Theor Appl Genet (1997) 95: 509–515

Y. Hisatomi · Y. Yoneda · K. Kasahara
Y. Inagaki · S. Iida

DNA rearrangements at the region of the dihydroflavonol 4-reductase gene for flower pigmentation and incomplete dominance in morning glory carrying the mutable *flaked* mutation

Received: 4 November 1996 / Accepted: 13 December 1996

Abstract The *a-3*^{flecked}[J] variegated line of Japanese morning glory bearing white flowers with normal-colored flecks and sectors has been shown to carry a 6.4-kb transposable element, *Tpn1*, inserted within the *DFR-B* gene, one of the anthocyanin biosynthesis genes encoding dihydroflavonol 4-reductase (DFR). The *a*^{flaked}[M] variegated line of morning glory also bears white flowers with normal-colored flakes and sectors, and it was shown to carry multiple DNA rearrangements, including insertions of mobile element-like sequences, *MELSIP1* and *MELSIP2*, in its *DFR* gene region. Unlike the *a-3*^{flecked}[J] mutation, the mutable *a*^{flaked}[M] allele exhibited incomplete dominance. Interestingly, not only intensely colored flakes but also white spots and sectors were often observed in lightly colored flowers of morning glory in the heterozygous state *A*[M]/*a*^{flaked}[M]. The interspecific F_1 hybrids between Japanese morning glory and morning glory carrying both *a-3*^{flecked}[J]/*A-3*[M] and *A*[J]/*a*^{flaked}[M] in the heterozygous condition bear lightly colored flowers with intensely colored sectors as well as white flakes. The results clearly demonstrated that the *DFR* gene in the *a*^{flaked}[M] line of morning glory is active and complements the *DFR-B* gene carrying *Tpn1* in the *a-3*^{flecked}[J] line of Japanese morning glory. Interspecific allelic interactions between the mutable *a*^{flaked}[M] gene of morning glory and the corresponding wild-type *A*[J] gene of Japanese morning glory resulted in incomplete dominance and the formation of white flakes and sectors. The appearance of the white flakes may be due to a somatic mutation of the *A*[J] gene.

Key words Allelic interaction · Dihydroflavonol 4-reductase gene · Mobile element-like sequences · Morning glory · Mutable allele

Communicated by I. Potrykus

Y. Hisatomi
Department of Biological Science and Technology, Science University of Tokyo, Yamazaki, Noda 278; Division of Gene Expression and Regulation I, National Institute for Basic Biology, Myodaiji, Okazaki 444; and Department of Molecular Biomechanics, The Graduate University for Advanced Studies, Myodaiji, Okazaki 444, Japan

S. Iida (✉)
Department of Biological Science and Technology, Science University of Tokyo, Yamazaki, Noda 278; and (✉) Division of Gene Expression and Regulation I, National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan

K. Kasahara · Y. Inagaki[1]
Department of Biological Science and Technology, Science University of Tokyo, Yamazaki, Noda 278, Japan

Y. Yoneda
Biological Institute, Department of Liberal Arts, Shizuoka University, Ohya, Shizuoka 422, Japan

Present address:
[1]Department of Bacteriology, National Institute of Health, Toyama, Shinjuku-ku, Tokyo 113, Japan

Introduction

Various plants bearing colored flecked, speckled or sectored flowers which reflect somatic instability in anthocyanin pigmentation have been described, and these mutable alleles are believed to be caused by mobile genetic elements (Nevers et al. 1986; Coen et al. 1989; Martin and Gerats 1993; Forkmann 1994). The *a-3*^{flecked} variegated line of Japanese morning glory (*Pharbitis nil* or *Ipomoea nil*) bears white flowers with normal-colored flecks and sectors (Fig. 1A), and its variegated pigmentation also occurs in leaves and stems (Inagaki et al. 1994). We have shown that the mutable *a-3*^{flecked} allele is caused by integration of the 6.4-kb transposable element *Tpn1* into the *DFR-B* gene, one of the three copies of the *DFR* genes in the genome of Japanese morning glory (Inagaki et al. 1994, 1996;

Hoshino et al. 1995). Since reversion of the variegated phenotype coincides with the excision of *Tpn1* from the *DFR-B* gene in the germinal revertants and their progeny, the *DFR-B* gene must be responsible for pigmentation in the flowers, leaves, and stems.

