A Review of Plants' Flowering Physiology: The Control of Floral Induction by Juvenility, Temperature and Photoperiod in Annual and Ornamental Crops

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ABSTRACT— ***Recent market demands have led to extended cropping seasons and off-season production of various plant species, especially ornamental plants. Understanding of genetic control of flowering, developmental stages and environmental requirements of crops is important to develop appropriate production guidelines and novel varieties. This paper presents a review of some main factors, including juvenility, vernalization, photoperiod and their interactions in control of flowering in plants. Length of juvenile stage may vary among species. Understanding of juvenility may help define appropriate time when they can perceive external stimuli for efficient flower induction and subsequent development. Based on the requirements and responses to temperature and photoperiods for floral initiation, plant species are categorized into different groups. Modifications of temperature range and/or vernalization, duration and regimes in accordance with day-lengths at receptive stage would be important techniques to produce flowers at a desired time of the year. In addition, genetic modifications applied in breeding together with some cultivation techniques to regulate flowering time in some crops are also discussed in this paper.***

Keywords - Flowering, juvenility, photoperiod, temperature, vernalization.

1. INTRODUCTION

Plants experience a number of developmental phases during their life cycles. The transitions start from germination to juvenile vegetative stage, at which plants are insensible to environmental inductive signals. It is followed by an adult vegetative phase where plants are receptive to external inductive cues for flowering. The shift to reproductive stage is marked by a transition to flowering (Bäurle & Dean, 2006). Flower induction is a phase change from vegetative to floral production (McDonald & Kwong, 2005). Flowering commences from "floral induction signal" which induces "floral evocation". This is followed by "floral initiation", "flower development" and eventually "anthesis" (Hopkins & Huner, 2004; McDonald & Kwong, 2005).

Flowering is an important step in a plant cycle which shows adaptability of plants to seasonal changes and decides subsequent reproductive success (Bäurle & Dean, 2006; Kim *et al.*, 2009). There are several reasons for timing of flowering among plant species. Plants that require cross pollination need to regulate flowering synchronously with other individuals within the same species and/or with presence of pollinators. For many species, flowering must occur at appropriate seasons for floral induction and following reproductive development (Kim *et al.*, 2009).

Floral development is controlled by both internal and external cues (Michaels & Amasino, 2000). Plants have developed sophisticated mechanisms with complex genetic network in regulation of flowering. Four flowering pathways have been determined as reviewed by various authors. Those include photoperiod, autonomous, vernalization and gibberellin-induced pathways (Bäurle & Dean, 2006; Lee & Lee, 2010; Sung & Amasino, 2004). There are common set of genes which define the signaling pathways in flowering among plant species (Putterill *et al.*, 2004), while photoperiod and vernalization and/or their interactions are reported as main external factors influencing flowering responses and behaviors (Jung & Müller, 2009; Kim *et al.*, 2009; Mouradov *et al.*, 2002; Searle & Coupland, 2004).

Manipulation of growing environments such as vernalization and day-lengths in accordance with the use of new technologies (e.g. genetic modifications, hybridization, grafting techniques, and plant growth regulators) have recently brought about successes in developing effective production guidelines and novel varieties for commercial production of many annual and horticultural crops (Wilkie *et al.*, 2008). This paper provides a review on some main factors in relation to flowering of plants, including juvenility, temperature, photoperiodism, and their interactions in controlling flowering.

2. JUVENILITY AND FLOWERING

A plant experiences different development phases in its life cycle, which can be classified as juvenile, adult vegetative (competent) and adult reproductive (determined). These changes can be observed by transition at the shoot apical meristem. The flowering occurs while the meristem is competent to flower (McDonald & Kwong, 2005).

Juvenility can be defined as an inability to initiate flowers regardless of receiving stimuli that induce mature plants to flower (Damann & Lyons, 1993; Fisher, 1999; Hare *et al.*, 2001; Pillitteri *et al.*, 2004). Juvenile stage varies among species. In annuals, this phase can only take several weeks or months, whereas, in perennial crops this can last for several years. For example, *Arabidopsis* has two months in its life cycle; therefore it undergoes a very short juvenile period, followed by a reproductive developmental phase. By contrast, a relatively long period of juvenility can be seen in citrus (5 - 13 years) and poplar trees (7 - 10 years) (Hsu *et al.*, 2006; Pillitteri *et al.*, 2004).

