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Developmental Physiology of Chrysanthemum

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Abstract

Chrysanthemum cultivation involves a dynamic interplay of factors that shape both plant development and growth. Although development and growth are intertwined, they respond to distinct environmental cues: temperature primarily influences development, while light intensity and CO₂ concentration predominantly govern growth. This chapter provides a comprehensive exploration of chrysanthemum development, elucidating phenology, plant architecture, and the intricacies of root, leaf, stem, flower, and seed formation. We discuss the environmental factors influencing the growth and development of different plant parts and delve into the nuanced relationship between environmental conditions and the developmental physiology of chrysanthemum. Furthermore, we discuss grafting techniques as a strategic approach to increase the vigor of stock plants and enhance the rooting quality of cuttings. The developmental physiology in this chapter offers insights for optimizing cultivation practices for improved productivity and quality in the chrysanthemum industry.

Keywords

Chrysanthemum development, environmental factors, flower initiation, grafting, leaf development, phenology, plant architecture, root formation, seed formation, stem growth, stock plants

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5.1 Introduction

For understanding plant behavior, it is beneficial to distinguish between growth and development. Growth refers to the increase in size (e.g. weight, leaf area, and length), whereas development is the genetically defined progression through the life cycle stages (germination, juvenile phase, generative phase, flowering, and seed formation). Initiation of new organs (e.g. leaves, flower initiation, and formation of fruits and seeds) clearly represents plant development. Development and growth almost always occur simultaneously, and both are influenced by environmental factors. For example, temperature primarily influences development (Van der Ploeg and Heuvelink, 2006), whereas light intensity and CO₂ concentration predominantly affect growth. In this chapter, we focus on chrysanthemum development (e.g. phenology, plant architecture, root, leaf, stem, flowering, and seeds), whereas chrysanthemum growth (e.g. photosynthesis, biomass production, and partitioning) is largely presented in Chapter 08.

5.2 Plant Phenology, Rosette, and Juvenility

The cultivated chrysanthemum is an interspecific hybrid that has a long history of inbreeding, mutations, and selection (refer to Chapters 03 and 04). This leads to a highly diverse assortment, and it is not surprising that different physiological responses during the life cycle of chrysanthemum exist. The life cycle of a plant can be divided into a vegetative or juvenile phase, followed by a mature reproductive phase, where the plant is receptive to floral stimuli. The existence of a juvenile phase in chrysanthemum is illustrated in the work of De Jong (1981), who showed that the number of leaves in a seedling population from crosses between all-year-round chrysanthemums varied from 15 to 32 leaves before seedlings became receptive to short days. Therefore, the existence of a juvenile phase might be of importance for breeders but does not affect chrysanthemum production, as this starts from cuttings that have no juvenile phase.

The phenology of autumn-flowering chrysanthemums is given in Fig. 5.1 (Sumitomo *et al.*, 2008). Flower bud initiation is induced by decreasing day lengths under cooler temperatures in late summer. The main stem flowers during October–November and dies before winter after the seed set. Suckers emerge from the basal zone from mid to late autumn. These suckers become dormant and form rosettes with an inability to flower, even under short-day inductive conditions. After fulfillment of the chilling requirements, the shoots will elongate and respond again to flower-inducing triggers. Also, Cockshull (1985) reported that certain cultivars form a rosette in autumn and winter and require vernalization to flower. After passing through the rosette stage, the plants grow rapidly and flower early in a heated greenhouse at temperatures of 15–20°C. However, this is not always the case, as, for certain Japanese chrysanthemums exposed to low temperatures, shoot extension will

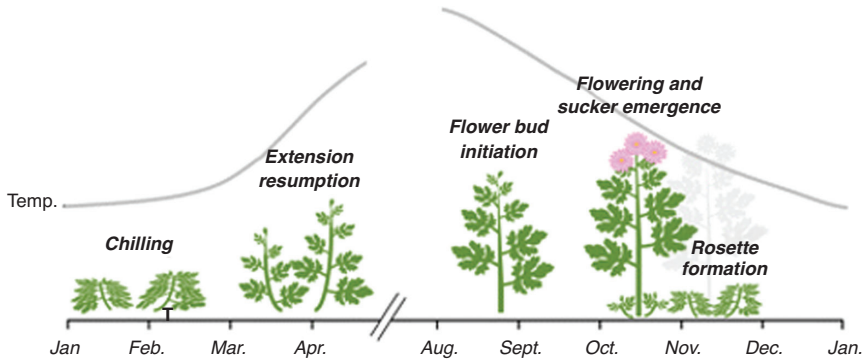


Fig. 5.1. Phenological events in herbaceous perennial chrysanthemums.
(Reproduced from Sumitomo *et al.*, 2008, with permission from Oxford University Press.)

occur, but flowering is delayed compared with non-chilled plants in a heated greenhouse (Higuchi and Hara, 1974). Additionally, Kawata (1987) showed that this pattern is also present in other Japanese autumn-flowering cultivars. Likewise, exposure of mother plants of certain autumn-flowering cultivars, such as “Jinba,” to temperatures lower than 10°C for an extended period did result in delayed flowering of the cuttings from these plants (Kunitake *et al.*, 2009). From these studies, it is clear that low temperatures result in different responses, depending on the genotype.

Hisamatsu *et al.* (2017) analyzed the effects of these temperature responses on rosette formation and floral induction for chrysanthemum cultivars with different genetic backgrounds. In certain genotypes, such as “Kinyo” and “City,” high summer temperatures induced shorter internodes in cuttings rooted and grown under a 12 hour photoperiod leading to rosette-like types that could be considered as a dormant condition. After a sufficient chilling period, extension growth resumed. However, these chilling temperatures might result in two contrasting effects on flowering. In certain genotypes, the chilling acts as a “vernalization” and leads to flowering, while in other genotypes, the chilling has an inhibitory effect, and plants do not respond immediately to flowering stimuli (this is called a form of juvenility in older literature). The study also included a “Reagan”-type (The Netherlands), but for this cultivar, flowering was always obtained irrespective of the treatments, and it did not respond to inhibitory temperature treatments (Hisamatsu *et al.*, 2017). In Europe, all-year-round genotypes for cut flower production are required; therefore, autumn-flowering lines have been discarded during the breeding process (De Jong, 1981). Likewise, European breeding companies of autumn-flowering pot chrysanthemums discard lines with rosette formation. However, in Japan and China, flower forms are important characteristics for the floricultural market, and autumn-flowering cultivars, such as “Jinba,” are still cultivated.

