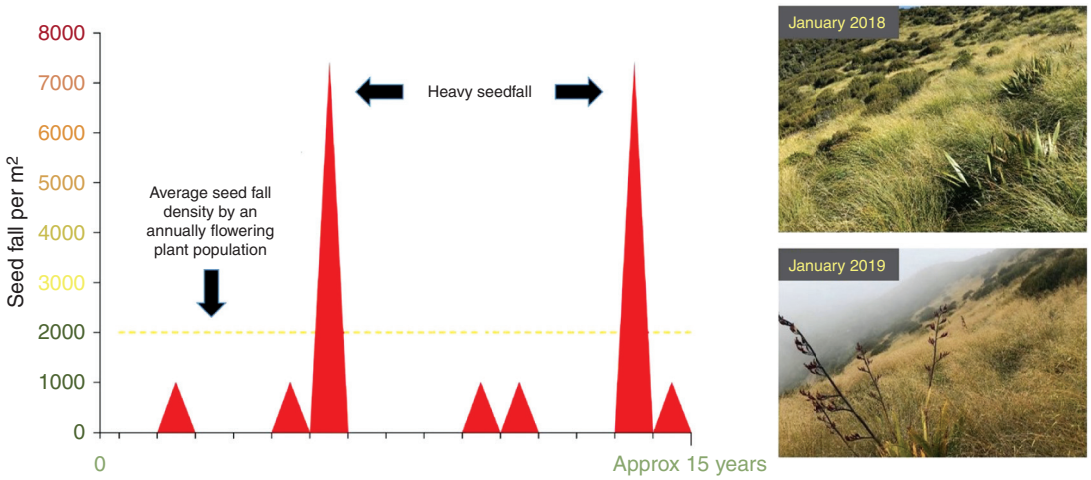

FIG. 1. On the left-hand side, the graph illustrates the flowering pattern of a masting plant population. A masting plant flowers heavily at irregular intervals but synchronized within the population. The plant population remains vegetative for most of its life and flowers only when the inductive signals are perceived. On the right-hand side, masting in *Chionochloa pallens* can be seen. These plants are present at 1070 m on Mt Hutt in Canterbury, New Zealand.

acting at the trophic level driving the ultimate cause of masting in plants, including predator satiation and higher pollination efficiency (Tachiki and Iwasa, 2012; Pearse *et al.*, 2016). Masting, as a reproductive strategy, is also interesting because plants delay reproduction during what would otherwise appear to be favourable conditions which results in a more density-dependent mortality (Kelly, 1994). Individually, each plant ought to suffer higher rates of intraspecific competition and seed predation or other forms of biotic attack (Kitzberger *et al.*, 2007). Moreover, the cost of heavy flowering as a reproductive strategy is very high. The event can force a plant to exhaust its resources, and reduce future vegetative growth (Kelly and Sork, 2002; Sala *et al.*, 2012) or produce non-viable seeds in the next season (Allen *et al.*, 2014). However, individual plants can increase their reproductive productivity by synchronizing their reproductive timing with the timing of reproduction in other plants of the same species (Kelly, 1994). This increases the chances of survival of the offspring and decreases the cost for each surviving offspring (Kelly and Sork, 2002).

To be advantageous for a masting plant, both high individual variability (i.e. the individual flowers at irregular intervals) and high synchrony among these individuals (each individual flowering at the same time) is required (Koenig *et al.*, 2003). Many hypotheses have been proposed to account for the masting phenomenon. These include predator satiation, wind pollination, environmental prediction, animal dispersal and weather cues (Pearse *et al.*, 2016).