The transition from juvenile to reproductive phases takes place when a plant reach a certain age, at which the plants are capable of perceiving environmental signals such as vernalization, photoperiod or both to initiate flowers. Cineraria, for instance, will not be able to perceive chilling treatments (at 3 and 6°C) to flower until it reaches 6 - 7 leaves in cultivar 'Cindy Blue' and 7 - 8 leaves in 'Cindy Dark Red' cultivar (Yeh & Atherton, 1997). Similarly, celery can only perceive vernalization when it has at least 17 leaves (cultivar 'New Dwarf White') and 17 - 20 leaves (cultivar 'Celebrity') (Ramin & Atherton, 1991). Moreover, *Petunia hybrida* 'Fantasy' was reported to end its juvenility at 6-leaf stage when the plants are receptive to inductive photoperiods (Hu *et al.*, 2007). *Brunonia australis* is responsive to long day (16h) after 18-22 days from germination, while it is receptive to vernalization at the age of 4-35 days (Cave *et al.*, 2011). Yuan *et al.* (1998) postulated that leaf and node numbers are the commonly used to measure the juvenile phase and/or age in herbaceous plants since these indicators are more stable than other such as time. Some flower cultivars such as *Coreopsis grandiflora* (Hogg ex Sweet.), *Gaillardia grandiflora* (Van Houtte), *Heuchera sanguinea* (Engelm.) and *Rudbeckia fulgida* Ait.) were found to reach adult stages after having 8, 16, 19 and 10 nodes, respectively.

The long juvenile phase in some species might be a constraint in production and breeding practices. The long juvenile trait associated with high levels of Terminal Flowering (TFL) genes have been found in *Arabidopsis*, a herbaceous species, and some perennial crops such as citrus. TFL prevents floral development by blocking the expression and activities of LEAFY (LFY) and APETALA1 (AP1) (the two main genes stimulating flowering) in the central dome of shoot apexes (Pillitteri *et al.*, 2004). In addition, Flowering Locus T (FT) genes have been investigated to reduce juvenility in some crops such as *Arabidopsis*, gentian plant (Nakatsuka *et al.*, 2009) and poplar trees (Hsu *et al.*, 2006). For these reasons, gene transformation techniques could be performed to down-regulate TFL genes, while at the same time increase the level of FT to force flower initiation in a desired time of year. For example, transgenic ornamental gentian plants contain high level of FT gene, which encodes a major component protein of the flowering hormone "florigen", form flower buds after 4 months of transformation, while the normal plants initiate flowers after one year. In addition, grafting techniques can be used to eliminate the long juvenile trait in some species by grafting scions from mature trees onto juvenile stocks. These mature scions can be able to perceive chilling treatment to initiate flowers early (Malik & Bradford, 2004).

Besides, early manipulation of environmental conditions for flowering before the end of juvenile phase may induce poor flower quality and uniformity since plants require a certain period to accumulate plant mass for effective photosynthesis and thus floral development (Cave *et al.*, 2011; Cavins & Dole, 2001).

Overall, understanding of juvenility and inductive stimuli is important for manipulating crop environments to promote synchronous and effective flowering in commercial production.

3. TEMPERATURE AND FLOWERING

Temperature has a strong influence plants' flowering behavior (Ha & Johnston, 2013; Ha *et al.*, 2013; Hopkins & Huner, 2004). Each plant species or a cultivar requires a suitable temperature range for flower induction and development as illustrated in a study by King *et al.* (2008). Crowea cultivar 'White Star' produced 100 or more flowers per plant under 11°C in combination with high light exposure (700 µmol m^{-2} s^{-1}), while 81% of plants kept under the same lighting regime at 21°C remained vegetative. The remainder had only 1-2 flowers. In contrast, the *Crowea exalata* 'Bindelong Compact' did not require cool temperature. Moreover, temperature also affects the duration to floral development. *Arabidopsis thaliana* flowered within 31 days at 22°C while flowering was delayed until 63 days when the temperature was reduced to 14°C (Lokhande *et al.*, 2003). The results suggest that temperature affects both time and rate of flower development.

