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

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学位論文題目：　Analysis of mechanism of flower color mutations in bud-sported carnation cultivars
Title of Dissertation　（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）

学位論文要約：
Dissertation Summary

Carnation is one of the most important ornamental flowers and well-known as a main item for Mother's Day. Carnation cultivars have various flower colors, pink, red purple, white, green, and so on. The diversity of flower color is attractive for flower market. Carnation cultivars having various flower colors have been produced by conventional cross breeding and spontaneous somatic mutation, namely bud sport. Bud sport is a scientific term that a branch or a few branches having different phenotypic traits from the original branches. The bud sport has contributed to the expansion of the cultivar number of horticultural plants including carnations. However, molecular-based knowledge of flower color mutations by bud sport has remained inadequate in carnations.

'Poly Minami' having pale-yellow flower has been cultivated in Kagawa prefecture. Several sports showing different flower colors from the original color were discovered in 'Poly Minami' cultivation field. At present, 'Poly Minami' and its sports are cultivated and shipped as namely "MINAMI series". The "MINAMI series" cultivars have various flower colors; pale yellow, yellow, off-white, orange, yellowish orange, pinkish orange, deep pink, pinkish red. Additionally, the several cultivars of the "MINAMI series" display bicolor such as chintz and variegation. The directions of bud sports have been recoded accurately. Therefore, the "MINAMI series" cultivars should be suitable for studies on flower color mutations by bud sport. In this research, nine cultivars of the "MINAMI series" were used to clarify the mechanism of the flower color mutations by bud sport in carnations.

Identification of gene associated with flower color mutations

To clarify causative genes associated with flower color mutations, flavonoid pigments and gene expression analyses were performed. In this research, flower-bud development was divided into five stages as follows; stage 1, closed bud; stage 2, bud break; stage 3, top of inflorescence opened; stage 4, vertically elongated flowers; stage 5, fully-opened flowers. Based on flavonoid composition and expression patterns of flavonoid biosynthesis-related genes, genes associated with flower color variation were identified.

Pale-yellow petals of 'Poly Minami' accumulated chalcononaringenin 2′-glucoside without anthocyanin due to the very low expression levels of dihydroflavonol 4-reductase (DFR) genes. Considering that only non-functional *DFR2* carrying footprint which leads frameshift mutation was isolated from 'Poly Minami', 'Poly Minami' might be a periclinal chimera whose L1 of shoot apical meristem (SAM) lacks functional *DFR1*. The three cultivars, 'Lemon Minami', 'Vanilla Minami' and 'Orange Minami' were derived from 'Poly Minami' by bud sports. Yellow petals of 'Lemon Minami'

