

## Flowering Control Mechanisms in Plants and Its Importance in Crop Production and Breeding

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### ABSTRACT

*Flowering time is a major life-history trait that contributes to fitness in annual plants. Depending on abiotic viz., photoperiod, temperature, nutrients and biotic viz., competition, pollinators, herbivores conditions and different flowering time strategies can be adopted by different plant species. Floral induction is a key developmental switch in plants that leads to the production of flowers, fruits and seeds to meet the demands of several crop harvests per year, or the growth of crop plants in regions with short vegetation times and for the production of ornamental plants, the timing of the floral transition plays very important role. The discovery of genes that are involved in flowering time control in model plants such as Arabidopsis should allow the modulation of this developmental switch also in plants with economic value. Genetic and molecular understanding of floral transition opens new horizons for plant breeding. However, many of the current examples are restricted only to a few key regulators, although our increasing knowledge of regulatory pathways might also help to adjust flowering time in response to specific environmental challenges and to avoid unwanted pleiotropic effects. Conflicting breeding goals, such as high biomass yield and efficient seed production can be approached by new strategies.*

**Key words:** Flowering, Arabidopsis, Vernalization, Photoperiodism

### INTRODUCTION

The flowering plants (angiosperms) go through a phase of vegetative growth and flowering phase where they produce the organs for sexual reproduction. There are three types of plants based on completion of life cycle. Annuals, vegetative phase begins with germination of the seed then flowering and it ends with the senescence and death of

the plant. In biennials, the vegetative phase takes up the first year and flowering followed by death occurs in the second year and in perennials flowering typically occurs year after year when conditions are appropriate. Flowering involves the conversion of the apical meristem into a floral meristem from which all the parts of the flower will be produced<sup>13</sup>.

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Signals that change the fate of the apical meristem are maturity of the plant, temperature, production of gibberellin hormones and photoperiod<sup>7</sup>.

### **The importance of flowering time for crop production**

There is widespread concern that we are approaching the yield barriers of many crop species, thus necessitating new technologies and visions for crop improvement, both to safeguard food supply and to satisfy increasing demands for plant biomass as a source of renewable energy. For generative and vegetative crops alike, the phenological development of a plant is highly important in crop production. The timing of flowering for plant's requirement and responsiveness to vernalization are major factors in regional climatic adaptation of elite germplasm. Genes that control flowering time affect hybrid vigor and thus are likely to impact on yield. In all seed crops, floral transition is a key developmental switch that determines the production of dry matter. Examples in vegetative crops like cabbage (*Brassica oleracea* L.) and sugar beet (*Beta vulgaris* L.) early bolting and flowering can limit the potential for yield increases or interfere with harvest operations<sup>8</sup>. In many trees and other perennials, breeding progress is severely hampered by the late onset of flowering.

### **Pathways controlling flowering time in model plant *Arabidopsis***

#### **Flowering time control in model species:**

In *Arabidopsis*, many of the key genes have been identified and functionally characterized. Mutant analyses under various environmental conditions, genetic and molecular interaction studies allowed many of the genes involved to be assigned to distinct regulatory pathways, which are generally referred to by the exogenous or endogenous cues that they respond to the vernalization, photoperiod, gibberellin and autonomous pathways. These pathways converge to regulate a set of floral integrator genes that integrate the outputs of the various pathways and under favorable conditions, directly activate floral meristem identity genes<sup>9</sup>.

#### **Vernalization:**

To ensure flowering in spring or summer, plants in temperate climates have evolved a signal perception and transduction pathway that senses prolonged periods of cold during winter and translates this environmental cue into an increased competence to flower, a process known as Vernalization. Vernalization requirement and response possess two intriguing features are (1) Temporal separation between the plant's exposure to cold in winter and the onset of flowering in spring or summer. (2) The renewed Vernalization requirement for flowering in subsequent generations.

#### **Vernalization regulates flowering in plants**

A gene *Flowering Locus C* (FLC) encodes a transcription factor that blocks the expression of the genes needed for flowering. The level of FLC mRNA is high in the fall. But with the onset of cold temperatures, production of an antisense transcript of *FLC* (called *COOL AIR*) increases as does later, a sense transcript of part of the *FLC* gene<sup>11</sup>. Both of these RNAs are non-coding; that are not translated into protein. But they cooperate in suppressing the production of FLC mRNA and its translation into FLC protein. With the arrival of spring, there is no FLC protein remaining to suppress flowering so flowering can begin<sup>8</sup>.