Cultivars within a species may have different flowering responses to temperature. Cultivars of *Pimelea ferruginea* (Thymelaeaceae) in cooler and southern latitudes (33-35°S) of Australia can flower at 12-15°C; however these cultivars

remain vegetative at 18°C. In contrast, northern cultivars (29-30°S latitude) flower at 18-21°C (King *et al.*, 1996). These physiological differences in flowering might reveal the ecotypic adaptation among different cultivars. Moreover, Warner & Erwin (2006) showed various responses to temperatures among 12 pansy cultivars (*Viola x wittrockiana*), although flower numbers and diameters of all cultivars decreased when the temperature was raised from 20 to 30°C, some cultivars were more heat-tolerant than other. For example, flower bud number of cultivar 'Crystal Bowl Purple' was reduced by only 20%, while that of 'Majestic Giants Red and Yellow' was reduced by 77%.

In addition, both high and low temperatures can inhibit floral development. For example, species *Hardenbergia violacea* requires a temperature range between 15 and 20°C for its flowering. Nonetheless, if the temperature is greater than 22°C, it will not flower, and the already formed flowers will abort (King *et al.*, 2008). In addition, temperature at 35°C delayed flowering of chrysanthemum for 30 days (Schwabe 1950, cited in Tanigawa *et al.*, 2009). According to Sharman *et al.* (1990), *Helipterum roseum* did not flower at 25°C, but the plants initiated flowers at 20°C. These authors found that the steady-state cell cycling was detected in the apical meristems under 20°C, but that was not found at 25°C. Meanwhile, low temperature might retard time to flowering in chrysanthemum. Ploeg *et al.* (2005) reported that flowering of all 25 chrysanthemum cultivars was delayed from 4 to 13 days at 16°C under short days (SD) (9h 30min.) compared to plants grown at 20°C under the same photoperiod. This result showed that 20°C would be more suitable for flowering of chrysanthemum since the optimum for growth and development of chrysanthemum was reported at a range of 17-22°C (Ploeg & Heuvelink, 2006).

3.1. Vernalization

Flowering in response to prolonged exposure to low temperature is a positive adaptation for some plant species that flower in spring season. This promotion is called vernalization (Eckardt, 2005; Finnegan *et al.*, 1998; Finnegan *et al.*, 2005; Ratcliffe *et al.*, 2003). The vernalization requirement occurs for both monocarpic species (the plants that flower, set seeds and then die) and polycarpic species (the perennials that can flower repeatedly over many years) (Amasino, 2005).

There are two types of vernalization responses, facultative and obligate (Finnegan *et al.*, 1998; McDonald & Kwong, 2005). Winter annuals, for instance, have facultative vernalization response; it means flowering does not require cold exposure; however flowering will occur more rapidly after cold treatment. By contrast, biennials have an obligate requirement for cold treatment which cannot flower without earlier cold exposure (Michaels & Amasino, 2000). Imbibed seeds and shoot apical meristems are reported to be the perceptive organs of a plant to vernalization (Hopkins & Huner, 2004; McDonald & Kwong, 2005).

In the absence of cold exposure, flowering is delayed or does not occur in plants that require vernalization. In this case, plants usually grow as rosettes (Taiz & Zaiger, 2006).

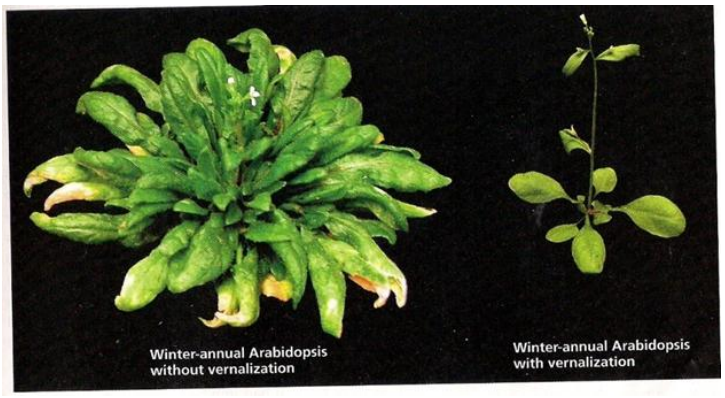

Figure 1. Vernalization induces flowering in the winter-annual types of *Arabidopsis thaliana*. The plant on the left is a winter-annual type that has not been exposed to cold. The plant on the right is a genetically identical winter-annual type that was exposed to 40 days under 4°C as a seedling. It flowered 3 weeks after the end of cold treatment with 9 leaves (Source: Taiz & Zaiger, 2006).