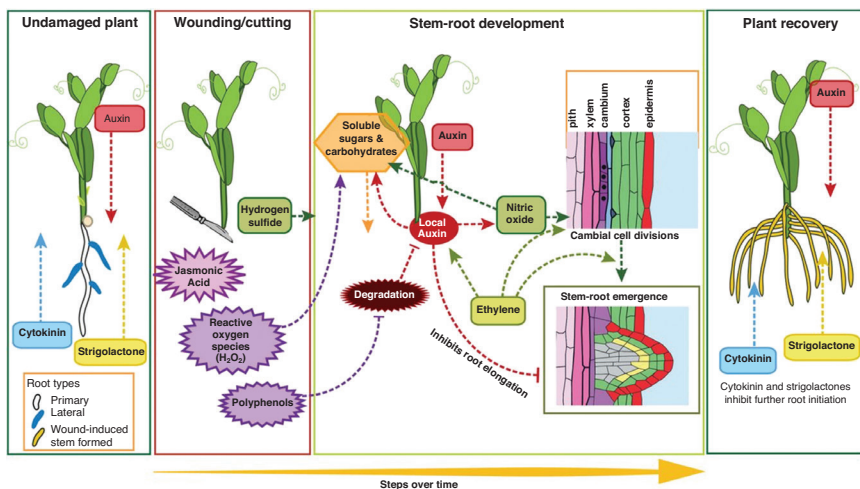


Fig. 5.2. Wound-induced adventitious root formation. (Reproduced from Steffens and Rasmussen, 2016, with permission from Oxford University Press.)

5.3 Root

Chrysanthemums are propagated asexually by leafy stem cuttings. Roots that are formed on non-root tissue, such as stem tissue in the case of *chrysanthemums*, are adventitious roots. Different triggers induce these roots (for an overview, see Steffens and Rasmussen, 2016; Drüge *et al.*, 2019), including wound-induced adventitious root formation, which is the basis of cutting propagation (Fig. 5.2). The process of adventitious root initiation and development is highly regulated by a suite of environmental and endogenous (hormones) factors, including physiological status of the mother plant (see Section 5.8).

In short, immediately after taking the cutting, wound response signals (jasmonic acid, hydrogen peroxidase) increase, followed by an increase of phenolics that helps to protect against this H₂O₂ burst. Adventitious root induction depends highly on auxin levels, while the role of ethylene is contradictory and depends on the investigated species. In *chrysanthemum*, endogenous ethylene levels increased till the early phase of adventitious root formation (Liu *et al.*, 2013), suggesting that ethylene is important in rhizogenesis. Also, a higher carbohydrate pool will enhance root initiation. Adventitious roots will be initiated in the cambial zone, and here, cell divisions will develop into new root primordia. Histological studies showed that 5 days after cutting of *Chrysanthemum* “Jinba,” root primordia are already found (Fig. 5.3). Once the root system is formed, normal hormone balances are restored as both cytokinin and strigolactones are biosynthesized in the roots.

Auxins, predominantly synthesized in the shoot apical meristems, play a central role in regulating adventitious root formation; indole-3-acetic acid

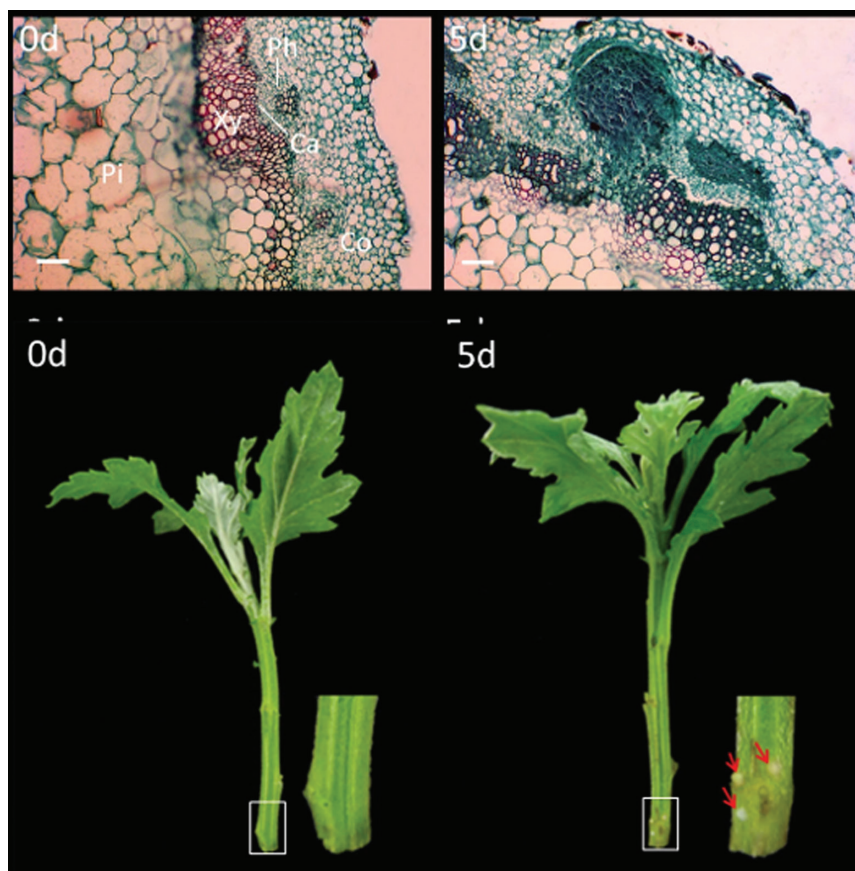


Fig. 5.3. Morphological and histological properties of adventitious root primordium formation in the stem base of *Chrysanthemum* “Jinba” cuttings. The typical stem anatomy consists of the cortex (Co), pith parenchyma (Pi), and a ring of vascular tissues comprising phloem (Ph), cambium (Ca), and xylem (Xy). Anatomy of adventitious root primordium formation at 5 days after cutting, where the first differentiated root primordia can be observed (top right). Scale bars = 500 μ m. Morphology of cuttings at 0 and 5 days (bottom panel). Arrows indicate adventitious roots, and the magnified image shows the cutting position. (Adapted from Liu *et al.*, 2013, CC BY 2.0.)

(IAA), a natural auxin and synthetic analogues, and indole-3-butyric acid (IBA, which is not only an endogenous hormone but also dominantly used as rooting hormone due to its higher stability than IAA) both have a dominant and stimulatory effect on adventitious rooting (Pacurar *et al.*, 2014). In roots, the polarity of auxin transport has been described as basipetal (from apex to base) and acropetal (toward root apex) (Rashotte *et al.*, 2000). With the help of auxin influx carrier proteins (AUXIN RESISTANT1/LIKE AUX1—AUX1/LAX) and efflux carrier proteins (PIN-FORMED-PIN), auxin is transported systemically via vascular tissues (Yang *et al.*, 2006; Swarup and Péret, 2012).

The members of PIN proteins are known for their auxin-related developmental processes, including adventitious root formation. For example, in chrysanthemum, the MADS-box transcription factor *CmANR1* positively modulates the development of numerous adventitious roots and lateral roots by directly regulating the auxin transport gene *CmPIN2* (Sun *et al.*, 2018). Furthermore, the proteomic profiling in chrysanthemum enlisted the root system development proteins belonging to the TCP (TB1-CYC-PCFs) family, which influence auxin biosynthesis and transport. *CmTCP20* gene may participate in phytohormone signal transduction with improved IAA content to promote both adventitious and lateral root development in chrysanthemum (Fan *et al.*, 2019).