While resources are often considered an important driver of masting (Smaill *et al.*, 2011; Miyazaki *et al.*, 2014; Koenig *et al.*, 2015; Monks *et al.*, 2016; Bogdziewicz *et al.*, 2018; Satake *et al.*, 2019), in terms of predictive models, change in temperature has been considered the most likely cue for masting in a number of plant species (Mark, 1968; Kelly *et al.*, 2008; Pearse *et al.*, 2016). Temperature has the advantage of being broadly uniform over large spatial areas (Kelly *et al.*, 2008). The generation of a high seed crop has been reported to be positively correlated with warm temperatures of the previous growing season (T_{n-1} model) in many New Zealand plant species (Schauber *et al.*, 2002). However, the warm temperature cue has some complications when applied to explain masting behaviour. First, statistical models based on warm temperatures of a previous year have been shown not to be completely effective in terms of predicting an upcoming masting year when simulations are run over several decades. Secondly, a warm temperature season alone cannot explain why the plants do not mast during two consecutive warm years. Thirdly, it is not at all clear how the plants are able to tailor their responses to mast synchronously over a large alpine area at diverse altitudes (Pearse *et al.*, 2016).

Since the warm temperatures of the preceding year do not explain the flowering anomaly effectively, Kelly *et al.* (2013) proposed the ΔT model. The ΔT model states that mast flowering is induced when plants experience a positive differential mean summer temperature, i.e. the temperature difference between the previous summer and the summer before that (Kelly *et al.*, 2013). Mathematically, it is expressed as:

$$\Delta T = T_{n-1} - T_{n-2}$$

here, ΔT = the change in mean summer temperature over two previous summer seasons, T_{n-1} = the mean summer temperature in the previous year and T_{n-2} = the mean summer temperature 2 years preceding the current season.

Kelly *et al.* (2013) studied 15 different species belonging to five different families over 30 years and showed that the ΔT model better predicted seed fall compared with the absolute temperatures in the previous season. The T_{n-1} model displays a proximal response to a high seed crop but is unable to show the variation in the flowering output during a low seed year, while the ΔT model significantly improves the fit for masting species including the variation in the reproductive output during both low and high seed years. The ΔT model has also been shown to be a better statistical predictor of seed crops than the previous year temperature alone for *Quercus lobata* (Pearse *et al.*, 2014), *Picea glauca* (Krebs *et al.*, 2017), *Cryptomeria japonica* (Kon and Saito, 2015), *Acer saccharum* and *Fagus grandifolia* (Cleavitt

and Fahey, 2017), although proving that ΔT is the underlying mechanism requires more than observational studies.

The ΔT model has several advantages over the T_{n-1} model. The model improved the best fit significantly when three decades of seed fall data were added compared with the T_{n-1} model (Kelly *et al.*, 2013). It also solves the conundrum of how plants at distinct altitudes are able to alter their threshold local mean temperature in order to flower synchronously. Further, it explains why the plants do not flower during two consecutive warm summer years as the second warm summer has a low temperature differential (Kelly *et al.*, 2013). The implication of ΔT is that masting plants may have a mechanism to sense and respond to two different, and temporally well separated, summer temperatures.

At the same time, an increase in the global mean temperatures may disturb the biological balance within a population, between species and at the ecosystem level where species function in a co-operative manner (Melillo *et al.*, 1993; Cao and Woodward, 1998) by altering the timing of reproduction (Franks *et al.*, 2007). With global climate change, the increase in variability in the weather conditions from year to year may lead to a greater variability in the ΔT values over the years, resulting in greater differences between high and low seed years (Kelly *et al.*, 2013).

Mechanistically, the ability of a perennial plant population to undergo masting using the ΔT cue over multiple years would require the presence of a plastic memory. We suggest that this is an 'epigenetic summer memory' which allows the plants to 'remember' the differential summer temperatures over successive years. Differential epigenetic marks on the flowering time genes, in response to the ΔT cue, may then explain how synchronized flowering is driven by the epigenetic summer memory. Consequently, it is crucial to understand the molecular expression pattern of flowering pathway genes to better understand the complexity of the putative epigenetic summer memory in masting plants.

