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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：　Theeraniti PUANGKRIT
Name

学位論文題目：
Title of Dissertation　Mechanism of Temperature Dependent Petal Coloration in Chrysanthemum
(キクにおける温度依存的花弁着色機構)

学位論文要約：
Dissertation Summary

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most popular ornamental plants as cut flowers, pot plants, landscape plants, and hobby plants. Chrysanthemums occupies an important position in world flower production, which is the second largest cut flower in the world flower market (Royal Flora Holland, 2016). Flower color is the most important trait in ornamental plants. Anthocyanins are responsible for flower color pink, red to purple, and orange to brown colors appears in the combination of anthocyanins and carotenoids. Anthocyanin biosynthetic enzymes are synthesized in the process of flower development. The anthocyanin biosynthesis in the epidermal cell of petals is induced during petal development through the increase of biosynthesis enzymes (Martin and Gerats, 1993). The facts indicate that it is very important to understand the influence of the environmental factors on petal coloration from the relationship with the developmental stage of petals. To understand the mechanism of poor coloration of pink flowered chrysanthemums under high temperature conditions and propose countermeasures in practical cut chrysanthemum flower production, this study was carried out.

The main anthocyanins in pink flowered cultivars of chrysanthemum are Cyanidin 3-O-(6″-O-monomalonyl-β-glucopyranoside); (Cy 3-6″-MMG) and Cyanidin 3-O-(3″,6″-O-dimalonyl-β-glucopyranoside); (Cy 3-3″,6″-DMG) (Nakayama et al., 1997). The content of main anthocyanins under both high (30°C) and low temperature (20°C) conditions was determined by HPLC. It has been reported that the contents of these two anthocyanins decrease under high temperature conditions (Nozaki et al., 2006b). The results showed the content of pigments at 20°C was much higher than those at 30°C. Two

anthocyanin contents reduced under 30°C from vertical stage to 2-weeks-old petals stage (2W), while anthocyanin contents increased from vertical stage (VE) to 1-week-old stage (1W) and then reduced rapidly at 2W stage at 20°C. The colorimeter was also used to evaluated petal color. Petals at 30°C showed low a^* value that represents the degree of red color compared with those at 20°C. a^* value in both 20° and 30°C decrease from vertical stage to 2W stage. To reveal the effect of high temperature in various developmental stages of inflorescence on pigmentation of petals, inflorescence development was divided into five stages: bud break stage (BB), petal extension starts stage (PE), VE, 1W, and 2W stage. Inflorescence were exposure to 20 or 30°C in different developmental stages. When 30°C was given during PE to VE, anthocyanin contents reduced drastically regardless temperature condition during BB to PE stage. Even though 30°C was given during BB to PE start, sufficient pigmentation occurred when the inflorescence was placed at 20°C during PE start to VE stage. The results indicate that pigmentation in the petals occurs mainly in the PE to VE stage. When the inflorescence was placed at 30°C during BB to VE, pigmentation did not enhance even though 20°C was given from VE to 1W stage. The results suggest that quick decomposition of anthocyanin occurred at 30°C. The results of colorimeter were coincident with the accumulation of anthocyanins. On the other hand, when 30°C was given during VE to 1W, pigment contents reduced drastically even though the inflorescence was kept at 20°C from BB to VE stage. The results indicate that PE to VE stage is the most temperature sensitive and important developmental stage for pigmentation.

To clarify the mechanism of low anthocyanin content under high temperature conditions, comprehensive gene expression by microarray experiment as conducted by using samples taken from four developmental stages of chrysanthemum flower. All anthocyanin biosynthesis-related genes were down-regulated at 30°C in comparison with those at 20°C though the time of a decrease of gene expression was different with each gene, and no novel candidate genes which are involved in anthocyanin synthesis pathway and showed significant changes by high temperature could be detected. ESTs showing high homology for anthocyanin biosynthesis-related genes showed that many ESTs were down-regulated. In chrysanthemum ESTs derived from Genbank, numbers of up-regulated ESTs at 30°C was much larger than the numbers of