Environmental factors might modulate auxin levels. For example, in the shade avoidance response, a low red:far-red (R:FR) ratio increases the biosynthesis of auxin (Halliday *et al.*, 2009). The increasing use of light-emitting diodes (LEDs) in glasshouse horticulture opens the possibilities for applying different light ratios and thus influencing endogenous auxin levels. The addition of FR to red light (lower R:FR ratio) resulted in faster rooting, higher root dry mass, and an increased number of adventitious roots in chrysanthemum. However, a low R:FR ratio will also lead to stem elongation, which reduces the quality of the cuttings. This effect on internode length can be counteracted by adding blue to the spectrum (Christiaens *et al.*, 2019). Besides auxins, *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)* genes also regulate root development. In chrysanthemum, *CmLBD* transcripts were earlier upregulated under blue light compared with red and fluorescent light, which correlated with a higher root mass under blue light (Gil *et al.*, 2020). Also, Christiaens *et al.* (2015) found a beneficial effect of blue light on the number of roots compared with red light; however, these effects were less pronounced than those observed under low R:FR ratios.

5.4 Leaf

Leaf characteristics (leaf shape, size, color, and organelles—mesophyll and palisade cells) affect growth as well as the external appearance of leaves in chrysanthemums. This is part of the ornamental value of cut chrysanthemums and contributes to the postharvest quality. Therefore, changes in leaf characteristics will affect photosynthetic capacity and ornamental value. In chrysanthemums, abundant morphological diversity in leaf phenotypes exists (leaf—base, color, shape, margin, tip, lobe, and vein distribution). Although leaf characteristics are also affected by environmental conditions, each genotype has relatively stable leaf characteristics (Ma *et al.*, 2020), which are the basis for cultivar identification and genetic variation.

The physiological status of leaves depends predominantly on the growing conditions, especially light and temperature. Leaves of plants grown under 25 and 15% irradiance were thinner with loose palisade tissue and irregularly arranged spongy mesophyll cells compared with leaves grown under full

sunlight (Han *et al.*, 2017). McMahon and Kelly (1995) described the importance of light quality that influences the growth of organelles in leaf cells in chrysanthemum. Leaves grown in CuSO_4 -filtered light (primarily absorbs FR light) had increased chlorophyll-a and -b and carotenoid contents per unit area; this appeared to be the result of more densely arranged palisade cells with reduced intercellular space. In addition, palisade cell numbers per unit area increased under CuSO_4 filters. Light through CuSO_4 filters reduced the leaf area, palisade cell length, and intercellular space but not leaf thickness. The stomatal density was not affected, but the number per leaf was reduced due to decreased leaf size by CuSO_4 -filtered light (McMahon and Kelly, 1995).

In chrysanthemum, the commercial value depends not only on the external (visual) quality but also on postharvest longevity, primarily determined by the growing conditions. Chrysanthemum grown under monochromatic red light showed enhanced stomatal ability (the rate and the degree of stomatal closure) to regulate water loss upon desiccation (Seif *et al.*, 2021). Another critical factor that affects postharvest longevity in chrysanthemum is vapor pressure deficit (VPD). Chrysanthemums grown under lower VPD have a disturbed stomatal functionality due to a reduced sensitivity of stomata toward desiccation, leading to premature leaf wilting and leaf yellowing (Aliniaefard and van Meeteren, 2016).

Ornamental value is affected by flower color as well as leaf color. Leaf coloration largely depends on the leaf pigments (chlorophyll, carotenoids, and anthocyanins). Mutations in the genes involved in chloroplast development can also influence leaf color. Variegated chrysanthemum can be of substantial ornamental value owing to leaf color characteristics. A recent transcriptomic study in variegated chrysanthemum variety “NAU04-1-31-1” gave molecular insights into the leaf color variation involving two genes (*CmChlH* and *CmFtsH*) responsible for yellow and green leaf tissues (Chang *et al.*, 2013). Additionally, biochemical analysis indicated that the yellow leaf tissue's chlorophyll content is lower than that in green tissue, and chloroplasts in the yellow leaf tissue were vacuolated and lacked thylakoid membrane structures (Chang *et al.*, 2013).

Temperature also influences chrysanthemum leaf characteristics. Kozáowska *et al.* (2011) observed a positive correlation between day temperature and leaf chlorophyll content. Berg and Cutter (1969) studied leaf initiation rate in chrysanthemum at 20°C. After removal of the terminal bud, the top two lateral buds produced leaves at a rate of 1.4–1.6 leaves/day for the first 9 or 10 days. Around the 10th day, there was an abrupt shift to a lower initiation rate of 0.7–0.8 leaves/day. Defoliation by periodic removal of leaves larger than 1 cm showed an increase in leaf initiation rate, but a negative effect on leaf initiation rate from maturing leaves could not explain the drastic drop in initiation rate. Berg and Cutter (1969) also concluded that the rate of leaf emergence (leaf larger than 1 cm) was equal to the rate of leaf initiation.

When the apex has been initiated to form flowers (refer to the following section), the production of true leaf primordia ceases and is initially followed

by the formation of bracts. Based on data collected from chrysanthemum “Pert” plants grown in greenhouses and daylight chambers at temperatures ranging from 9 to 27°C and light integrals ranging from 0.8 to 7.8 mol m⁻² day⁻¹, Larsen and Hidden (1995) concluded that leaf unfolding rate shows an optimum response to temperature and does not depend on light integral. This optimum temperature response was shown to be stronger when considering day temperature alone instead of the average 24 hour temperature (Carvalho *et al.*, 2002). Only when the light integral was below 2 mol m⁻² day⁻¹, leaf unfolding rate was reduced. At temperatures between 15 and 25°C, about one leaf was unfolded (reached 3 mm length) every second day. The finding that leaf unfolding rate did not depend on light integral (when above 2 mol m⁻² day⁻¹; Larsen and Hidden, 1995) suggests that other factors influencing assimilate availability (planting density, CO₂ concentration) will also not affect leaf unfolding rate.

5.5 Stem

Stem length depends on the number of internodes (same as the number of leaves, see Section 5.6) and their length (Fig. 5.4). Since chrysanthemum has a determinate growth pattern under short-day conditions, new internodes are formed only until the start of flower initiation. The longer the duration of the long-day period, the taller the plants, as more time exists to form new internodes (Carvalho and Heuvelink, 2003).