also showed very low expression levels of *DFR* as well as its derivative parent. Additionally, the petals of ‘Lemon Minami’ showed lower expression levels of chalcone isomerase 2 (CHI2) genes at stages 1 and 2, resulted in promoting of Ch2´G accumulation by suppressing the conversion of chalcononaringenin into naringenin. Additionally, *CHGT3/4/5* were expressed much highly in ‘Lemon Minami’ petals in comparison with ‘Poly Minami’. Off-white petals of ‘Vanilla Minami’ accumulated very small amount of anthocyanins due to very low expression levels of *DFR* through flower-bud development. The petals of ‘Vanilla Minami’ expressed chalcononaringenin glycosyltransferases (CHGT) genes lowly as compared with ‘Poly Minami’, resulting in decrease of Ch2´G and increase of flavonoids. Orange petals of ‘Orange Minami’ accumulated pelargonidin 3,5-cyclicmalyl-glucoside (Pg3,5cMdG). The bud sport from ‘Poly Minami’ to ‘Orange Minami’ was resulted by the reversion of *DFR1*. Three cultivars, ‘Minami’, ‘Passion Minami’ and ‘Feminine Minami’ were derived from ‘Orange Minami’ by bud sports. Yellowish orange petals of ‘Minami’ showed lower expression levels of *CHI2* at stage 1 and higher expression levels of *CHGT3/4/5* through flower-bud development as well as the bud sport of ‘Poly Minami’ to ‘Lemon Minami’, resulting in accumulations of a larger amount of Ch2´G and a smaller amount of anthocyanin. Pink color of distal parts of ‘Passion Minami’ petals was deeper than ‘Orange Minami’. The petals of ‘Passion Minami’ showed higher expression levels of *CHI2* at stage 1 and lower expression levels of *CHGT2* through flower-bud development than ‘Orange Minami’, resulting in a larger amount of Pg3,5cMdG and a smaller amount of Ch2´G. Deep-pink petals of ‘Feminine Minami’ showed lower expression levels of *CHI2* at stages 1 and 2 and very lower expression levels of *CHGT2/3/4/5* through flower-bud development than ‘Orange Minami’. As the result, ‘Feminine Minami’ accumulated a larger amount of Pg3,5cMdG and a very smaller amount of Ch2´G in the petals. The two cultivars, ‘Tommy Minami’ and Pinkish red-flowered carnation ‘Tommy Minami’ derived from ‘Feminine Minami’ accumulated pelargonidin 3-malylglucoside (Pg3MG), a precursor of Pg3,5cMdG, as major flower pigment in the petals. Additionally, the accumulation of Pg3MG in ‘Tommy Minami’ was resulted by low transcriptional levels of acyl-glucose-depended anthocyanin glucosyltransferase (AA5GT) gene. Furthermore, the expression pattern of *AA5GT* in ‘Tommy Minami’ suggested that ‘Tommy Minami’ was a periclinal chimera. ‘Sakura Minami’, derived from ‘Feminine Minami’, has pale-pink-colored flowers variegated with sectors and flecks showing deep pink. The expression analysis for two glutathione *S*-transferase-like 2 (GSTF2) genes associated with anthocyanin transportation into vacuole was carried out. The functional *GSTF2* and non-functional *GSTF2* having nonsense mutation were expressed highly in sectors and flecks of petals of ‘Sakura Minami’ and ‘Feminine Minami’, whereas the expression levels of functional *GSTF2* but not non-functional *GSTF2* were very low in pale-pink-colored parts of petals of ‘Sakura Minami’.

These results clarified that cultivar-specific flavonoid compositions in the “MINAMI series” were determined by the expression levels of *CHI2*, *DFR*, *AA5GT*, multiple *CHGTs*, and *GSTF2*. Especially, *DFR*, *AA5GT* and *GSTF2* were key genes of three bud sports from ‘Poly Minami’ to ‘Orange Minami’, from ‘Feminine Minami’ to ‘Tommy Minami’ and from ‘Feminine Minami’ to ‘Sakura Minami’, respectively.

Elucidation of mechanisms of two bud sports by tissue-specific

The pigment and gene expression analyses suggested the causative genes of the bud sports from ‘Poly Minami’ to ‘Orange Minami’ and from ‘Feminine Minami’ to ‘Tommy Minami’ were *DFR* and *AA5GT*, respectively. Additionally, these cultivars are likely periclinal chimeras. Therefore, layer-specific genomic PCR targeting causative genes was performed to clarify causes of the bud

sports. For genotyping in L1 cells, the L1-derived surface and L2+L3-derived interior tissues were separated from carnation stem.

Genomic PCR indicated that L1 of ‘Poly Minami’ and its sports having acyanic flowers had only *DFR2* but not functional *DFR1* and L2+L3 had *DFR1* and *DFR2*. On the other hand, L1 and L2+L3 of ‘Orange Minami’ had both *DFR1* and *DFR2*. The flower color genotype was further determined through survey of partial petal color mutation, namely “sinus blotch”. Sinus blotch is known as histological marker predicting flower color genotype in inner cell layer. The flower color of sinus blotch refers to flower color genotype of inner cell layer of the plant having sinus blotch. The results of the survey indicated that a few ‘Poly Minami’ flowers had sinus blotch whose flower color is similar to that of ‘Orange Minami’. Additionally, major anthocyanin in the sinus blotches was also Pg3,5cMdG as well as ‘Orange Minami’. These results concluded that the bud sport was involved in displacement of L1 by inner cell layer having ‘Orange Minami’ genotype.