MOLECULAR CONTROL OF FLOWERING

The developmental transition from a vegetative meristem to a reproductive meristem is regulated by various internal and external factors (Andres and Coupland, 2012; Romera-Branchat *et al.*, 2014). These factors act as input signals integrated into a feedback network regulating the timing of floral induction. Studies of flowering time control in *Arabidopsis thaliana* (arabidopsis) have revealed seven distinct pathways regulating the reproductive transition. These comprise the autonomous, photoperiod, vernalization, hormonal, age, sugar and ambient temperature pathways (Fig. 2). Detailed reviews of each of these pathways can be found in Andres and Coupland (2012), Khan *et al.* (2014), Cho *et al.* (2017) and Susila *et al.* (2018).

In arabidopsis, the molecular network regulating the transition from a vegetative meristem to a reproductive meristem converges on the floral integrator genes (He, 2009) which are at the epicentre of the flowering mechanism. These include *FT* (FT protein is regarded as the mobile 'florigen' (Liu *et al.*, 2016)), *TWIN SISTER OF FT* (*TSF*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), *AGAMOUS-LIKE 24* (*AGL-24*), *FRUITFULL* (*FUL*), *FLOWERING LOCUS C* (*FLC*), *SHORT VEGETATIVE PHASE* (*SVP*) and *LEAFY* (*LFY*) (Khan *et al.*, 2014; Bouche *et al.*, 2016) (Fig. 2). Once the expression of the integrator genes exceeds a threshold level (or is reduced below a certain level in the case of the repressor *FLC*), the transition from a vegetative meristem to an inflorescence or floral meristem is activated (Fig. 2) (Huijser and Schmid, 2011; Bouche *et al.*, 2016). The final control of the transition is by the floral meristem identity genes which encode transcription factors that are involved in the initiation of floral development in the shoot apical meristem and include *APETALA1* (*AP1*), *LFY*, *AGL-24* and *FUL* (Fig. 2).

AMBIENT TEMPERATURE PATHWAY

As warm summer temperatures promote mast flowering, it is important to note that several genes have been reported to induce flowering in plants via the activation of the transcription of floral promoter genes in response to warm ambient temperature (Song *et al.*, 2013; Capovilla *et al.*, 2015*a*; Susila *et al.*, 2018). *FT* can be silenced by the deposition of the H2A.Z histone variant in nucleosomes by the SWR1c at the transcriptional start site. However, with a rise in ambient temperature, the H2A.Z nucleosome is evicted, leaving the promoters of the floral integrator genes, including *FT*, more accessible to transcription factors such as PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) to bind and initiate the floral transition (Kumar *et al.*, 2012).

Additionally, *FCA*, encoding an RNA-binding protein, produces four distinct alternatively spliced transcripts, α, β, γ, and δ, in response to temperature change (Macknight *et al.*, 1997). Under ambient temperature control, only γ transcripts are produced which code for a mature full-length protein with a WW protein interaction domain. The full-length protein is able to repress the activity of *FLC* at ambient temperatures (22–27 °C) (Quesada *et al.*, 2003).

FLOWERING LOCUS M (FLM), a DNA-binding protein, interacts with SVP to repress flowering at low temperature. As the temperature increases, an alternative isoform of the FLM transcript is produced which is unable to bind to SVP and is thus incapable of inhibiting the expression of the floral transition genes (Capovilla *et al.*, 2015*b*). MADS AFFECTING FLOWERING2 (MAF2), a MADS-box transcription factor, ensures depression of flowering until a sufficient period of cold has been experienced by the plant. Airoldi *et al.* (2015) showed that at lower temperatures a splice variant of *MAF2*, *MAF2var1*, represses flowering by interacting with SVP. At high temperatures, another variant of *MAF2*, *MAF2var2*, is produced which is unable to bind to the SVP to form a repressor complex and thus the floral transition is induced (Airoldi *et al.*, 2015). If their function is conserved, these genes may have a role in the induction of flowering in masting plants by activating floral promoter genes in response to the summer temperature change.

