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論文内容の要旨

Title: Molecular genetic analysis of flower coloration in the Japanese morning glory

The genus *Ipomoea* includes approximately 600 species distributed on a worldwide scale that are characterized by a diversity of floral morphologies and coloration pattern. Most *Ipomoea* species can be found in the Americas, particularly in Mexico, and those widely distribution are covered with the Pacific basin and Africa, Asia and Australia. The genus *Ipomoea* can be divided into following three subgenuses, *Eriospermum*, *Ipomoea* and *Calystegia*. Among the subgenus *Ipomoea*, three morning glories, *I. nil* (the Japanese morning glory) with blue flowers, *I. purpurea* (the common morning glory) with dark purple flowers, and *I. tricolor* with blue flowers, were domesticated well as ornamental plants, and many mutants displaying various flower colors were isolated. The blue flowers of *I. nil* and *I. tricolor* contain a polyacylatyed cyanidin-based anthocyanin, termed Heavenly Blue Anthocyanin (HBA), as a major pigment, whereas the dark purple flowers of *I. purpurea* contains a cyanidin derivative that lacks a methyl residue and one glucose molecule from HBA.

The Japanese morning glory had been introduced into Japan from China as a medical herb approximately in the 8th century, and a number of spontaneous mutants displaying various flower colors have been isolated since the 17th century. According to the classical genetic studies, mutations affecting flower pigmentations can be classified mainly into three categories: mutations conferring white flowers, mutations affecting blue flower coloration, and mutations influencing flower hue. Mutations conferring white flowers can be classified into four groups, *a*, *c*, *ca*, and *r*; the *a* and *r* mutants exhibit white flowers with green stems and normal-colored black seeds, and the *c* mutants display white flowers with red stems and normal-colored seeds, while the *ca* mutants produce white flowers with green stem and ivory seeds. Blue flower coloration was regarded to be mainly controlled by two genetic loci, *Magenta* and *Purple*. Recessive *magenta* and *purple* mutants bloom magenta and purple flowers, respectively, and double mutants carrying both *magenta* and *purple* alleles display red flowers. Three loci, *Dusky*, *Duskish* and *Dingy*, control flower hue, and recessive mutations in one of these loci confer dull colored flowers.

The anthocyanin biosynthesis pathway is well documented, and the genes for anthocyanin biosynthesis can be divided between structural genes for enzymes involved in anthocyanin biosynthesis and regulatory genes for transcription factors acting on the structural genes. The structural genes encoding enzymes to produce anthocyanidin 3-*O*-glucsides, which are the first major stable colored pigments in the anthocyanin biosynthesis pathway have been identified and characterized. Further modifications of anthocyanidin 3-*O*-glucsides including glycosylation, acylation, and methylation can occur in a species-specific manner, although only limited information about the genes responsible for these modification processes is available. The transcriptional regulators encoded by the regulatory genes are known to include members of proteins containing an R2R3-MYB domain, a bHLH (basic helix-loop-helix) domain, and conserved WD40 repeats (WDRs), and the combinations of the R2R3-MYB, bHLH, and WDR

factors and their interactions determine the set of genes to be expressed. Among the mutations affecting flower pigmentations, *a* and *r* such as *a-3, r-1* and *r-3* are mutations in the structural genes encoding dihydroflavonol 4-reductase (DFR), chalcone synthase (CHS) and anthosyanidin synthase (ANS), respectively. For the genes controlling blue flower coloration, the *Purple* gene was shown to be *NHX1* encoding a vacuolar Na^+/H^+ exchanger, which is responsible for increasing vacuolar pH in the petals during flower opening.

In this dissertation, he briefly introduced flower pigmentation of three morning glories, *I. nil*, *I. purpurea,* and *I. tricolor,* the genes for biosynthesis of anthocyanin pigments, and the *I. nil* spontaneous mutations that control the flower coloration. In Chapter 2, he described that spontaneous mutations in *F3'H*, *magenta, pink,* and *fuchsia,* conferring reddish flowers are shown to be a nonsense mutation caused by a single C to T base transition generating the TGA stop codon in *I. nil*, an insertion mutation caused by 0.55-kb DNA transposon *Tip201* belonging to the *hAT* superfamily in *I. purpurea*, and a single T insertion generating the stop codon TAG in *I. tricolor*, respectively. Although various plants exhibiting reddish flowers are postulated to carry mutations controlling the *F3'H* activity, none of them have been identified and only a few *F3'H* mutations identified are those affecting seed coloration; the *tt7* mutation in *Arabidopsis* conferring pale brown seeds and reduced anthocyanin content to the whole plant is caused by a single C to T base transition generating the stop codon TAA, and the recessive *t* mutant in soybean affecting pigmentation in its seed coats and trichome hairs is a frameshift (a single C deletion) mutation. Therefore, the characterization of the *magenta, pink,* and *fuchsia* mutations in these three morning glories were the first report on the mutation in the *F3'H* gene conferring reddish flowers. In Chapter 3, he described that the *dusky* mutation in *I. nil* conferring reddish-brown or purplish-grey hue in the petals are frameshift mutations caused by 4-bp insertions at an identical position near the 3' end of the *3GGT* gene for a novel glucosyltransferase, UDP-glucose:anthocyanidin 3-*O*-glucoside-2"-*O*-glucosyltransferase, which mediates the glucosylation of anthocyanidin 3-*O*-glucosides to yield anthocyanidin 3-*O*-sophorosides. Except for the *3GGT* gene described here, there has been only one gene, whose mutation is characterized in the genes mediating an addition of a sugar residue to the glucose molecule of anthocyanidin 3-*O*-glucosides; the petunia *Rt* gene encoding UDP-rhamnose:anthocyanidin 3-*O*-glucoside-6"-*O*-rhamnosyltransferase (3RT), which controls the conversion of anthocyanidin 3-*O*-glucosides into anthocyanidin 3-*O*-rutinosides. In Chapter 4, he first characterized the tissue-specific expression of three *MYB* genes, three *bHLH* genes, and two *WDR* genes in *I. nil*. Subsequently, he showed that the *c* and *ca* mutations are frameshift mutations caused by a 2-bp deletion and 7-bp insertions in the genes for the R2R3-MYB and WDR transcriptional regulators designated as MYB1 and WDR1, respectively. In addition to defects in flower, stem, and seed pigmentations, he also found that the *ca* mutants show reduced trichome formation in seeds, which is a novel epidermal traits associated with the transcriptional factors.