Next, the tissue-specific genomic PCR targeting *AA5GT* was performed in ‘Feminine Minami’ and its sport ‘Tommy Minami’. The genomic PCR and expression analysis of *AA5GT* carrying retrotransposon *Ty1dic1* showed that L1 of ‘Feminine Minami’ was heterozygous for functional *AA5GT* and *AA5GT/Ty1dic1*, whereas L1 of ‘Tommy Minami’ was homozygous for had only *AA5GT/Ty1dic1*. However, the L2+L3-derived tissues of both cultivars had *AA5GT* and *AA5GT/Ty1dic1*. Furthermore, the bud sport was not involved in a new *Ty1dic1* insertion into functional *AA5GT* of L1 of ‘Feminine Minami’. To determine flower color genotype of inner cell layer of ‘Feminine Minami’, partial petal color mutation was also investigated as ‘Poly Minami’. The results showed that petals of ‘Feminine Minami’ occasionally had red-pigmented streaks, accumulating Pg3MG majorly. Considering that streak is also known as histological marker predicting flower color genotype of inner cell layer, inner cell layer of ‘Feminine Minami’ had red flower genotype, which was likely homozygous for *AA5GT/Ty1dic1*. These results concluded that the bud sport from ‘Feminine Minami’ to ‘Tommy Minami’ was also caused by the displacement of L1 by inner cell layer having ‘Tommy Minami’ genotype as well as the bud sport from ‘Poly Minami’ to ‘Orange Minami’.

This research indicated that flower color mutations by two bud sports in the “MINAMI series” were involved in cell layer displacement but not transposition.

Analysis of mechanism of bud sport from a bicolor carnation to a single-colored carnation

Flower color pattern is one of important traits for flower market. Thus, the molecular mechanisms of formation of flower color pattern have been studied in several ornamental flowers but not carnations. It has known that carnation cultivars having bicolor flower are sported from single-colored carnation cultivars, *vice versa*. However, few reports noted the molecular mechanism of the bud sport containing cultivars with bicolor flower. The “MINAMI series” cultivars also contain the bud sports from bicolor flowers to single-colored flowers. Here, the mechanism of the bud sport from ‘Orange Minami’ to ‘Feminine Minami’ was investigated by topographic analysis of petal coloration. Pigment analysis showed that the amount of anthocyanins and the total amounts of flavonols and Ch2′G in distal parts of petals of ‘Orange Minami’ were larger than proximal ones, but the expression levels of flavonoid biosynthesis-related genes except *CHGT* genes did not differ between the distal and proximal parts of the petals. Thus, RNA-seq analysis was performed to explore the other genes affecting the amount of flavonoids. As a result, the expression levels of two cinnamoyl-CoA reductase-like (CRL) genes annotated as Dca460 and Dca470 in carnation DB were remarkably

different between both cultivars. The CRL is known as enzyme associated with lignin biosynthesis. The qRT-PCR analysis indicated *CRL1/2* were expressed highly in the proximal parts of 'Orange Minami' petals as compared with the distal parts and 'Feminine Minami' petals accumulating large amounts of flavonoids. These results indicated that topographic transcriptions of *CRL1/2* changed flavonoid composition within petals, resulting in flower color pattern. Therefore, *CRL1/2* might be key genes of the bud sport from bicolor carnation 'Orange Minami' to single-colored carnation 'Feminine Minami'.

In conclusion, through studies on the spontaneous flower color mutations of the "MINAMI series" having various flower colors, *CHI2*, multiple *CHGT* genes and *CRL1/2* were newly identified as key genes associated with flower color variation. Furthermore, tissue-specific genomic PCR analysis clarified cell layer rearrangement was also involved in flower color mutation as well as transposition. And then, topographic analysis within petals showed trade-off relation between flavonoid and lignin biosyntheses is responsible for flower coloration. The outcomes obtained in the studies will surly contribute to the provision of the valuable information for flower color modification and further development of bud sport breeding.