EPIGENETIC REGULATION OF FLOWERING

Plants appear to have a plastic memory, enabling them to remember variable environmental conditions and seasonal changes. The memory is not only passed cell to cell after mitotic cell division during the growth of a plant, but can also be transgenerational (Murgia *et al.*, 2015). Plants can erase the memory in order to re-establish sensitivity to external

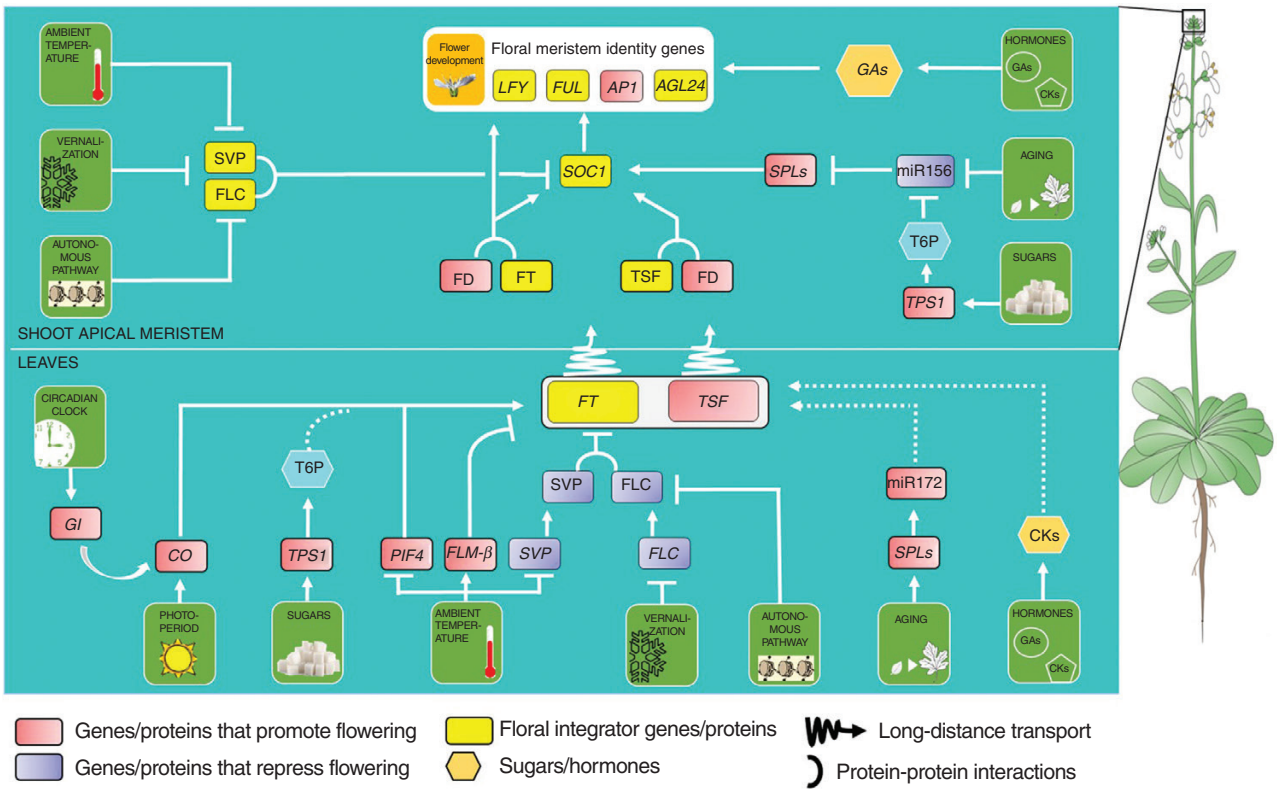

FIG. 2. Floral integrator pathway. FT is the mobile flowering hormone activating the transcription of the floral meristem identity genes which transform the shoot apical meristem into a floral meristem. The activation of flowering is regulated by the various pathways shown in the figure. These include photoperiod, vernalization, sugar, hormonal, ageing and thermosensory pathways. Under inductive conditions, *CONSTANS* (*CO*), encoding a zinc finger transcription factor protein, activates the transcription of *FT* by physically interacting with the promoter of *FT*. FT protein then travels via the vasculature to the shoot apical meristem to bind with FD and initiate the transcription of floral meristem identity genes. *GIGANTEA* (*GI*) is a photoperiodic activator of *CO*, which stabilizes the CO protein. FLC is a MADS-box transcription factor which physically interacts with SHORT VEGETATIVE PHASE (SVP) to form a floral repressor complex. SVP–FLC then physically interacts with *FD*, *FT* and *SOC1* to repress their expression. Prolonged cold exposure regulates *FLC* expression. Endogenous gibberellin induces the expression of *LEAFY*, a floral meristem identity gene. *MiR156* and *miR172* are found to have an antagonistic role in the vegetative to reproductive transition. During early development, *miR156* is highly abundant. With subsequent growth, the concentration of *miR156* decreases, and that of *miR172* increases. *MiRNA156* targets *SQUAMOSA BINDING PROTEIN LIKE* (*SPL*) genes (promoters of flowering) and downregulates their expression, whereas *miR172* negatively regulates the expression of *APETALA2* (*AP2*) family genes (repressors of flowering). Sugars, including sucrose, glucose and maltose, can act as endogenous signals to initiate the reproductive phase. *TREHALOSE PHOSPHATE SYNTHASE 1* (*TPS 1*) decreases the levels of *miR156* and promotes flowering by increasing the transcript levels of *SPL* genes. Under high temperature, PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) binds more strongly to the promoter of *FT* to activate its transcription. At low temperature, FLOWERING LOCUS M-β (FLM-β) binds to SVP to repress flowering. Adapted from www.flor-id.org (Bouche *et al.*, 2016).