#### **The expression of FLC itself is tightly controlled by a plethora of both positive and negative regulators**

The principal activator of FLC is FRI (FRIGIDA) which accounts for much of the natural variation in vernalization requirement among *Arabidopsis* accessions. Activation of FLC by FRI and FRI-like genes is dominant over FLC repression by autonomous pathway genes, a largely developmentally controlled group of negative regulators of FLC but can be overcome by vernalization. The response to vernalization is facilitated by a cascade of gene regulatory processes that is initiated by induction and gradual up regulation during prolonged cold exposure of the plant homeodomain finger gene VIN3 (Vernalization insensitive 3) and results in

chromatin-based and mitotically stable repression of FLC. In the following generation, FLC expression is reset around the time of early embryogenesis thus ensuring a renewed requirement for vernalization<sup>15</sup>.

#### **Photoperiodism:**

Many angiosperms flower at about the same time every year. This occurs even though they may have started growing at different times. Their flowering is a response to the changing length of day and night as the season progresses. The phenomenon is called photoperiodism. Some plants like Spinach, Arabidopsis, Sugar beet and Radish flower only after exposure to long days and hence are called long-day plants. Some other plants like chrysanthemums, rice, Poinsettias, Morning glory and cocklebur (*Xanthium*) flower when exposed to short day conditions *i.e.*, long night condition and hence they are called short day plants<sup>11</sup>. The site of perception of the photoperiodic stimulus are leaves. The leaves produce a chemical signal called florigen, which is transmitted to the apical meristems to start their conversion into floral meristems.

#### **Applications of flowering control in plant breeding**

##### **Lessons from natural Variation**

Although genetic and molecular analyses have linked numerous genes in Arabidopsis to the regulation of floral transition, only a relatively small number of genes was shown to contribute to natural variation in flowering time, including genes controlling vernalization (e.g. FLC, FRI and VIN3) and photoperiodic responses (CO and several photoreceptors, including phytochromes and the cryptochrome CRY2)<sup>6</sup>. Besides mutant phenotype analyses, the occurrence of allelic variants associated in natural populations with a given trait, such as flowering time, is arguably another good indicator of genes or specific alleles, which can be selected for or modified to alter flowering time without significant negative effects on general fitness and yield. In cereals: Natural allelic variation was exploited from early on during domestication and selective breeding for flowering time-*VRN1-3*, *Ppd1*<sup>1</sup>.

#### **Flowering time and selection for adapted breeding material**

Selection for flowering time traits in the past was based exclusively on phenotypic characteristics and relied on natural variation present in the primary and secondary gene pool of a crop species. Now that many of the floral regulatory genes have been identified, their sequences can be used by breeders as functional markers for selecting favorable genotypes, for quality control of seed lots or for targeted manipulation of flowering traits by genetic modification<sup>5</sup>.

#### **Seed yield:**

The timing of flowering is a major determinant of seed yield and varies depending on the local environment and crop production system. In cereals, flowering should be as early as possible to extend the corn filling phase, to avoid harsh environmental conditions which endanger seed production or harvest (e.g. drought, heat, frost) or to escape pathogen attack<sup>12</sup>. By contrast, delayed flowering might be desirable to realize high yields in biomass for energy production. Current examples of strategies for increased seed yield potential in cereals include the use of both Vernalization and photoperiod pathway genes.

Some of the Examples are:

In barley, two major genes affecting flowering time, *Vrn-H1* and *Ppd-H1* (Photoperiod-H1), have a significant effect on several agronomic traits, including yield. *Ppd-H1*, a homolog of the circadian clock and photoperiod pathway gene *PRR7* in Arabidopsis, is the major determinant of long-day response in barley and possesses photoperiod-responsive and non-responsive alleles. The importance of flowering time for the yield potential of barley was also demonstrated by Aqtl analysis which showed that two QTL for grain yield under Mediterranean growing conditions colocalize with the positions of two major QTL for heading date<sup>3</sup>. In rice, much of the phenotypic variation in flowering time can be explained by allelic variation of the CO ortholog *Hd1*. Another photoperiod pathway gene in rice, *Ghd7*, colocalizes with a QTL for major effects on an array of traits, including plant

height, heading date and number of grains per panicle. Selection for phenotypic effects that Ghd7 is causally involved which has helped increasing productivity and adaptability of rice in traditional breeding programs<sup>16</sup>.