Morning glory (*Pharbitis purpurea* or *Ipomoea purpurea*), often called common morning glory, bears purple flowers (Fig. 1B) and is closely related to Japanese morning glory although their interspecific hybrids were difficult to obtain (Yoneda 1990). Several genetic studies mainly on flower color variations of morning glory have been conducted, and these include reports of variegated pigmentation in flowers and leaves (Barker 1917; Imai 1927; Imai and Tabuchi 1935; Kasahara 1956; Ennos and Clegg 1983; Schoen et al. 1984; Epperson and Clegg 1987, 1988, 1992). The best-studied allele in morning glory is the mutable allele called *flaked* (Barker 1917; Imai and Tabuchi 1935; Kasahara 1956). The *flaked* mutant bears white flowers with colored flakes and sectors (Fig. 1C), and colored variegation also occurs in the stems and leaves. The *flaked* mutation was localized in the *W* locus, which controls anthocyanin biosynthesis, and the *flaked* mutable allele was termed *w′* by Kasahara (Kasahara 1956). Later, probably the same locus was independently termed *A* and the corresponding mutable allele *a** (Epperson and Clegg 1987, 1992).

Here we have adopted the term *anthocyanin*^{flaked} (a^{flaked}) or a^{f} for the mutable *flaked* allele in the *A* locus instead of either *w′* or *a**. In this paper, moreover, the symbol [M] was added to the alleles derived from morning glory and [J] to those from Japanese morning glory in order to avoid confusion.

One of the characteristics of the *A*[M] locus of morning glory is that the defective allele *a*[M] as well as the mutable allele a^{flaked}[M] show incomplete or partial dominance (Imai 1927; Imai and Tabuchi 1935; Epperson and Clegg 1987, 1992). Thus, morning glory in the homozygous state a^{flaked}[M]/a^{flaked}[M] produces flowers with colored flakes in a white background (Fig. 1C), whereas the plant in the heterozygous state bears lightly colored flowers with intensely colored flakes and sectors (Fig. 1D). Like the *a-3*^{flecked}[J] mutation of Japanese morning glory (Imai 1931, 1934; Inagaki et al. 1994, 1996), the variegation in the a^{flaked}[M] allele of morning glory is regarded to be caused by recurrent somatic mutations from the white to the pigmented allele producing anthocyanin; its germinal reversion is also known to occur (Imai and Tabuchi 1935; Epperson and Clegg 1987, 1992). Unlike the *a-3*^{flecked}[J] mutation, white spots and sectors are often observed in the heterozygous *A*[M]/a^{flaked}[M] flowers (Imai and Tabuchi 1935; Kasahara 1956; Epperson and Clegg 1987, 1992) (Fig. 1D).

These observations suggest the possible involvement of a mobile genetic element in the generation of the mutable a^{flaked}[M] allele in one of the genes for anthocyanin pigmentation. To elucidate molecular mechanisms for the formation of the variegated phenotypes in morning glory, we are attempting to characterize the mutable a^{flaked}[M] gene. The present paper reports the results of our investigation on the *DFR* genes in the mutable a^{flaked}[M] line of morning glory.

