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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
Analysis of mechanism of flower color mutations in bud-sported carnation cultivars
（カーネーション枝変わり品種群における花色変異機構の解明）

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The flower color diversity in carnation (*Dianthus caryophyllus* L.) cultivars is one of the attractive traits as commercial products. Carnation cultivars with various flower colors have been produced by conventional cross breeding but also bud sport. Bud sport breeding have contributed to the expansion of the number of carnation cultivars. However, the scientific knowledge of flower color mutation by bud sport in carnations remain inadequate.

A group of bud-sported carnation cultivars, namely “MINAMI series”, consists of pale yellow carnation ‘Poly Minami’ and its eight derivative cultivars having various flower colors, and the four cultivars show bicolor such as chintz and variegation. Additionally, the genealogy of bud sports has been recorded accurately. The “MINAMI series” is suitable for studies on flower color mutations by bud sport. Thus, this thesis aimed to clarify the mechanisms of the flower color mutations in the “MINAMI series”.

First, cultivar-specific flavonoid compositions were clarified by HPLC analysis. The eight cultivars except pinkish red carnation ‘Tommy Minami’ accumulated pelargonidin 3,5-cyclicmalyl-diglucoside (Pg3,5cMdG) and chalcononaringenin 2′-glucoside (Ch2′G) as major flower pigments in the petals. ‘Tommy Minami’ petals accumulated pelargonidin 3-malylglucoside (Pg3MG) majorly. The qRT-PCR analysis clarified that cultivar-specific flavonoid composition was determined by the expression levels of flavonoid biosynthetic genes; *CHI2*, *DFR*, *AA5GT*, *CHGTs* and/or an anthocyanin-transportation-related gene *GSTF2*. Especially, *DFR*, *AA5GT* and *GSTF2* were identified as causative genes of the bud sports.

The bud sports from ‘Poly Minami’ to ‘Orange Minami’ and from ‘Feminine Minami’ to ‘Tommy Minami’ were thought to be caused by change of chimeric structure of shoot apical meristem consisting three cell layers based on the genetic results. It has known that the frequent flower color mutations of periclinal chimera by irradiation is resulted by displacement of L1, which differentiates petal epidermis accumulating anthocyanins, by inner cell layer having different flower color genotype from that of the L1. This event is namely “cell layer displacement”. Here, to confirm whether cell layer displacement is involved in the two bud sports, the genotypes of *DFR* or *AA5GT* at each cell layer were determined. For genotyping, the L1-derived surface was separated from stems, and the rest of the stem was identified as L2+L3-derived tissues. Then, genomic PCR using the separated stem tissues was performed. Additionally, anthocyanins and petal color of the partial petal color mutations were also investigated to predict the flower color genotype of inner cell layer. These results concluded that the two bud

sports were attributed to spontaneous cell layer displacement.

There are few molecular-based reports regarding flower color patterns except variegation in carnations. Thus, the mechanism of the bud sport containing a bicolor carnation was investigated using ‘Orange Minami’ and its sport. Pigment analysis postulated that the bud sport from bicolor flower ‘Orange Minami’ (petal color of distal and proximal parts is pink and yellowish orange, respectively) to single deep pink flower ‘Feminine Minami’ is involved in the alternation of the upstream metabolism of flavonoid biosynthetic pathway. To explore causative genes of the bud sport, RNA-seq analysis was performed. As a result, the transcriptional levels of two genes encoding cinnamoyl-CoA reductase-like (CRL), which is likely associated with lignin biosynthesis competing with flavonoid biosynthesis, were remarkably different between the two cultivars. Furthermore, the qRT-PCR analysis showed that the expression levels of *CRL1/2* genes were very low in the distal part of the petals of ‘Orange Minami’ and a whole petal of ‘Feminine Minami’, whereas *CRL1/2* were expressed highly in the proximal parts of ‘Orange Minami’ petals. These results indicated that topographic expression of *CRL1/2* is important for chintz formation. In addition, the suppression of *CRL1/2* transcription might be involved in the bud sport.

These studies newly clarified that *CHGTs*, *CHI2* and *CRL1/2* are involved in flower color variation. This outcome will surly provide the valuable information for molecular breeding of flower color with genome editing and transgenic experimentation. Additionally, molecular biological and histological analyses demonstrated that cell layer displacement is also involved in spontaneous mutation as a cause of bud sport except transposition. Diagnosis of chimerism of carnations by targeting flower color mutation-related genes such as *DFR* and *AA5GT* will contribute to the production of new cultivars with bud sport breeding.