Based on these results, he also discusses in Chapter 5 that the three morning glories, *I. nil*, *I. purpurea,* and *I. tricolor*, can collectively serve good models for the elucidation of anthocyanin pigmentation in the flowers, because the identified mutations affecting flower pigmentation in these

Ipomoea are comparable with or slightly exceed the characterized mutations for flower coloration in petunia, which is regarded to be a model plant for flower pigmentation.

論文の審査結果の要旨

サツマイモ属（*Ipomoea*）は約600の植物種よりなり、世界各地に広く分布し、花の色や形態も様々であるが、その中のアサガオ（*I. nil*）、マルバアサガオ（*I. purpurea*）、ソライロアサガオ（*I. tricolor*）は園芸植物として早くから栽培化され、多彩な花色の自然突然変異体が多数分離されている。これらアサガオとその近縁種の野生型の花の色素は、一般的には赤い色を呈するシアニジン系のアントシアニン色素であるにもかかわらず、青色もしくは濃紺色であり、これは複雑に配糖化やアシル化された色素構造と液胞ｐＨが例外的に高いためと考えられている。花色の変異に関する研究は、主にペチュニア、次いでキンギョソウで行われてはいるが、まだ未解明の問題も多い。

申請者は、主にアサガオの自然突然変異体を用いて色調、特に赤系の花を咲かせ、P450系のflavonoid 3'-hydroxylase (F3'H)をコードする遺伝子の変異や、鼠色ないしは茶色がかった色調の花を咲かせてanthocyanidin 3-glucosideをanthocyanidin 3-sophoroside に換える配糖化酵素をコードする*3GGT*遺伝子の変異の同定、白色花を賦与するR2R3-MYBやWD40 repeatsをもつ転写調節因子をコードする遺伝子と変異の同定及び解析を行い、アサガオとその近縁種を従来最もよく研究されているペチュニアをも凌ぐ花色発現に関するモデル植物の地位にまでに引上げる成果をあげている。

第１章序論に引続き、第２章でアサガオ、マルバアサガオ、ソライロアサガオの各*F3'H*遺伝子の自然突然変異を同定し、各変異の*F3'H*遺伝子発現への影響を詳細に検討して国際専門誌に発表している。この論文は、*F3'H*遺伝子の変異に関する最初の論文となった。次いで、第３章ではアサガオの*3GGT*遺伝子とその変異を初めて同定し、anthocyanidin 3-glucoside以降の配糖化過程を確定し、自然突然変異の生成過程や色調との関係を論議している。第４章では、先ずアサガオの３種類の*R2R3-MYB*遺伝子*MYB1*、*MYB2*、*MYB3*、３種類の*bHLH*遺伝子*bHLH1*、*bHLH2*、*bHLH3*、２種類の*WDR*遺伝子*WDR1*、*WDR2*を分離同定し、各遺伝子の組織特異的発現を詳細に解析している。次いで、花色だけを白色化して茎や種子の色は野生型と変らない*MYB 1*遺伝子の自然突然変異を同定し、さらに白花色を咲かせて茎も緑色で種子も象牙色となる*WDR1*遺伝子の変異も同定して、この変異では種子表面のtrichome形成にも影響を与えることも見出している。このような種子trichomeの形成はペチュニアでは見出されず、アサガオで初めて見出された遺伝形質である。申請者はさらにこれらの変異体を用いて、花色発現に係わる遺伝子発現への影響を経時的に解析し、ペチュニアなどで得られつつある知見との類似点と相違点についても詳細な議論を展開している。第５章は、本研究で得られた知見を基に総合的討論を行っている。

以上の如く、アサガオとその近縁種の花色発現に関する申請者の研究の意義は極めて大きく、今後の当該分野の研究を拓くものであり、審査委員会は全員一致で学位論文として十分な内容を持つものと判定した。