5.5.1 Shoot architecture and branching

Plant architecture is shaped by its branching pattern, and shoot branching depends on the formation of axillary buds at the shoot apex and their subsequent outgrowth. Therefore, the control of axillary bud outgrowth will shape the plant architecture. If axillary meristems or buds remain paradormant, new leaves will only be initiated in the apical zone, leading to a single stem. The paradormant state of the axillary bud is regulated by signals in the plant (not in the bud itself) (Lang *et al.*, 1987). Decapitation will release axillary buds from their paradormant state but with the potential to inhibit the outgrowth of axillary buds. Floral transition will also release the dormant state of other axillary buds. This phenomenon is described as apical dominance (for a review, see Cline, 1991). The regulatory system for the outgrowth of axillary buds is under both endogenous and environmental control. Endogenous control is based on the cross talk of auxins, cytokinins, and strigolactones, while endogenous sugar levels also control bud outgrowth. In particular, auxins, strigolactones, and their transport play a central role in the regulation of axillary bud outgrowth associated with apical dominance (Liang *et al.*, 2010). Branching signals from auxins and strigolactones are integrated within axillary buds by transcription factors from the TB1, CYC, and PCFs (TCP) family, *BRANCHED 1*

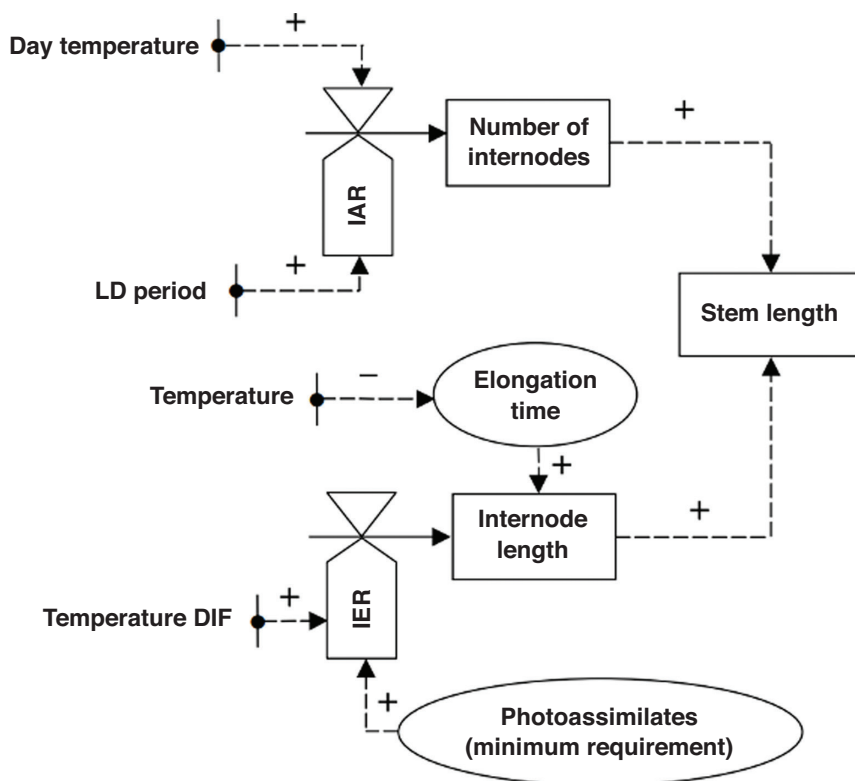


Fig. 5.4. Relational diagram for stem length in cut chrysanthemum. Boxes are state variables, ellipses are parameters, and valves are rate variables. Dashed arrows represent information flow: (+) indicates positive influence, and (-) indicates negative influence.

Note: DIF = difference between day and night temperatures, IAR = internode appearance rate, IER = internode elongation rate, LD = long day. (Reproduced from Carvalho and Heuvelink, 2004, with permission from International Society for Horticultural Science (ISHS).)

(*BRC1*) to influence bud outgrowth. *BRC1* regulates shoot branching through phyB as a response to changes in R:FR ratio (González-Grandío *et al.*, 2013). *BRC1* inhibits floral transition and stimulates outgrowth of buds by interacting with *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) (Niwa *et al.*, 2013; Rameau *et al.*, 2015). In chrysanthemum, the involvement of *BRC1* in the regulation of branching was elucidated (Chen *et al.*, 2013; Dierck *et al.*, 2015). Furthermore, the sugar transporter gene, *CmSWEET17*, may be involved in the process of sucrose-induced axillary bud outgrowth by mediating the auxin transport pathway genes (Liu *et al.*, 2020). Auxin export is key to the outgrowth of buds, and Liu *et al.* (2022) showed that sucrose treatment



Fig. 5.5. Morphotypes of chrysanthemum. (A) Standard (disbud) with single flower. (B) Spray type with multiple flowers on single stem. (C) Cushion type with multiple branching. (Adapted from SharathKumar, 2023.)

of two-node stems of chrysanthemum promoted the expression of *CmPIN1* and *CmAUX1*, which induced auxin export from buds. Furthermore, plant nutrient status also influences shoot branching by influencing the phytohormones such as auxins, cytokinins, and strigolactones. For example, phosphate deficiency inhibits the outgrowth of axillary buds with the relocation of auxin and increased strigolactone level (Xi *et al.*, 2015). Also, in a chrysanthemum field experiment, shoot branching was reduced at low levels of phosphorus fertilization (Satar *et al.*, 2016).

Commercial chrysanthemums can be grouped into different morphotypes/phenotypes, varying from upright single-stem types (cut flowers, spray chrysanthemums) to highly branched growth habits (cushion types, potted chrysanthemum) (Fig. 5.5). Branching is an important quality trait in the breeding and production of chrysanthemums. Indeed, axillary bud outgrowth in cut flower types needs manual labour to disbud the stems, while in the cushion types, well-branching genotypes are needed. Therefore, knowledge of the hormonal control and genetic markers could help the breeding efforts. In chrysanthemum, markers associated with key genes in the biosynthetic pathway of strigolactones could explain the phenotypic shoot branching traits (Klie *et al.*, 2016; Dierck *et al.*, 2018). Sun *et al.* (2019) used a quantitative trait loci (QTL) approach to identify the branching pattern of chrysanthemum, grown at two planting densities, but found a strong genotype by environment ($G \times E$) effect; thus, the search for stable QTLs linked to branching patterns is still ongoing.

5.5.2 Environmental control

In chrysanthemum production, shoot architecture is controlled by pruning, disbudding (manual decapitation or removing the axillary buds), and the use of chemical growth retardants. Recently, LED lighting technology has been explored to regulate bud outgrowth by changing the R:FR ratio. Shoot branching and outgrowth of axillary buds are interlinked with shade avoidance syndrome (SAS). A lower R:FR ratio, perceived by phyB, leads to unwanted

shoot elongation and less branching. Planting density will directly influence the light intensity in the canopy and also filter wavelengths, resulting in an enrichment of FR for axillary buds. Shading and low R:FR ratio negatively regulate shoot branching in many species. Growing cut chrysanthemum in a low R:FR or high R:FR environment during their developmental stage showed that the effect of light quality was influenced by the bud position (Yuan *et al.*, 2018). In the upper stem zone, the bud length of plants grown under low R:FR was much shorter than that of the plants grown under high R:FR. Yet, this was reversed for the basal buds, indicating that a low R:FR not only inhibits bud outgrowth but this response is also influenced by bud position.