conditions either in the next generation or, in the case of a polycarpic plant, in the same individual over the next reproductive cycle (Bossdorf *et al.*, 2008).

Active gene expression is often associated with histone modifications involving histone acetylation, histone 2B mono-ubiquitylation (H2Bub1), H3 lysine 36 di/trimethylation (H3K36-me/me2/me3) and histone H3 lysine 4 trimethylation (H3K4me3) (Xu *et al.*, 2008). These epigenetic marks of active gene transcription are rendered by a heterogeneous class of enzymes collectively known as the Trithorax group (TrxG) proteins (He, 2012). Inactive or inert gene expression is mediated by epigenetic marks associated with the histones H3K27me3, H3K9me and H2A mono-ubiquitylation (H2Aub1). The polycomb group (PcG) of proteins is responsible for depositing these epigenetic marks leading to the repression of a target gene at the transcriptional start site. These epigenetic modifications can be modulated by changes in temperature in response to fluctuating environmental conditions.

The most well studied epigenetically regulated plant gene is *FLC*, encoding a MADS-box transcription factor which acts as a floral repressor in the Brassicaceae (Mateos *et al.*, 2015). FLC physically interacts with SVP to form a floral repressor complex repressing the expression of *FT*, *FD* and *SOC1* (Mateos *et al.*, 2015). In arabidopsis, active expression of *FLC* is regulated by the ATX1 H3K4 methyltransferase and the EFS H3K36 methyltransferase to prevent precocious flowering (Xu *et al.*, 2016). After exposure to an extended period of cold, *FLC* expression is downregulated through chromatin remodelling by VERNALISATION INSENSITIVE 3 (VIN3) homeodomain finger protein (Sung and Amasino, 2004), co-transcriptional RNA processing through an antisense transcript, *COOLAIR*, generated from the 3'-downstream region of *FLC* (Liu *et al.*, 2010), and polycomb silencing through the PHD–PRC2 complex (Bastow *et al.*, 2004). Together, histone modifications including H3K9me3, H3K27me, H3K4 and H3K36 demethylation occurred at the *FLC* chromatin to maintain the

suppression of *FLC* after the winter (Bastow *et al.*, 2004), the so-called 'winter memory' (Bratzel and Turck, 2015).