down-regulated ESTs at 20°C, in all stage and from stage 2 to stage 4. Anthocyanin accumulation usually occurs at later stage of petal development (Weiss, 2000) and its biosynthesis genes expression started from early stage in petal development (Jackson et al., 1992; Martin et al., 1991; Gonzalez et al., 2008). The candidate genes involved in anthocyanin biosynthesis pathway could not detect in this analysis because ESTs annotated as anthocyanin biosynthesis related did not show consistent change although some ESTs showed specific gene expression patterns. To investigate real gene expression pattern by high temperature, anthocyanin biosynthesis-related genes showed expression variations in microarray profiling need to isolate. The anthocyanin biosynthesis-related genes (three types of *CmplCHS*, *CmplCHI*, and *CmplF3'H*, and two types of *CmplF3H*, *CmplDFR*, and *CmplANS*) were isolated. These sequences were slightly different from the known gene sequences of chrysanthemum because the different of genotype. The gene specific primers were designed between the ORF and 3' UTR and qRT-PCR was performed under various temperature conditions. All the isolated genes were contained with the ORF and 3' UTR and had a conserved region the same as the known genes. Chrysanthemum *CHS*, *F3H*, and *DFR* have been known as one type only (Sanjari et al., 2015; He et al., 2013); however, three types of *CHSs* were isolated, and two types each of *F3Hs*, and *DFRs* were isolated from 'Pelican' which slight differences. Recently, five *CHSs*, two *CHIs*, two *F3Hs*, two *F3'Hs*, and three *DFRs* were discovered through whole-transcriptome analysis of chrysanthemum ray and disc florets (Liu et al., 2016). These sequences have not yet been published, and the gene expression analyses were not performed by qRT-PCR. In this study, three types each of *CHSs*, *CHIs*, and *F3'Hs* and two types of *F3Hs*, *DFRs*, and *ANSs* were isolated from 'Pelican'. Expression analysis at 25°C showed that *CmplCHS1* and *CmplCHI* expressed as early biosynthetic genes and *CmplDFR1* and *CmplANS* expressed as late biosynthetic genes as reported in other plants. Previous studies using pink, red, and purple cultivars of 'Lijin' showed a development stage with a maximum gene expression level in *CmCHS*, *CmCHI*, *CmF3H*, *CmF3'H*, *CmDFR*, and *CmANS*, and were different in each cultivar (He at al., 2013). In particular, *CmF3H* showed a high expression level from the early stage, although some cultivars showed a constant high expression level until the fully opened stage and others showed a decrease in expression level from the inflorescence vertical stage. Furthermore, *CmDFR* showed a high expression level from the early development

stage, and then it showed half the high expression level at the fully opened stage, and *CmANS* reached a maximum gene expression level at the inflorescence vertical stage (He at al., 2013).

To reveal developmental stage-specific temperature effects on pigmentation and gene expression of flavonoid biosynthetic-related genes, between PE and 1W stage was subdivided into two new stages, namely early vertical stage and full open stage. Only *CmplCHS* was expressed considerably at the PE stage as an early gene in the anthocyanin biosynthesis pathway. According to petal development and anthocyanin accumulation, other gene expressions were up-regulated at 20°C. On the other hand, all gene expression was depressed at 30°C compared with those at 20°C.

As practical countermeasure on poor pigmentation of chrysanthemum petal under high temperature conditions application of plant growth regulators was investigated. The results showed that applying Benzyl aminopurine (BA) was effective to enhance pigmentation at both 20 and 30°C and restored petal coloration and pigmentation in chrysanthemum petals at 25°C. Applying 100 mg/L BA produced higher anthocyanin accumulation compared with the inflorescences treated with 50 mg/L BA at 20°C. The effect is BA-dose dependent and application-stage specific. The optimal developmental stage of inflorescences for BA application was the period of petal appearance to the petal elongated vertical position, which is the period when pigment accumulates most intensely in the petals of chrysanthemum.

The accumulation of anthocyanin in plant cells can be affected by many factors such as light (Sato et al., 1996), temperature (Nozaki et al., 2006a), phytohormone (Weiss, 2000), and sugar (Pasqua et al., 2005). Chrysanthemum is one of the important cut flowers, and production in Asia countries has been increasing in recent year. I focus on the fact that the flower color is poor in the high temperature period, and studied its mechanism and countermeasure. This study showed that poor coloration is occurred by decreases in anthocyanin accumulation and the lowered accumulation was brought by lowered gene expression of anthocyanin biosynthesis-related genes due to high temperature. Present study has revealed that early developmental stage of petal is the most high-temperature sensitive, the pigmentation is controlled by flavonoid biosynthesis related gene expression and BA treatment enhance pigmentation of petal under high temperature conditions. Through this

study, it was revealed that anthocyanin biosynthesis is suppressed at the transcriptional level by high temperature at the early stage of petal development, and the poor coloration is improved by application of BA in practice.

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