The relative progression of lateral shoot elongation from pinch to flower of chrysanthemum “Bright Golden Anne” plants grown under 2–22 mol m⁻² day⁻¹ photosynthetic photon flux, at a temperature range of 10–20°C, has been modeled using a Richards function (Karlsson and Heins, 1994). The relative time (first short day set to 0.0 and flowering to 1.0) required to attain 50% of the shoot length at flowering was lower at higher average daily temperature during plant development.

Stem length increased linearly with temperature (15–24°C) in four cultivars; however, for one cultivar, the slope of the line was steeper than that for the other three cultivars (van der Ploeg *et al.*, 2007). This increase in stem length resulted from an increase in both internode length and the number of internodes. Internode appearance rate shows an optimum around 25°C (Larsen and Hidden, 1995; Carvalho *et al.*, 2002). Chrysanthemum starts to initiate flowers soon after the start of the short-day period (Van Ruiten and De Jong, 1984), and once the plant has induced the apical flower bud, it loses the ability to initiate new internodes. Therefore, during the long-day period, only temperature influences the final leaf number. After the flower buds have been initiated, stem elongation is solely the result of internode elongation.

Besides the average temperature, the temperature regime (or temperature pattern) is very important for stem elongation. The difference between day and night temperatures is called “DIF” (short for difference). An inversed temperature regime with a higher night than day temperature is called a “negative DIF.” Growers, especially of potted plants, use negative DIF to reduce elongation and obtain more compact plants. Carvalho *et al.* (2002) showed that DIF can predict the final internode length only within a temperature range, where the effects of day temperature and night temperature are equal in magnitude and opposite in sign (18–24°C). In that range, the stimulation in internode length because of higher day temperature is mitigated by the reduced internode length as a result of the higher night temperature (Fig. 5.6).

Not only the day/night temperature regime is important, but also the regime during the day. In particular, a drop in temperature during a brief period (up to a few hours; called DROP) can reduce plant elongation. The point during the day at which this DROP occurs plays a significant role. In the first hours of the light period, elongation growth is most sensitive to temperature: a low temperature during those hours reduces internode length. In the last hours of

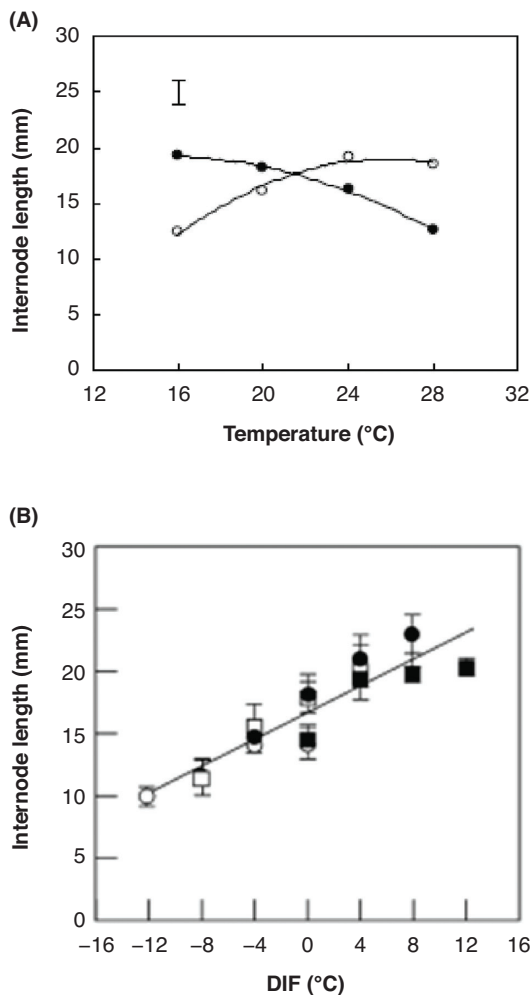


Fig. 5.6. (A) Final length of internode 10 as a function of day temperature (o: averaged over four night temperatures) or night temperature (●: averaged over 4 day temperatures) 27 days after temperature treatments started in chrysanthemum "Reagan Improved." Sixteen treatments (day length 12 h) were all combinations of 4 day temperatures (16, 20, 24, and 28°C) and four night temperatures (16, 20, 24, and 28°C). (B) Relationship between the final length of internode 10 and DIF (°C) for these 16 treatments. Symbols represent day temperatures of 16 (o), 20 (□), 24 (●), and 28°C (■). Regression line, $y = 16.57 + 0.547x$, $R^2 = 0.81$. Vertical bars indicate standard error of the mean (s.e.m.) ($n = 2$) and are shown only when larger than the symbol. (Adapted from Carvalho *et al.*, 2002.)

the light period, DROP does not affect elongation. Potted plant growers use this phenomenon to keep their plants compact (Carvalho *et al.*, 2008). In a greenhouse, DROP is easier to create than a negative DIF (in late spring and summer), as day temperature only needs to be lower than night temperature during the first hours of the light period, and not throughout the whole light period. DROP in the early-morning hours often has an effect similar to that of DIF.

5.6 Flowering

Most chrysanthemums are short-day flowering plants. Nevertheless, the wide range of chrysanthemum cultivars results in a flowering range that extends from summer-flowering to autumn- and winter-flowering genotypes under natural conditions. Flower buds can thus be formed under long days, and the time to flower under long day is assessed by the number of formed leaves (long-day leaf number). Even late-flowering cultivars can start bud formation after several months under long day, although anthesis will not be reached. Therefore, flower initiation in chrysanthemum has a quantitative short-day response in early-flowering genotypes and a qualitative response in late-flowering types (Cockshull, 1985). In Asia, summer-to-autumn flowering types are commercially produced as cut and loose flowers, and in certain cultivars, unwanted flower initiation under long day occurs before the inductive photoperiod. This unwanted “premature” budding results in short-length cut flowers and the outgrowth of axillary vegetative buds. A cold pretreatment of stock plants generally reduced the incidence of premature budding in rooted cuttings (Sumitomo *et al.*, 2014). Also, in the European pot mum production, “premature budding” can occur in the early-flowering types. Here, chemical pinching (e.g. Ethrel) can be a method to avoid the negative effect of premature budding.

In Europe, for all-year-round production, autumn- and winter-flowering cultivars are used. Here, the vegetative phase continues when long days (natural light or photoperiodic lighting) are provided such that the plant can initiate a certain number of leaves before the start of the floral inductive phase under short-day conditions. The time it takes for a plant to flower (up to anthesis) from the onset of short day is called reaction time or response time. Each cultivar has its own response time, which varies between 6 and 15 weeks (see Box). Under natural conditions, cultivars with an early reaction time are induced faster than cultivars with a late response, and the duration of all stages becomes shorter with shorter reaction times. For example, Van Ruiten and De Jong (1984) showed that early response types needed 3–7 short days to transform from a vegetative to an inflorescence meristem, intermediate cultivars were induced in 10–14 days, and late response types needed 14–17 days when grown at 19/16°C (day temperature/night temperature (DT/NT)). Once the apical meristem was induced, the axillary meristems followed; the first axillary meristem needed 3 days, but the other axillary meristems followed quickly. Short day has to be maintained till commercialization to allow the floral transition in the axillary buds. In cut flowers, this leads to the spray type of flowering stems.