According to Hopkins and Huner (2009), plants that require vernalization are usually long-day (LD) flowering plants. Nonetheless, some biennials can flower in both LDs and SDs after vernalization. For instance, *Chrysanthemum morifolium* is a SD plant. Some Chrysanthemum cultivars response as quantitative SD plants after being exposed to cold treatment.

The effective range of temperature for vernalization of most plant species is 1-7°C. However, this may vary depending on species. For example, species of warm climatic zones such as olive, the optimum temperature for

vernalization is 13°C (Michaels & Amasino, 2000), whereas in Petkus rye (*Secale cereal*) the temperatures range from -5 to +15°C (Hopkins & Huner, 2009). In Australian native plant species, vernalization requirements ranges from 5°C to 20°C, in which some species have an obligate response such as *Acacia pycnantha, Boronia serulata* and *Pimelea ferruginea,* while other have facultative or quantitative response such as *Chamelaucium uncinatum, Crowea exalata* 'White Star', *Eucalyptus lansdowneana, Helichrysum cassinianum and Helipterum craspedioides* (King *et al.*, 1992).

Duration of vernalization is another aspect which influences flowering of plants (Ha & Johnston, 2013; Ha *et al.*, 2013). This duration varies widely from several days to several months. Flowering can be promoted after 8 days of exposure to low temperature in celery; however, for maximum acceleration of flowering this plant requires more than one month of vernalization (Thompson 1944, cited from Michaels & Amasino, 2000). In *Arabidopsis*, maximum response of flowering can be obtained when seedlings are exposed to 4°C for 6 weeks, meanwhile much lesser effect are resulted under 2 weeks of exposure to the same temperature (Samach & Coupland, 2000). Furthermore, *Pimelea ferruginea* obtained more than 8 flowers/plants after 7 weeks of vernalization at 15/10°C (day/night) compared to about 1 flowers/plants in 5 week cool treatment or no flower in non-vernalized plants at 24/19°C (King *et al.*, 1992). Moreover, a range of Australian native species, reviewed by King et al. (1992), required at least 30 days of exposure to cool temperature for flowering. Nonetheless, some species such as *Matthiola* 'Column Lavender' requires only a short spell of continuous cool temperature for 3 days for flower initiation (Emsweller & Borthwick, 1937), while, 21 days of chilling treatment at 20/10°C (day/night) were found to be sufficient for floral development of *Pycnosorus thompsonianus* (Ha *et al.*, 2013).

Horva´th *et al.* (2003) found that the 3-week vernalization treatment for winter wheat (*Triticum aestivum* L., cv. Martonva´sa´r 15) at 2°C resulted in 100% flowering after 56 days, whereas, the treatments at 1 and 2 weeks did not have this effect on floral initiation. The results show that a certain cold period is required for the plants to perceive vernalization induction leading to floral development of the cultivar. However, Ortiz-Ferrara *et al.* (1998) elucidated that there are differences in response to vernalization and the time to anthesis between different cultivars of wheat. This implies that, though in the same species, each cultivar has its own vernalization requirement. A similar result on different responses to cold treatment can be seen in two crowea cultivars, 'White Ctar' and 'Bendelong Compact'.

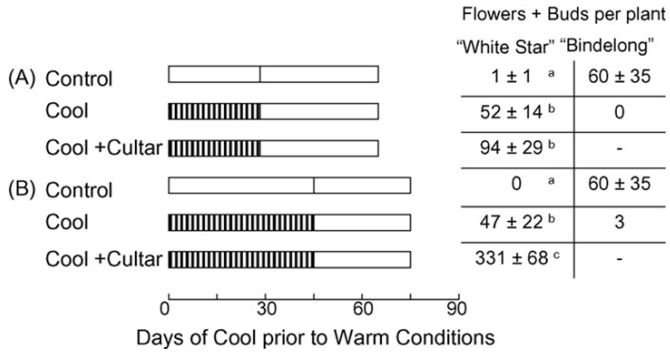

Figure 2. Flowering response of 2 cultivars of Crowea to cool temperatures at the time of shifting plants to high light intensity (10h photoperiod at 380 µmol m^{-2} s^{-1}). Cool treatment at 11°C; Control treatment and subsequent temperatures of cool treatments are at 18°C. The letters (a, b, c) indicate statistically significant differences between the treatments (Source: King *et al.*, 2008).