Materials and methods

Plant materials

The line KK/FP-36 of morning glory bears wild-type purple flowers (Fig. 1B). The morning glory line KK/VR-37 carrying the mutable *flaked* allele (a^{flaked}[M]/a^{flaked}[M]) produces white flowers with red variegation (Fig. 1C). Both of these lines are from K. Kasahara's private collection. The line KK/SSB-4 of Japanese morning glory carrying the mutable *flecked* allele (*a-3*^{flecked}[J]/*a-3*^{flecked}[J]) caused by the insertion of *Tpn1* within the *DFR-B* gene was described by Inagaki et al. (1994) (Fig. 1A). The line Seiwa SU2001 carrying the mutable *flaked* allele (a^{flaked}[M]/a^{flaked}[M]) is a interspecific hybrid between an African variety of Japanese morning glory and morning glory producing the flower variegation (Yoneda 1976, 1979) (Fig. 1E). Crossings between the lines KK/SSB-4 and Seiwa SU2001 as well as between the lines KK/VR-37 and Seiwa SU2001 were carried out as described previously (Yoneda 1976,1979). Plants were grown in pollinator-free greenhouses.

Isolation and analysis of genomic DNA from morning glory

Plant DNA was extracted from leaves by the CTAB (hexadecyltrimethylammonium bromide) method (Murray and Thompson 1980). Approximately 10 μg of DNA was digested with appropriate restriction enzymes, separated on 0.8% agarose gels, and subjected to Southern hybridization (Sambrook et al. 1989; Inagaki et al. 1994). The 1.5-kb petunia *DFR-A* gene cDNA (Beld et al. 1989) was used as a probe.

Genomic cloning and characterization of cloned fragments

General methods for genomic cloning were according to Sambrook et al. (1989). Approximately 100 μg of plant DNA was completely digested with the restriction enzyme B*gl*II and fractionated by sucrose density gradient centrifugation (Inagaki et al. 1994). The genomic 17-kb B*gl*II fragments containing the *DFR* gene of morning glory from lines KK/FP-36 and KK/VR-37 were cloned into the B*am*HI site of λGEM11 (Promega) to yield λF-36Dfr and λV-37Dfr, respectively. Two clones obtained from KK/FP-36 carried an identical fragment, and it was cleaved out by *Xho*I and recloned into the plasmid vectors pSHI901 (Bilang et al. 1991) to yield pDRA1001. Similarly, two clones obtained from KK/VR-37 contained the same fragment, which was recloned into the plasmid vector pBluescript II SK^{-} (Stratagene) to yield pDRA2001. Appropriate segments on these plasmids were subcloned into pBluescript II SK^{-} for further molecular analysis. The host *Escherichia coli* K12 strains used for λ phage and plasmid vectors are LE392 and JM109, respectively (Sambrook et al. 1989). For digestion of plasmids with restriction enzymes affected by DNA methylation, the plasmids were also grown in *E. coli* K12 MG48 *dam dcm* (Marinus 1973).

To map the *DFR* sequences in the cloned fragment, we used the 1.5-kb *DFR-B* cDNA of Japanese morning glory (Y. Inagaki, unpublished) and its exon 1 (Probe 5′) or exons 4 and 5 (Probe 3′) as hybridization probes. DNA sequencing was performed by the chain termination method (Sambrook et al. 1989).