The *Chrysanthemum* response types (early, medium, and late response types)

Response group refers to the number of weeks it takes for the chrysanthemum to flower from the time they begin receiving short-day treatments to reach anthesis at optimum conditions. Although response groups range from 6 to 15 weeks, most potted chrysanthemums fall into the 9- or 10-week response groups, while most cultivars grown as cut chrysanthemums have a response time of 7–9 weeks although Santini types have a shorter response time, ranging from 5 to 7 weeks.

Based on their response time, chrysanthemums are classified into the following:

- Early response: flowering 7–9 weeks after the start of short days
- Medium response: flowering 10–12 weeks after the start of short days
- Late response: flowering 13–15 weeks after the start of short days

5.6.1 Floral induction

Photoperiod-induced flowering is controlled by phytochromes, with the perception of day length occurring in the leaves. Up to five phytochromes (PhyA–PhyE) exist in most dicotyledonous plants, and they largely differ in their response to spectral light quality (Bae and Choi, 2008). Three phytochromes are identified in *Chrysanthemum* spp. (PhyA, PhyB, and PhyC) (GenBank). Phytochromes exist in two forms that are reversibly interconvertible by light. Pr absorbs maximally in the red region of the spectrum (660 nm) and converts into the biologically active Pfr that has its absorption peak in the FR region (735 nm). Under darkness, Pfr gradually falls back to the inactive Pr form. PhyA is light labile and responds to FR, including the very low fluence response (VLFR) and the FR-high irradiance responses (FR-HIR). The other phytochromes (PhyB–PhyE) are responsible for the red/FR reversible low fluence response (LFR). Photoconversion of Pr to Pfr mediates an array of morphogenic responses, including the flowering response in photoperiodic plants, whereas reconversion of Pfr to Pr cancels the induction of those responses. The main photoreceptor mediating photomorphogenesis in red light is phyB.

In chrysanthemum production, day length is controlled by applying dark screens for an inductive short-day treatment or night interruption to maintain the vegetative stage and prevent flowering. It was elucidated that photoperiodic chrysanthemum flowering is primarily mediated by florigen-*FTL3* and antiflorigen-*AFT*. Under inductive short days, the flower inducer *CsFTL3* in leaves is highly expressed, which correlates with the floral evocation in the apical meristem (Oda *et al.*, 2012). On the other hand, antiflorigen-*CsAFT* is highly expressed under non-inductive long days and night break conditions with red light to prevent flowering (Higuchi *et al.*, 2013). Grafting experiments of wild-type plants on a rootstock overexpressing *CsAFT* clearly showed that *CsAFT* protein delays flowering under short days and is thus a floral repressor. Higuchi *et al.* (2012) dissected the responses of night interruption with red

light at different timepoints after dusk and found that the induction peak for CsAFT occurred 8–10 hour after dusk. Dusk is thus critical for the dark-time measurement, and 8–10 hour after dusk, a light-sensitive phase or gating mechanism is present in this species. Furthermore, by evaluating different light qualities (blue, green, red, FR) for night interruption, the highest expression of CsAFT occurred under red light and was reversed by FR light, indicating the negative regulation of PhyB on floral induction (Higuchi *et al.*, 2013).

The increasing use of LED light opens possibilities to exploit the interaction between day length and light quality on floral induction in chrysanthemum. For example, when chrysanthemums were grown under 11 hour of red/blue light followed by 4 hour of blue light alone (i.e. a total of 15 hour of light, constituting a long day), they showed normal flowering—similar to what is typically seen under short-day conditions (SharathKumar *et al.*, 2021). However, an extension of the natural sunlight during the first 11 hour of the photoperiod with monochromatic red or blue LED light inhibited flowering (SharathKumar *et al.*, 2021). In addition, a 7-h day extension with green light after a 9 hour natural light period retarded flowering, although a dose–response relationship between the green photon flux density and flowering time was present (Meng and Runkle, 2019). Yet, the specific role of cryptochrome in the flowering response is still under investigation.

5.6.2 Development of the floral primordium

The flower meristem is undetermined in the first phase: reversion to the vegetative state can still occur in the absence of the inductive signals. In the second phase, when the inductive signals have acted on the apex for a sufficiently long time, flowering determination occurs, and four types of floral organs are initiated: sepals, petals, stamens, and carpels. These are formed in whorls on the flanks of the meristem. The identity of each of these organ whorls is based on the interaction of the so-called “MADS-box” genes (Theißen *et al.*, 2016).

A chrysanthemum flower head is called capitulum, and it contains many flowers. In chrysanthemum, different MADS-box genes, belonging to the APETALA1/FRUITFULL and SEPALLATA3 subfamilies, were identified (Shchennikova *et al.*, 2004). The different developmental stages of the floral transition are illustrated in Fig. 5.7.

When the plant assimilate status becomes more favorable (e.g. wider spacing and/or higher light intensity), chrysanthemum responds by producing more flowers and flower buds, rather than larger flowers (Carvalho and Heuvelink, 2003; Carvalho *et al.*, 2004). However, it has been clearly demonstrated that the reason for this response is not that flowers would not be able to grow bigger, as actual flower dry mass is only 42% of potential flower dry mass (Carvalho *et al.*, 2004). A positive linear relationship between the number of flowers per plant, including flower buds, and the total aerial plant dry mass was found (Carvalho and Heuvelink, 2003). A single linear relationship could adequately describe this number of flowers for highly diverse

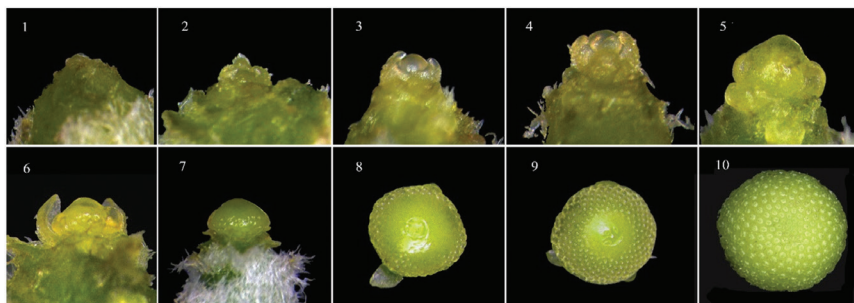


Fig. 5.7. Developmental stages of floral transition in *Chrysanthemum morifolium* cv. Radost. Stage-1 (vegetative phase)—the flat shoot apical meristem (SAM). Stage-2—between the leaf primordia, a barely perceptible bulge of SAM occurs. Stage-3—the bulge is larger, and the leaf primordia begin to deviate. Stage-4—the first bracts are visible under the leaf primordia. Stage-5—the bracts cover the dome. Stage-6 (floral initiation phase)—first floral primordia visible. Stage-7 (floral development phase)—one to four rings of floral primordia visible. Stage-8—multiple rings of floral primordia visible (less than half of area of flower head). Stage-9—multiple rings of floral primordia visible (greater than half of area of flower head). Stage-10—entire bud covered with floral primordia (visible bud emerges). Images were taken from a stereoscope with 80 × magnification. When the diameter of apex was more than 2 mm (images 8–10), a lower magnification (from 30× to 70×) was used to fit whole apex in the field of view. (Adapted from SharathKumar *et al.*, 2021, CC BY 4.0.)