The period of 30-35 day vernalization seems to be suitable for the cultivar "White Star', while 'Bindelong Compact' flowered better in the absence of cold exposure. Interestingly, the duration and combination of vernalisation and a plant growth retardant (Cultar™ or Paclobutrazol) resulted in more flowers for cultivar 'White Star'. This suggests that in some cases, use of plant growth retardants (GA inhibitors) might help accelerate flower production in some cultivars and species (King *et al.*, 2008).

Furthermore, the rate of flowering can be influenced by duration of vernalization and temperature regime (Ha & Johnston, 2013). For example, two species of *Pimelea*, *P. rosea* and *P. ferruginea*, were treated with different cool temperatures for 7 weeks then transferred to 24/19°C. Days to flowering of *P. rosea* and *P. ferruginea* were 133 ± 1and 126 ± 1, respectively in 12/7°C (day/night) chilling treatment, while these were only 23 and 40 days respectively at 15/10°C. This implies that temperature at 12/7°C might be too low compared to their optimum temperature range of vernalization for these species and thus it delayed growth and development. In addition, time to flowering of ripgut brome (*Bromus diandrus*) reduced with the increase in vernalization duration at 5°C; six week vernalization treatment resulted in the shortest number of days to flower. However, a longer period (8 weeks) did not further reduce the time to flowering (Gleichsner & Appleby, 1996). This suggests that a saturated vernalization requirement had been achieved after 6 weeks. Therefore, long exposure to low temperature beyond the requirement of each species might slow plant

growth and thus delay the flowering time.

Nevertheless, the cold induction can be eliminated by changes of external cues. The transition period from completion of vernalisation treatment to flower initiation can be divided into two phases. The first phase is a stage (usually the first five days) right after vernalisation when flower induction can be reversed by exposure to hot temperatures, low irradiance and/or SDs. The reversal is called "devernalisation". Phase two begins when flower induction is stable and cannot be reversed (McDonald & Kwong, 2005).

According to Michaels & Amasino (2000), vernalizing effect can be partially or completely nullified by some days of hot temperatures, usually 30-40°C. For instance, floral development of winter wheat can be totally eliminated if the plants are subject to 30°C for 3-5 days (Hopkins & Huner, 2009). However, Wiebe (1994) found that leeks (*Allium porrum* L.) have optimum vernalisation effect at 5°C, temperatures above 18°C can induce devernalisation in this species.

Devernalization is more achievable when the hot treatment immediately follows vernalization (Hopkins & Huner, 2009; McDonald & Kwong, 2005; Michaels & Amasino, 2000). Additionally, the effect of devernalization decreases along with the increase of vernalization period (Michaels & Amasino, 2000; Taiz & Zaiger, 2006).

Cape daisy 'Pink Whirls' could only flower after 2 weeks of cool temperature at 12°C, the plants grown at continuous 22°C or those were treated at 12°C for 1 week did not initiate flower (Pearson *et al.*, 1995). The result might suggest that 1 week of venalization might not be sufficient to induce a stable vernalized stage and the vernalizating effect might have been lost when the plants were moved to higher temperature at 22°C (devernalization).

Interestingly, in *Arabidopsis* and some chrysanthemum cultivars, devernalization can only be successful where the plants have been vernalized in the dark (Bernier 1981, cited in Michaels & Amasino, 2000). The authors advocate that this might indicate a prerequisite for a level of metabolic activities or cell division to stabilize the vernalized phase.