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases

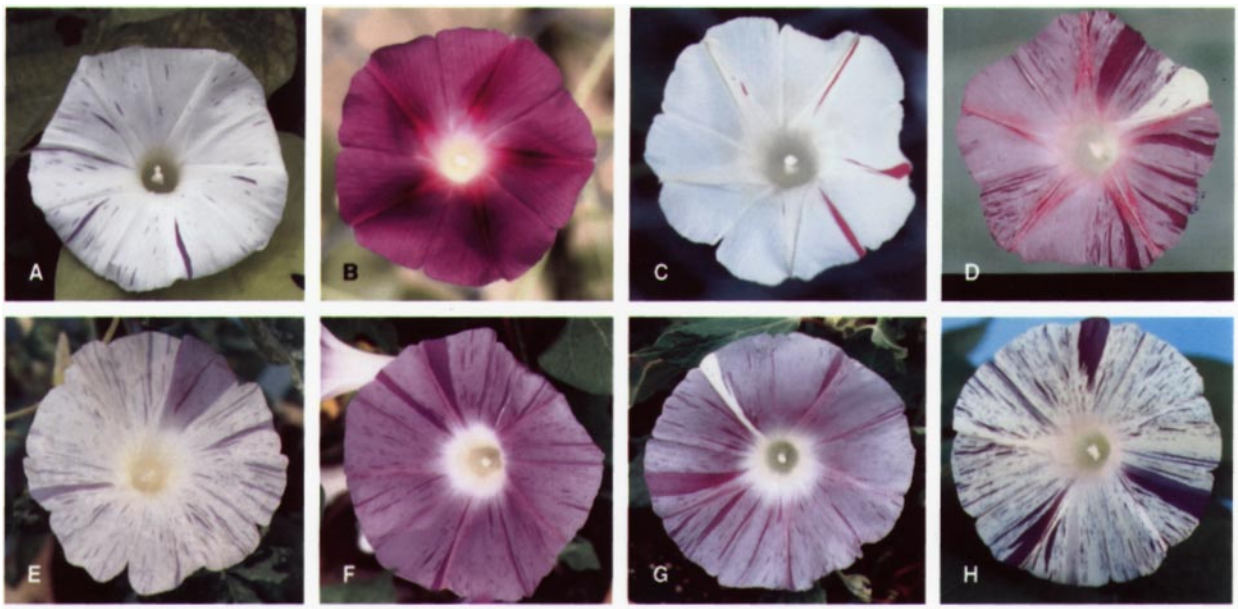

Fig. 1A–H Variegated flowers of Japanese morning glory, morning glory, and their F_1 hybrid. **A** Line KK/SSB-4 of Japanese morning glory carrying the mutable allele (*a-3*^{flecked}[J]/*a-3*^{flecked}[J]), **B** line KK/FP-36 of morning glory carrying the wild-type allele (*A*[M]/*A*[M]), **C** line KK/VR-37 of morning glory carrying the mutable allele (*a*^{flaked}[M]/*a*^{flaked}[M]), **D** F_1 hybrid of morning glory carrying the mutable allele in the heterozygous condition (*A*[M]/*a*^{flaked}[M]), **E** interspecific hybrid line Seiwa SU2001 carrying the mutable allele (*a*^{flaked}[M]/*a*^{flaked}[M]), **F** and **G** F_1 hybrid between line KK/SSB-4 of Japanese morning glory carrying the mutable allele (*a-3*^{flecked}[J]/*a-3*^{flecked}[J]) and the interspecific hybrid line Seiwa SU2001 carrying the mutable allele (*a*^{flaked}[M]/*a*^{flaked}[M]), **H** F_1 hybrid between the mutable line KK/VR-37 and the interspecific hybrid line Seiwa SU2001

with the following accession numbers D85723, D85724, D85725, D85726, D85736.

Results

Characterization of the *DFR* regions in morning glory lines

Since the *flaked* phenotype of morning glory is similar to the *flecked* phenotypes of Japanese morning glory which is caused by integration of *Tpn1* into the *DFR* gene (Inagaki et al. 1994, 1996), we have examined the region containing the *DFR* sequence in the genome of morning glory. DNAs were isolated from the wild-type line of morning glory KK/FP-36 and from the mutable *a*^{flaked}[M] line KK/VR-37, and these were subjected to Southern hybridization analysis using the petunia *DFR* cDNA (Beld et al. 1989) as a probe. As can be seen on Fig. 2, distinct RFLPs (restriction fragment length polymorphisms) were detected at the *DFR* region in *Bam*HI, *Sac*I and *Xba*I digests. RFLPs were also observed in *Dra*I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Pst*I, and S*pe*I digests (data not shown).

