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学 位 論 文 要 旨
Dissertation Abstract

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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)

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Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the most popular ornamental plants and it has various flower type and flower color. Flower color is the most important trait in ornamental plants. Anthocyanins are major flower pigments. It is known that the change of petal color is caused by environmental conditions. 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. First, the content of main anthocyanins under both high (30°C) and low temperature (20°C) conditions was determined by HPLC. The colorimeter was also used to evaluated petal color. HPLC analysis show main anthocyanins in *C. morifolium* 'Pelican' were cyanidin 3–O– (6"–O–monomalonyl–β–glucopyranoside) and cyanidin 3–O– (3",6"–O–dimalonyl–β–glucopyranoside). 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, while anthocyanin contents increased from vertical stage to 1-week-old stage and then reduced rapidly at 2-weeks-old petal stage at 20°C. 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 2-week-old 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), vertical stage (VE), 1-week-old (1W), and 2-week-old stage (2W). 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. 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. 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. The results indicated that anthocyanin biosynthesis-related genes were down-regulated under 30°C condition, and no novel candidate genes which are involved in anthocyanin synthesis pathway and showed significant changes by high temperature could be detected. 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. 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. All genes 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^{-1} BA produced higher anthocyanin accumulation compared with the inflorescences treated with 50 mg L^{-1} BA at 20°C. The effect is BA-concentration 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.

Present study has revealed that early developmental stage of petal is the most high-temperature sensitive, the pigmentation is controlled by anthocyanin biosynthesis-related gene expression and BA treatment enhance pigmentation of petal under high temperature conditions.