McDonald and Kwong (2005) argue that most of cold-demanding plants can, theoretically, be devernalzed such as winter rye, cauliflower, kohlrabi, cineraria and celery. Further, Hopkins and Huner (2009) state that there is a "neutral" temperature where most of plant species do not have vernalization or devernalization responses. For example, 15°C is reported as a neutral temperature for Petkus rye. For this reason, the use of a neutral temperature for several days is necessary to prevent devernalization. For instance, cineraria cultivars 'Cindy Blue' and 'Cindy Dark Red' need to be placed under at 15°C for 3-6 and 6-9 days respectively to avoid devernalization at 21°C (Yeh *et al.*, 1997).

In short, temperature is one of the most important external cues which influence flowering time, number of flowers/inflorescences as well as the rate of floral development. A species requires a certain optimum range of temperature for floral production. Vernalization plays an important role in cold-requiring species for effective floral initiation and development. Vernalization can either be facultative where cold temperature treatment is not compulsory but it can help accelerate flowering or obligate requirement where flowering can only take place after a cold treatment. Plants that require vernalization are usually LD plants; however some plant cultivars such as chrysanthemum are day-length indifferent. Cold requiring flowering plant species demand an effective range of cold induction, in which this ranges widely among Australian native species, from 5 to 20°C. In addition, duration of vernalization can affect both quantity and quality of flowers/inflorescences. This duration varies from several days to months depending on species. Moreover, vernalization can be lost when the vernalized plants are immediately exposed to devernalizing conditions such as high temperature. To ensure the effectiveness of vernalization induction, a longer duration of cold treatment and/or placement of plants under neutral temperatures, where vernalization and devernalization might not occur, are recommended.

4. PHOTOPERIODISM AND FLOWERING

Besides light quality and intensity, photoperiod and/or day-length is known to affect growth and development of many plants, influencing flower development and many other traits (Ha *et al.*, 2013; Han *et al.*, 2005; Hopkins & Huner, 2004).

Plants can be divided into several different groups regarding photoriodic response: short-day (SD), long-day (LD), day-neutral (DN), intermediate-day plants and ambiphotoperiodic plants (Gavino, 2005; Hopkins & Huner, 2004; McDonald & Kwong, 2005; Wareing & Walston, 1963). "SD plants" are those species which only flower under SD length, or in other words, when the period of light is less than critical period. These species are generally from low latitudes such as coffee, cotton, rice, fall chrysanthemum, tobacco and soybean (ACPET, 2007; Gavino, 2005; McDonald & Kwong, 2005). "LD plants" flower only under LDs or when the daily light period exceeds the critical light duration. Those species are normally from high latitudes such as temperate grasses, radish, spinach, spring wheat, and spring rye

(ACPET, 2007; Gavino, 2005; Hopkins & Huner, 2004; McDonald & Kwong, 2005). "DN plants" are known to flower irrespective of daily light period. Those species are distributed widely at various latitudes such as potato, tomato, cucumber and sunflower (ACPET, 2007; Hopkins & Huner, 2004; McDonald & Kwong, 2005). "Intermediate-day plants" are those species flower only when the day-length is neither short nor long, usually 12-14 hours. Some species of this type are sugarcane, coleus and *Echinacea purpurea* (Gavino, 2005; Runkle *et al.*, 2001). Finally, "Ambiphotoperiodic plants" flower under short or long irradiance period, usually 8 hours or 18 hours. Under intermediate light period (12-14 hours) the plants remain vegetative. This group is illustrated by tarweed (*Madia elegans*) (Anderson, 2007; Hopkins & Huner, 2004; McDonald & Kwong, 2005).

Interestingly, within the SD and LD plant groups, the plants are categorized according to optional (quantitative) or compulsory (qualitative) responses. The species with quantitative response will flower under any photoperiod; nonetheless, under the described photoperiod flowering is promoted. In contrast, the plants with qualitative response will not flower until they obtain the desired photoperiod (Anderson, 2007; Hopkins & Huner, 2004). Cultivars of *Chenopodium rubrum* L. collected from different latitudes have various ranges of photoperiodic responses for floral development (Erwin, 2009). This suggests a variation among ecotypes and cultivars within a species.