FT expression in arabidopsis is also controlled by epigenetic marks induced by chromatin modifiers including SWR1c, PRC2, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), REF6 H3K27 demethylase and the PKDM7B (also known as AtJMJ4 or AtJMJ14) H3K4 demethylase (Xu *et al.*, 2016). The structure of the *FT* chromatin is bivalent, constituting active as well as repressive epigenetic marks (He and Amasino, 2005). The active epigenetic marks involve H3K4 trimethylation and repressive marks have H3K27 trimethylation. The balance between these epigenetic marks at the *FT* locus determines whether FT protein is produced (Jeong *et al.*, 2015). The expression of *FT* is repressed by PcG activity depositing H3K27 trimethylation in both long and short days. *REF6* demethylase removes the methylation at H3K27 to elevate the expression of *FT* in the vasculature under inductive conditions.

Reports suggest that PKDM7B binds to the *FT* chromatin and catalyses H3K4 demethylation to suppress *FT* expression. Loss of PKDM7B activity results in a decrease in H3K27 methylation marks and an increase in the H3K4 methylation marks, leading to FT protein production and early flowering (Jeong *et al.*, 2009).

Expression of *SOC1*, another key floral integrator gene, was found to be activated by MSI1 protein via deposition of active histone marks at the H3K4 position in response to elevated temperatures, allowing arabidopsis to undergo flowering without induction of *FT* (Bouveret *et al.*, 2006). As both *FT* and *SOC1* expression can be regulated by epigenetic modifiers in response to elevated temperature, either (or both) could be considered candidates for the 'summer memory'.

MOLECULAR REGULATION OF FLOWERING IN MASTING PLANTS

Masting is a complex phenomenon. The very nature of masting, with flowering synchronized even though the timing is highly irregular, acts as a barrier to the dissection of the relevant pathways. Flowering time is a quantitative trait associated with multiple signalling pathways (Fig. 2). There is a major gap in our molecular understanding of the masting syndrome, and relatively few researchers have attempted to demonstrate the role of flowering pathway genes in the regulation of flowering in masting plants.

For example, Kobayashi *et al.* (2013) identified homologous floral genes including *FT*, *SVP*, *SPL* and *FLC* in the tropical mast flowering tree *Shorea beccariana*. Differential expression of the flowering genes was shown in plants induced to flower by drought conditions. Most of the differentially expressed genes before the floral induction were induced by sucrose (Kobayashi *et al.*, 2013). Recently, cold spring temperatures along with drought served as the synchronizing cue for floral induction in individual trees of *S. curtisii* and *S. leprosula* (Yeoh *et al.*, 2017).

Miyazaki *et al.* (2014) identified the key floral identity genes, *FcAP1*, *FcFT* and *FcLFY*, from the masting plant *Fagus crenata* and showed a correlation between expression of these genes and the floral transition and initiation of flowering. They also demonstrated the effect of nitrogen availability on the reproductive transition by manipulating the nitrogen levels in the field. The plants treated with nitrogen showed a significant increase in the expression of *FcFT* along with a second round of flowering in the next year (Miyazaki *et al.*, 2014).

More recently, Satake *et al.* (2019) used a non-linear time-series analysis (CCM analysis) to study causative mechanisms for the induction of flowering in *F. crenata* in the field. The study suggested a synergistic non-linear relationship between nitrate accumulation and activation of the floral transition by *FcFT*, based on the statistical analysis. The non-linear causal relationship does suggest the presence of a complex activation mechanism operational in *F. crenata* to induce masting (Satake *et al.*, 2019). The approach used by Satake *et al.* (2019) is a powerful method enabling detection of gene regulatory networks and their causal relationship between environmental variables. A similar approach should help in the dissection of the molecular regulation of synchronized flowering and aid our understanding of masting phenology.

HYPOTHETICAL MECHANISTIC MODEL FOR MASTING PLANTS

Here we propose an 'epigenetic summer memory' which would allow differential temperature to be used as the actual mechanism whereby masting plants respond to environmental signals to induce masting (Fig. 3). In this model, we suggest that temperature is acting as an activator leading to changes in methylation patterns of the flowering gene(s) and thus controlling developmental changes (Fig. 3). We suggest that the 2 year summer temperature requirement is necessary for the plant to fully commit to undergoing the floral transition by modulating the expression of the flowering gene(s) through changes in the histone marks at the nucleosome level.