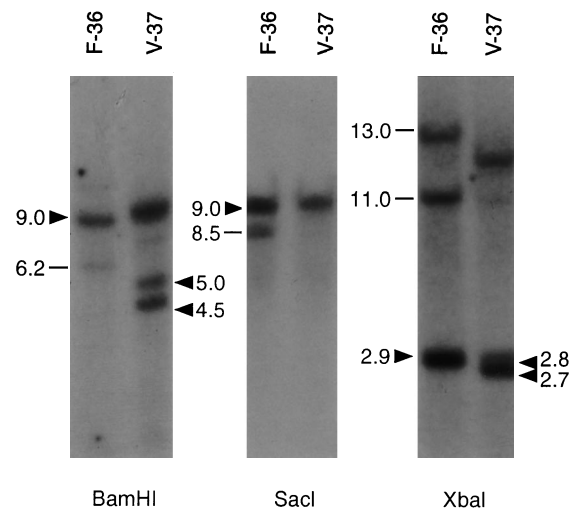

Fig. 2 Southern hybridization analysis of the *DFR* region of morning glory. Genomic DNAs were cleaved with the restriction enzymes indicated and subjected to Southern hybridization analysis using the petunia *DFR* cDNA. *F-36* indicates the wild-type line KK/FP-36 and *V-37* the mutable line KK/VR-37. Estimated lengths of hybridizing fragments are indicated in kilobases. *Arrows* indicate internal fragments derived from the cloned segments in pDRA1001 and pDRA2001

To characterize the RFLPs found at the *DFR* regions in lines KK/FP-36 and KK/VR-37, we cloned their B*gl*II fragments (about 17 kb long). Figure 3 shows the physical maps of the cloned *DFR* regions. All of the restriction site alterations detected in the mutable line were found in an approximately 12-kb region located to the left of the rightmost *Bam*HI site on the cloned fragment. Hybridization analysis of the cloned fragments with the petunia *DFR* cDNA probe revealed that segments homologous to the *DFR* sequence were

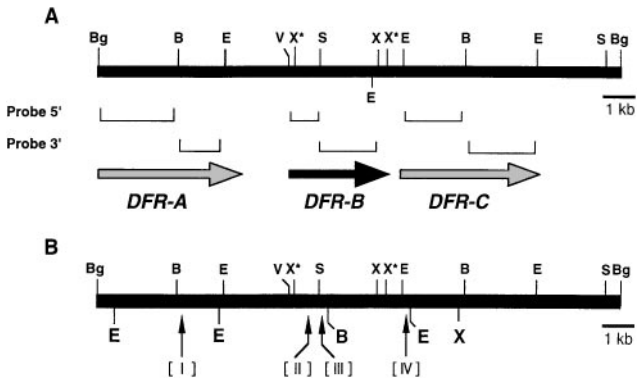

Fig. 3A, B Physical maps of the *DFR* region of morning glory. **A** Restriction map of the cloned *Bgl*II fragment from KK/FP-36 (pDRA1001). The *brackets* under the map indicate the regions hybridized with the 5′ part (probe 5′) or the 3′ part (probe 3′) of the *DFR-B* cDNA of Japanese morning glory. The *horizontal arrows* represent the approximate location and orientation of the *DFR* genes. The *DFR-B* gene showing the strongest homology to the *DFR-B* cDNA of Japanese morning glory is shown by the *filled arrow*, the *DFR-A* and *DFR-C* genes less hybridizable with the *DFR-B* cDNA probe than *DFR-B* are indicated by the *hatched arrows*. **B** Restriction map of the cloned fragment from KK/VR-37 (pDRA2001). The sites of DNA rearrangements in pDRA2001 are indicated by the *vertical arrows* with the *Roman numerals* I to IV below the map. Restriction sites present in both pDRA1001 and pDRA2001 are represented by the *vertical lines* above the *thick horizontal lines*, and the restriction sites unique to each cloned fragment are below the *thick lines*. Restriction sites are: *B Bam*HI, *Bg Bgl*II, *E Eco*RI, *S Sal*I, *X Xba*I. *X** represents the *Xba*I sites cleavable when the plasmids were grown in *E. coli* K12 MG48 *dam*

dispersed in about a 14-kb region located to the left of the rightmost *Eco*RI site of the cloned fragments. Comparison of the physical maps with patterns of the genomic Southern hybridization (Fig. 2) indicated that all of the internal fragments that hybridized with the petunia *DFR* cDNA probe in Fig. 3 were also detected in the genomic Southern hybridization and that restriction sites in the flanking regions of the cloned fragments appeared to vary between the two lines.