Some species possess a facultative response to photoperiod. For example, *Bracteantha bracteata* (syn. *Helichrysum bracteaturn*), *Rhodanthe chlorocephala* subsp. rosea (syn. Helipterum roseum), *Schoenia cassiniana* (syn. *Helichrysum cassinianum*), *Helipterum craspedioides* and *Rhodanthe floribunda* are quantitative LD plants with flowering fostered in LDs (Bunker, 1995; Sharman *et al.*, 1989b). For example, time to first visible inflorescence bud of *R. floribunda* was 31.4 ± 2.1 days under LD (16h photoperiod) compared to 73.0 ± 2.5 days under SD (8h day-length) (Bunker, 1995); meanwhile *Chrysanthemum morifolium* and Geraldton wax-flower (*Chamelaucium uncinatum*) are quantitative SD plants with flowering promoted in SDs (Sharman *et al.*, 1989a). However, species such as *Brachycome halophila* is day neutral (Bunker, 1995).

5. INTERACTIONS BETWEEN JUVENILITY, PHOTOPERIOD AND VERNALIZATION

Plants need to reach a certain age to perceive cold induction for flowering (Ramin & Atherton, 1991). The juvenile length varies widely among species. Beet and radish for example, could be vernalized as imbibed seeds. On the other hand, in some species, seedlings need to reach a particular stage of development at which the meristems are able to perceive cold induction (McDonald & Kwong, 2005). For instance, the 3 year-old plants (average rhizome size: 20g) of *Blandfordia grandiflora* (Liliaceae) could obtain 80% flowering, while the 2 year-old ones (rhizome size: < 15g) achieved only 10% flowering after vernalization treatment at 9°C for 5 weeks (Goodwin *et al.*, 1995). In addition, Markowski & Ryka (1981) showed that winter rape with 5-10 developed leaves (53-64 days old) reached 93.3% of flowering plants after 56 days of vernalization treatment at 1°C, while plants were treated at germinated seeds and 27 days old (3-4 leaves) achieved only 13.3% of flowering plants. These results are in agreement with Wellensiek and Hakkaart (1955) who stated that the sensitivity with low temperature for floral development is increased when the plant age increases.

A study by Cave & Johnston (2010) showed a relationship between duration of vernalization and development stages of plants in flowering of two Australian native flower species, *Brunonia australis* and *Calandrinia sp.* The plants of both species were subjected to 0, 3 and 6 weeks of vernalization at two development stages of 17 days (1-4 leaves) and 35 days (8-14 leaves). Generally, the increase of vernalization duration resulted in shorter time to flowering, increases of flower/inflorescence number and production rates in both species. Time to first visible bud of plants with 8-14 leaves of *Brunonia* and *Calandrinia* were 11 and 3 days earlier than those of plants with 1-4 leaves, respectively. Also, the number of inflorescences and rate of floral production were significantly higher. In addition, there was also an interaction between vernalization duration and development stage in *Brunonia* where the increase in duration of cold treatment led to reduced days to first visible bud stage, greater number of inflorescences and higher rate of inflorescence production corresponding to the increase in plant ages before vernalization treatment. It can be concluded that both species have a facultative requirement of vernalization as non-vernalized plants still flowered. That means the species can still flower in the absence of cold temperature; however flowering is enhanced after cold treatment (Michaels & Amasino, 2000). Moreover, the result of more flowers produced in plants at 35 days old compared to 17 days old before vernalization is consistent with Wellensiek & Hakkaart (1955) who asserted that plants could be perceptive to vernalization at any ages. This sensitivity increases in accordance with the increase of age. Those were also consistent with a recent study of Ha and Johnston (2013) on flowering response of white paper daisy (*Rodanthe floribunda* (DC) Wilson), in which the species could be able to perceive chilling as one-day old seedlings. Additionally, the oldest seedling group (4 weeks old) prior to chilling had faster floral development rate and more inflorescences.

Similarly, mature plants might be more receptive to photoperiod induction. Two campanula cultivars, 'Champion

Blue' and 'Champion Pink', are facultative LD plants. The plants were grown under 8h photoperiod before being transferred to 16h day-length at 2-3 and 8-9 leaf stages. The percentages of flowering plants were 64% and 63% for 'Champion Blue' and 'Champion Pink' respectively with the plants transferred at 2-3 leaves. However, 100% flowering was resulted in both cultivars with the plants transferred at 8-9 leaves (Cavins & Dole, 2001). This suggests that two varieties have short juvenile phases which indicate the capability of perceiving photoperiod induction for flowering at early stages, but flowering is more pronounced when photoperiod treatment is applied at mature age.