Several floral integrator genes including *FLC*, *SOC1* and *FT* have been shown to be regulated by chromatin modifiers which are active in both annual and perennial plant species (He, 2012). The activity of the chromatin modifier genes to deposit trimethylation marks at H3K27 or H3K4 nucleosomes (Jeong *et al.*, 2015) of the floral integrator gene(s) is also modulated by temperature. These marks are associated with either the activation (H3K4me3) or the repression (H3K27me3) of the flowering pathway genes.

The *FT* locus has a bivalent chromatin structure which may act as a regulator to control the expression of *FT* and, in turn, the induction of the floral transition (Adrian *et al.*, 2010; Verhage *et al.*, 2014). Additionally, most perennial plants have undergone individual duplication events leading to the generation of multiple homologues of floral promoter genes (Hsu *et al.*, 2011; Karlgren *et al.*, 2011). For example, due to the presence of multiple homologues of *FT* (either orthologues or paralogues), the temperature requirement after one warm year may not be enough to efficiently activate the floral transition. The temperature of the next growing season may then additively provide sufficient signals for deposition of more trimethylation marks at H3K4/H3K36 nucleosomes, leading to the elevated expression of *FT* or *FT-like* genes and, thus, activation of the floral meristem genes.

In addition to *FT*, *FLC* is known to be epigenetically regulated. Vernalization-mediated repression of *FLC* is known as