growth conditions, that is, daily incident photosynthetically active radiation ($4.2\text{--}18.3 \text{ mol m}^{-2} \text{ day}^{-1}$), CO_2 concentration ($345\text{--}623 \text{ } \mu\text{mol mol}^{-1}$), and duration of the LD period (0–21 days). The temperature variation was smaller but remained between 19.1 and 22.6°C (Carvalho and Heuvelink, 2003).

5.6.3 Environmental control of flower development

Besides the photoperiodic response for floral induction, flower development in chrysanthemum is dependent on both temperature and light intensity. In northern countries, the short-day phase during winter conditions also results in a relatively low daily light integral (DLI). Quite often, a macroscopic scale is used to quantify flower development, ranging from no visible bud to ray florets fully open or first row of ray florets horizontal. Using these stages, flowering development reached saturation at $6 \text{ mol m}^{-2} \text{ day}^{-1}$ (Persson and Larsen, 1998).

For all-year-round production in greenhouses, shading with blackout curtains is commonly used to achieve short-day conditions to induce flowering during long-day seasons. This action raises the air temperature in greenhouses due to reduced ventilation (Shibata, 1997) and increased absorption of irradiance. During all-year-round cultivation in greenhouses, high temperature

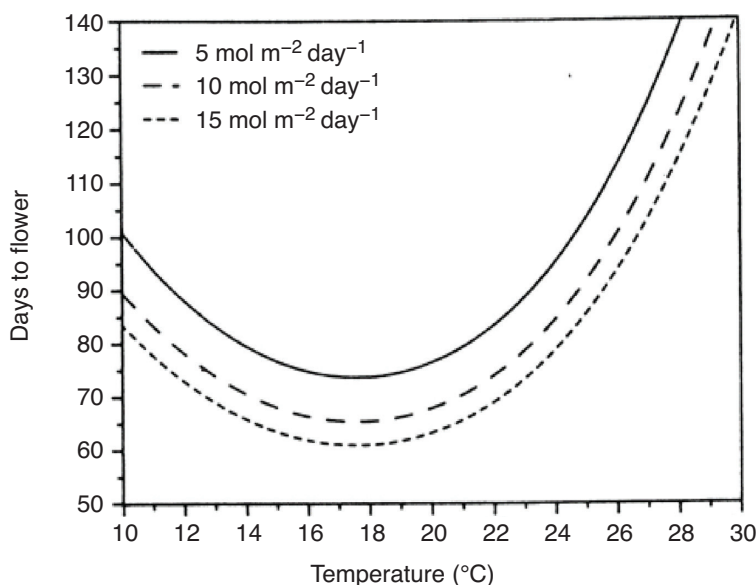


Fig. 5.8. Effects of temperature and daily light integral on flower development. (Adapted from Karlsson *et al.*, 1989, Elsevier STM agreement.)

caused by blackout shading in long-day seasons or by local climates at daytime delays the flowering of chrysanthemum, which is termed “heat-induced delay.” However, higher light intensities may partially overcome the negative influence of higher temperatures on the rate of development (Fig. 5.8) (Karlsson *et al.*, 1989).

Even in short-day seasons, the air temperature in the greenhouse could raise to an unfavorable level for chrysanthemum flowering, especially in the middle of days, depending on the local climate and daily weather. The optimal temperature for chrysanthemum flowering is reported to be approximately 20°C (de Lint and Heij, 1987; Adams *et al.*, 1998). Temperature elevation (especially >26°C) could not only influence flower quality (Carvalho *et al.*, 2005) but also delay flowering (Whealy *et al.*, 1987). Although heat-tolerant cultivars have been introduced in warm regions, these cultivars are generally of reduced quality and still cannot guarantee to flower on time when the temperature reaches above 30°C (e.g. Willits and Bailey, 2000; Nakano *et al.*, 2013). Recent molecular studies revealed that the heat-induced delay is also associated with the florigenic and antiflorigenic genes that regulate flowering under photoperiod. Furthermore, high temperature during capitulum development (after visible bud stage) has a larger contribution to flowering delay compared with floral induction and initiation phases (Nozaki and Fukai, 2008; Nakano *et al.*, 2013). The possible explanation could be that during the

early phases, the inhibited florigen-*FTL3* expression by high temperature is still sufficient to convert the shoot apical meristem into inflorescence meristems but becomes insufficient to promote the further stages of capitulum development and flower anthesis (Nakano *et al.*, 2015). However, Vaid and Runkle (2013) stated that high temperature during the first 2 weeks of short days has greater potential to delay flowering than later during production. Therefore, differentiation of the sensitivity of floral phases to heat-induced delay in chrysanthemum is still under investigation.

5.7 Seeds

Chrysanthemum is considered a self incompatible outcrossing species. Both pre- and postfertilization barriers are described (refer to Chapter 02). Wang *et al.* (2014) screened 24 chrysanthemum cultivars and found that most of the cultivars were self incompatible, but a small portion of cultivars was self compatible, demonstrating that chrysanthemum self incompatibility is cultivar dependent. Chrysanthemum flowers comprise ray and disk florets, and peripheral ray florets are largely responsible for ornamental value. However, decorative chrysanthemums contain a higher number of ray florets with very low fertility due to reduced receptivity of stigmatic tissues, and lack of fertilization results in poor seed set. Therefore, the ratio of disk to ray florets and the quality of pistils determines fertility and seed set in chrysanthemum. Seeds are only used to produce new cultivars, as seedlings do not come true or bear all the same characteristics as the parents. After successful pollination, it takes 10–15 days to reach the stage of the globular embryo. In most crosses, seeds become ripe within 5 weeks after self- or artificial pollination.

5.8 Developmental Physiology of Stock (Mother) Plants

5.8.1 Physiological background

Chrysanthemum production, whether cut flowers or cushion types, is a global industry with firms specialized in cutting production. The climate of the (sub) tropical highlands is excellent for producing the most popular ornamentals. Also, for chrysanthemum, the majority of the commercial cutting production takes place in Africa or Latin America, where light conditions during winter are more favorable, resulting in more vigorous cuttings compared with those in temperate climates. Also, the lower wages and other production costs are an advantage. This globalization results in extra phases in the production chain, namely the long-distance transport by air and trucks and a storage period of unrooted cuttings up to several weeks. Immediately after severing the cuttings from the mother plant, leaves maintain a high photosynthetic capacity (Hegewald *et al.*, 1999), so if this is followed immediately by rooting under sufficient light intensity, carbohydrates should not be the limiting factor. However, transport and dark storage will affect the carbohydrate pool of the cuttings.