There is an interaction between photoperiod and vernalization which influence flowering in some plant species. In *Coreopsis grandiflora*, SD treatment can be used to replace vernalization requirement. For instance, vernalizing temperature at 10°C for flowering of *Calceolaria x herbeohy brida* 'Zwerg Ziichterstolz' can be substituted by SD treatment at 15-20°C (Runger, 1975). Conversely, LDs in addition to vernalization accelerate flowering in some species such as *Lilium longiflorum*. Most of cold-demanding species require LDs after cold exposure (McDonald & Kwong, 2005). The number of LDs and/or critical daylength needed for floral development reduces as the vernalization duration increases (McDonald & Kwong, 2005; Runger, 1975). For example, *Calceolaria x herbeohy brida* 'Zwerg Ziichterstolz' is a LD plant with critical day-length of around 14-15h. Without vernalization treatment, the critical day-length of this species is 14 hours at 20-25°C. This threshold is 4 hours shorter when the plants are held at 15°C, and under 10°C the photoperiodic requirement is eliminated (Runger, 1978). Moreover, another experiment showed that flowering can only occur after a period of low temperature treatment at 10°C. After 40 days of cold treatment, flowering under SD (8h) is inhibited. However, flowering takes place in both LD (18h) and SD (8h) when the plants are preceded by 70 days of chilling at 10°C (Runger, 1975). It can be concluded that in some cases, SD can be applied to replace vernalization requirement and vice versa. Manipulation of photoperiod coupled with vernalization duration can create year-round production for some flower species.

According to Pearson *et al.* (1995), exposure of LD plants to low temperature at early stage of plant development can accelerate flowering. Some native Australian daisies such as *Helichrysum cassinianum* (syn. *Schoenia cassiniana*) and *Helipterum craspedioides* were also reported as facultative LD plants, in which low temperature during winter and spring promotes floral development, while high temperature and long photoperiod in summer accelerate growth and flowering (Mott & McComb, 1975). Furthermore, in *Brunonia australis*, quantitative requirement of vernalization and photoperiod are evident as reported by Cave and Johnston (2010). In general, increase in duration of vernalization and photoperiod reduced time to first visible bud. A period of cold treatment (3 or 6 weeks) led to higher inflorescence number compared to non-vernalized plants in both day-lengths, while the figures are all higher under LD (16h) compared to SD (11h). Moreover, these authors found that vernalization could be replaced by LDs on young plants (1-4 leaves). These results further support the conclusions of the above mentioned authors. It can be therefore concluded that for maximal flowering of a species, understanding of its environmental requirement together with manipulation of vernalization duration and photoperiod is necessary.

Studies on flowering of chrysanthemum reviewed by Kawata *et al.* (1987) have indicated that there are four developmental phases in the life cycle of chrysanthemum, namely rosette, juvenile, photoperiod-sensitive and ripening. To overcome the rosette stage, induction of cold treatment is needed. Subsequent high temperature is then required to pass through juvenile phase. And eventually a SD treatment is necessary to fulfill the photoperiod-sensitive stage.

6. CONCLUSION

In brief, the paper has presented different flowering pathways with a major focus on juvenility, photoperiod and vernalization and their interactions in control of floral initiation in plant species. Plants experience a juvenile phase before they can be capable of perceiving external stimulus for floral induction such as chilling temperatures and day-lengths. The juvenile phase varies with species, being very short for ephemeral species. There is more or less an interaction between duration of vernalization and developmental stages of some plant species in which the longer duration of cold treatment, the shorter time to flowering and the higher number of flowers and/or inflorescences. Species that demand cold induction for flowering are usually LD plants, flowering is promoted under LDs. Further, there is a relationship between photoperiod and vernalization in regulation of flowering in some species. The critical day-length reduces while the duration of vernalization treatment rises. In some cases, vernalization duration can totally nullify photoperiodic requirement. Understanding of plant developmental stages, genetic control and their environmental requirements for flowering is important to adjust growing environments to extend cropping seasons and address consumer demands. Recent advances in genetic modifications, hybridization and cultivation techniques, as reviewed by Jung and Müller (2009), have provided new directions for breeding and production of various food and horticultural crops.

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