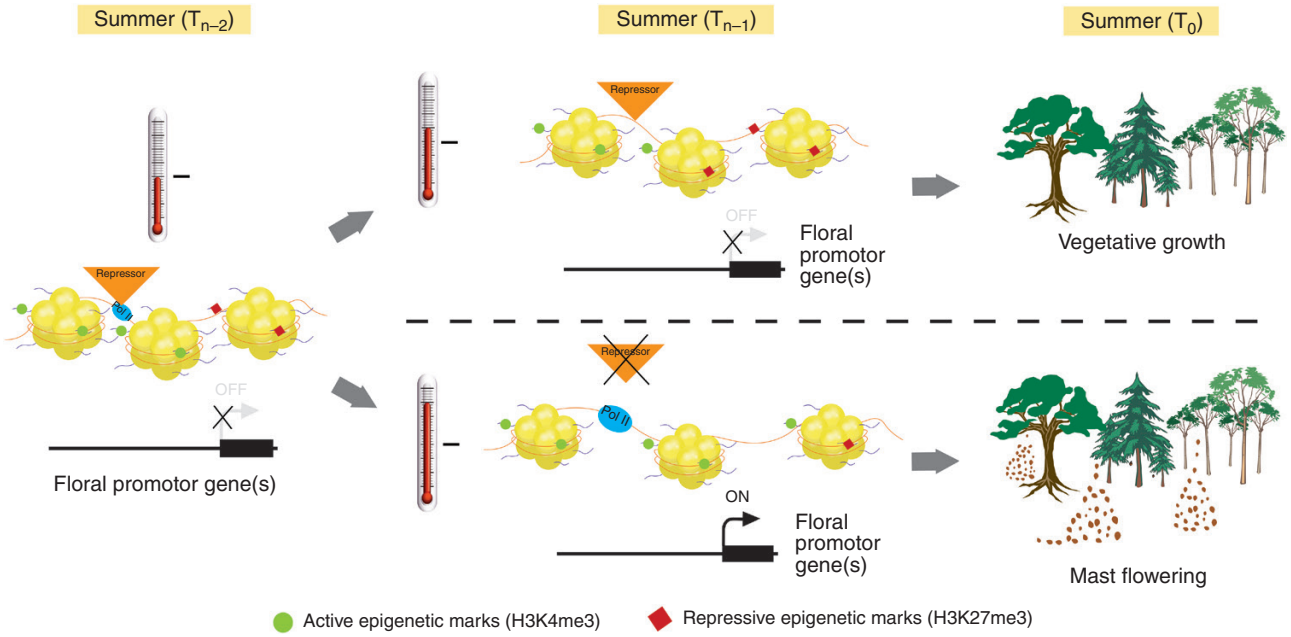

FIG. 3. Hypothetical mechanistic model responsible for imparting 'summer memory' in masting plants. The example given shows how the bivalent chromatin structure of *FT*, or *FT-like*, genes may be responsible for induction of flowering in response to the differential temperature cue. The balance between activating and repressive trimethylation marks determines whether *FT* is transcribed. The summer temperature of the T_{n-2} year may initiate the activation of the floral integrator genes such as *FT* and *SOC1*. However, an additional year of elevated summer temperature (T_{n-1}) is required to provide sufficient activation of these genes to allow the plant to fully commit to the reproductive transition. If the T_{n-1} summer temperature is not sufficiently elevated, the balance of repressive and activating marks is in favour of no or limited flowering. In addition, if the summer temperatures of the T_{n-1} year are elevated, suppression of floral repressors may also occur, thereby releasing *FT* and *SOC1* to be expressed.

the 'winter memory' (Satake and Iwasa, 2012). *FLC-like* repressors could also be critical in regulating masting in response to the ΔT cue. Elevated summer temperatures in the year preceding flowering (T_{n-1}) could activate the epigenetic modifiers to deposit repressive histone marks at the nucleosomes of the *FLC-like* repressor at H3K9/H3K27 loci. This will result in the suppression of *FLC* (or *FLC-like* repressors) which could then be maintained until the following year when the plant proceeds to flower. Consequently, this may further elevate the expression of the floral promoter genes such as *FT* or *SOC1*. Overall, we suggest it is the balance between activating epigenetic marks and repressive epigenetic marks on both promoters and repressors of flowering in response to the summer temperatures over 2 years which determines mast flowering.

This molecular network, being common across many species, where activation of floral integrator genes subsequently activates the floral meristem genes to initiate the floral transition, could provide for the strong synchrony of flowering observed during mast flowering years.

CONCLUSION

Even though various factors have been shown to be correlated with masting, few studies have probed the causative mechanism behind this mode of irregular but synchronized reproduction. Moreover, with the current rate of increase in global temperature, there is uncertainty about whether progressive warming could make masting stronger (McKone *et al.*, 1998; Pearse *et al.*, 2017) or weaker (Rees *et al.*, 2002; Koenig *et al.*, 2015), with downstream effects on higher rates of seed predation (Bogdziewicz *et al.*, 2020). Molecular studies have the potential to be used to forecast changes in flowering behaviour and to provide an understanding of how changes in natural conditions may lead to adaptation of flowering time genes under a changing global climate. To gain a better understanding of the mechanisms underpinning masting requires a critical evaluation and analysis of the molecular flowering pathway operational in masting plants.

An approach utilizing a combination of ecological transcriptomics and ecological epigenetics may allow us to dissect the flowering mechanism in masting plants, including those plants where we lack genomic information. Identification of potential floral-promoting orthologues which may respond to inductive summer temperatures, such as *FT*, *SOC1* and *LFY*, and floral repressors, similar to *FLC*, and their regulation under complex environmental situations, where conditions are constantly fluctuating, may then be correlated with the onset of flowering in masting plants. Epigenetic approaches such as bisulfite sequencing and methylation-sensitive amplified fragment length polymorphism (AFLP; MSAP) have shown the potential for the identification of trait-associated methylation patterns in plant species without a reference genome (Richards *et al.*, 2017). Similar approaches, including chromatin immunoprecipitation sequencing, can now be used to characterize the 'epigenetic summer memory' and the activation of flowering in response to the ΔT cue. Such research will shed light on the evolution of this novel mechanism of flowering time control in masting plants.

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