The quality of apical cuttings starts with the vigor of the stock plants, which is highly dependent on irradiance level and nitrogen availability (Zerche *et al.*, 1999). Drüge *et al.* (2000) studied the effect of nitrogen supply to stock plants (0.6, 1.5, or 4.0 g N m⁻² week⁻¹) on cutting quality, and this for increasing periods (2, 3, or 4 weeks) of dark cold storage (0.5 or 5°C) on the adventitious rooting of chrysanthemum. Internal nitrogen concentrations ranged from 2.7 to 6.5% of dry matter. Here, a positive relationship between the number of roots and root length and increasing nitrogen content was found, and this positive effect was found for both stored and unstored cuttings. In addition, the carbohydrate content was measured in this experiment; however, the conclusions were unclear as high nitrogen content correlated with high sucrose levels and decreasing starch levels. It is, however, clear that dark storage of cuttings results in depletion of carbohydrate reserves after $\pm$ 2 weeks (Drüge *et al.*, 2000).

A survey of chrysanthemum stock plants grown in Central America showed that the total nitrogen content of harvested cuttings ranged from 3.6 to 5.8% DM (average of 20 cultivars) (Zerche *et al.*, 2019). However, after shipping the cuttings to Germany, no correlation between nitrogen (total nitrogen ranged between 4.7 and 5.7% DM) and carbohydrates in leaves or stems was found, with an exception for glucose that positively correlated with N proteins. Shifts among the soluble and insoluble N pools after transport result from unpredictable events that affect carbohydrate content of the cuttings at harvest (DLI and temperature before harvest), followed by partial or total depletion of carbohydrates depending on the transport temperature and duration of dark storage. This can lead to a postharvest shift from N bound in proteins to soluble N pools, which can maintain respiration if carbohydrates are depleted. So, a sufficient and relatively high N content in the growing axillary shoots of the mother plant is needed before harvesting them as cuttings. A minimal 4.2% nitrogen content in chrysanthemum is advised to secure a qualitative rooted cutting, while lower values will increase the risk of reduced rooting (Drüge *et al.*, 2000; Zerche *et al.*, 2019).

5.8.2 Grafting to enhance stock plant's vigor and rooting quality of cuttings

Chrysanthemum apical cuttings are taken from healthy and vigorously growing stock plants. However, cutting vigor tends to decline over time after repeated harvests (multiple collections) from the same stock plants. Furthermore, the cutting homogeneity and quality (e.g. ease of root formation) also decline with the stock plant's age. This decline in vigor and quality may be associated with an internal imbalance in the hormone and metabolite levels and may also be associated with epigenetic changes (Drüge *et al.*, 2019); epigenetic changes are modifications arising from the growing environment that can potentially alter the genetic (inherent) properties of elite genotypes over time. The success of chrysanthemum propagation relies on adventitious

rooting ability and homogeneity of cuttings. Homogeneity of cuttings helps crop scheduling with the predictability of flowering time and harvest date (De Ruiter, 1996). The reduced number of cuttings due to lower vigor and insufficient rooting causes a significant economic loss for breeding companies and rooted cutting suppliers, despite intensive control over the growth factors (such as photoperiod, temperature, and relative humidity) in the chrysanthemum propagation industry. It is expensive and laborious to establish a new batch of stock plants to meet the need for vigorous and well-rootable cuttings; the development of techniques to extend the period for cutting collection is of significant economic interest (Zhang *et al.*, 2013).

To mitigate reduced productivity and rooting ability of cuttings, grafting of mother stock plants has been proposed. Grafting increased water and nutrient uptake, translocation and utilization efficiency with increased synthesis of endogenous hormones to improve physiological processes associated with growth, signal transduction, and development (Nawaz *et al.*, 2016). Hence, grafting is a way to increase the stock plant's cutting productivity and rooting ability. For example, the wild species used belongs to the herbaceous genus *Artemisia*, which, like chrysanthemum, belongs to the *Asteraceae* family and serves as a useful rootstock with a strong root system. Plants grafted by "hole insertion" grafting (described by Lee, 1994) on *Artemisia* showed higher vigor with enhanced rooting ability and tolerance to various biotic (*Alternaria* leaf spot—Deng *et al.*, 2012) and abiotic stresses (drought—Chen *et al.*, 2018). Cuttings from mother stock plants grafted onto *Artemisia scoparia* showed improved rooting ability, caused by higher auxin (IAA/IBA) levels, and improved growth compared with cuttings from non-grafted mother stock plants (Zhang *et al.*, 2013). Therefore, grafting to generate stock plants of elite genotypes can be expected in commercial practice eventually. However, grafting techniques and rootstock–scion relationships need to be optimized by selecting compatible rootstocks for thousands of chrysanthemum genotypes, in order to improve the vigor, cutting productivity, and rooting ability in chrysanthemum.

5.9 Conclusion and Future Prospects

The developmental physiology of chrysanthemums is shaped by a broad array of factors that influence their growth and development. The interplay between environmental cues—temperature, light intensity, and CO₂ concentration—and the plant's physiological responses critically determines the overall productivity and quality of chrysanthemums. Notably, different chrysanthemum genotypes exhibit distinct responses to temperature variations. The photoperiod plays a critical role in floral induction, while hormonal processes regulate adventitious rooting and shoot branching. These insights not only summarize current understandings but also pave the way for future research focused on optimizing these responses to environmental variables,

thereby enhancing cultivation practices and genetic selection for resilience and productivity. Utilizing grafting techniques has proven to enhance the vigor of stock plants and improve the rooting quality of cuttings. Optimizing these techniques and identifying compatible rootstock–scion combinations could further enhance propagation efficiency, leading to plants with improved health and stress tolerance. Long-term studies on grafting's effects are essential for refining commercial cultivation practices. Future research should focus on unraveling the genetic and molecular bases of chrysanthemum development. By understanding the regulation of key genes involved in phenology, root formation, and flowering, breeders can develop new varieties with desirable traits. Advances in genome editing, such as CRISPR/Cas9, offer opportunities for precise genetic enhancements to improve stress resistance, floral characteristics, and adaptability to environmental changes. As climate change continues to reshape agricultural practices, developing resilient chrysanthemum varieties is imperative. Breeding programs should prioritize traits that enhance tolerance to extreme temperatures and fluctuating photoperiods. Integrating advanced horticultural technologies, such as precision agriculture and controlled environment agriculture (CEA), can significantly improve the efficiency and sustainability of chrysanthemum production. Moreover, employing LED lighting to manipulate light quality and photoperiods could further optimize growth and flowering while minimizing energy consumption.

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