### **Biomass yield**

Maize is used for seed production and as a forage crop. Under northern European and northern American growth conditions, only early-flowering varieties are cultivated due to the short period with favorable temperatures for this tropical species. The recent demand for bioenergy, however, created new breeding goals for maize as an energy plant which is fermented to methane. Energy maize should produce as much biomass as possible and full seed development might best be avoided. This can be achieved by extending the vegetative phase and shifting the onset of flowering towards the end of the vegetation period<sup>4,14</sup>.

One sophisticated approach is to introduce tropical maize accessions (e.g. from Peru) which are adapted to short days. Under central European conditions, these accessions flower late and do not complete ripening. The major flowering time QTL in maize, Vgt1 (Vegetative to generative transition 1), was cloned recently and found to carry a cis-regulatory element of the floral repressor gene ZmRap2.7, which is located 70 kb downstream of Vgt1. The allele distribution at Vgt1 is highly correlated with geographical origin, suggesting that Vgt1 markers are suitable tools to assist gene introgression from short-day genotypes. New allelic variants for late flowering are presently identified from exotic maize accessions and combined with other desired traits, such as cold tolerance. The resulting recombinant types are expected to almost double the fresh mass yield when compared with conventional forage maize varieties.

### **Stress avoidance:**

Avoiding stress in new cultivation areas and under changing climate conditions by selecting genotypes with well adapted flowering times is another challenge for plant breeders worldwide. The timing of flowering often correlates with abiotic and biotic stress

avoidance, which is frequently scored as a component of yield, for example in maize<sup>2</sup>. In addition, QTL for adaptation to drought, which is a serious problem not only in arid and semi-arid regions, are often related to flowering time loci. In maize grown in temperate areas, drought stress occurs just before and during the flowering period.

### **Novel strategies for crop improvement by altering flowering time regulation**

To increase genetic variation for flowering time beyond the natural variation available from the primary and secondary gene pool of a plant species, two major strategies can be followed.

#### **1. New alleles can be identified after mutagenesis by TILLING**

As many flowering time genes have been cloned, mutations can be selected by their genotype rather than the traditionally applied phenotypic selection. Moreover, two or more mutations at different flowering time loci can be combined by crossing, and plants with novel flowering time behavior can be selected. For example, if flowering time repressors behave in an additive or synergistic manner, two mutant plants with delayed flowering phenotypes can be produce a non-flowering hybrid, a strategy followed in our laboratory to breed winter beets<sup>10</sup>.

(a) A simple model for suppression of flowering by transformation (red arrow) with floral repressor genes or by RNAi (orange arrow) or mutation (green arrow) of floral activator genes. If successful, transformants and mutants will not flower even when subjected to prolonged low temperature conditions.

(b) Non-flowering transformants or mutants might be induced to flower by transformation with a floral activator gene under the control of an inducible promoter (Tind), or alternatively by transformation with an inducible RNAi transgene targeting a floral repressor. Induction of the transgene would allow for flowering, breeding and seed production, whereas induction is not required and would have to be avoided for crop production.

(c) In standard biennial or perennial cultivars, flowering might be suppressed under controlled conditions by transformation with a floral repressor gene under the control of a repressible promoter (Trep), or alternatively by transformation with a repressible RNAi transgene targeting a floral activator.

(d) Synergistic effect model for the production of non-flowering genotypes in hybrid crops. Each of the hybrid parents is genetically modified by transformation or mutation to carry different dominant inhibitors of floral transition (R and S, respectively). R and/or S might also correspond to transgenic RNAi loci that suppress positive regulators of floral transition. In this model, the presence of a single inhibitory locus is not sufficient to abolish flowering, thus allowing production of seeds from the parents. Complete inhibition requires the presence of the dominant alleles at both loci in the hybrid progeny.

## 2. Targeted Genetic Modification through Transformation:

Novel flowering time characteristics can be generated through targeted genetic modification by transformation. Current knowledge of flowering time control has been exploited through either overexpression or suppression of gene activity. The target of genetic modification depends on the cropping system and reproduction type of the respective species. Suitable phenotypes might also be generated by selecting and effectively combining mutant and transgenic genotypes (as well as natural variants), for example for hybrid crop production<sup>7</sup>.

## CONCLUSION

Since flowering time is controlled by both temperature and photoperiod, as temperature changes, there will be changes in the flowering time of the plants. By knowing the temperature which alters the flowering time, we can modulate the pathway involved in flowering time control through mutation or genetic transformation. Genetic and molecular understanding of floral transition opens new horizons for plant breeding. Development of transgenic plants with controlled flowering

behavior opens the door for alternative production systems. Conflicting breeding goals, such as high biomass yield and efficient seed production, can be approached by new strategies.

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