To characterize the *DFR* sequences further, we hybridized the 5′ and 3′ probes containing the exon 1 and the exons 4 and 5 of the *DFR-B* gene of Japanese morning glory with the cloned fragment on pDRA1001 derived from the wild-type line KK/FP-36. As shown in Fig. 3A, the cloned fragment carried three copies of the *DFR* genes in direct orientation, and partial sequencing in the region (data not shown) supported this view. We have designated these copies as *DFR-A*, *DFR-B*, and *DFR-C*. While the three *DFR* gene regions were detected by hybridization analysis with the entire *DFR* cDNA of Japanese morning glory under low-stringency conditions, only the *DFR-B* area hybridized well under high-stringency conditions. Since the *DFR* cDNA probe used is derived from the active *DFR-B* gene among the three *DFR* genes in Japanese morning glory, the corresponding *DFR-B* gene of morning glory is likely to be an active one.

Most of the alterations in restriction sites between the cloned fragments on pDRA1001 and pDRA2001 (Fig. 3) were found to be caused by a few base-pair substitutions rather than associated with DNA rearrangements. For example, the two sequences at the *DFR-A* region on pDRA1001 corresponding to the *Eco*RI sites on pDRA2001 were GAATTA and GAGTTC instead of GAATTC. Analogously, those on pDRA1001 corresponding to the *Bam*HI and *Xba*I sites on pDRA2001 were GGTC and TCTAGG instead of GGATCC and TCTAGA, respectively. About 90% homology was found between partially sequenced discrete segments in the *DFR* regions on pDRA1001 and pDRA2001, and the differences could be mainly attributed to non-coding regions rather than the exon sequences of the *DFR* genes.

We also detected DNA rearrangements in at least four discrete regions, indicated by I to IV (Fig. 3B), and their sequence differences are summarized in Fig. 4. Microsatellite-like repeats of ATT with a poly-T sequence were inserted at region I on pDRA2001, while tandem repeats of a C_5A motif were deleted in region III. Two insertions of a 57-bp sequence named *MELSIP1* (mobile element-like sequence *Ipomoea purpurea* one) and a 415-bp element, *MELSIP2*, were found in regions II and IV on pDRA2001, respectively. An *Eco*RI site was localized within *MELSIP2*. The *MELSIP1* sequence of region II carried 7-bp inverted repeats near its termini and was flanked by apparent 5-bp target duplications with 1 bp (C at the leftmost) deleted. The element *MELSIP2* contained imperfect 23-bp terminal inverted repeats. Although no apparent target duplication was detected at the insertion site of *MELSIP2*, the 4-bp flanking sequences at the left of the element and the 8-bp ones at its right were not present on pDRA1001. The copy numbers of tandemly repeated $A_{10}TG$ motif sequences located to the right of the insertion site in region IV were different between the cloned fragments.

Phenotypes observed in hybrids between the *a-3*^{flecked}[J] and *a*^{flaked}[M] lines

Although Japanese morning glory and morning glory belong to the same genus, *Pharbitis* or *Ipomoea*, interspecific hybridization between these two species was unsuccessful. However, Yoneda (1976) succeeded in obtaining a few fertile hybrids between morning glory carrying the mutable *a*^{flaked}[M] allele and an African variety of Japanese morning glory bearing pale-blue flowers. An interspecific hybrid line carrying the *a*^{flaked}[M] allele in the homozygous state was called "Seiwa", which produces white flowers with blue flakes and sectors (Fig. 1E). Seiwa can be crossed with Japanese morning glory to produce fertile progeny (Yoneda 1979).

To test whether the mutable *a*^{flaked}[M] allele falls in the *DFR* gene, we attempted to cross the hybrid line

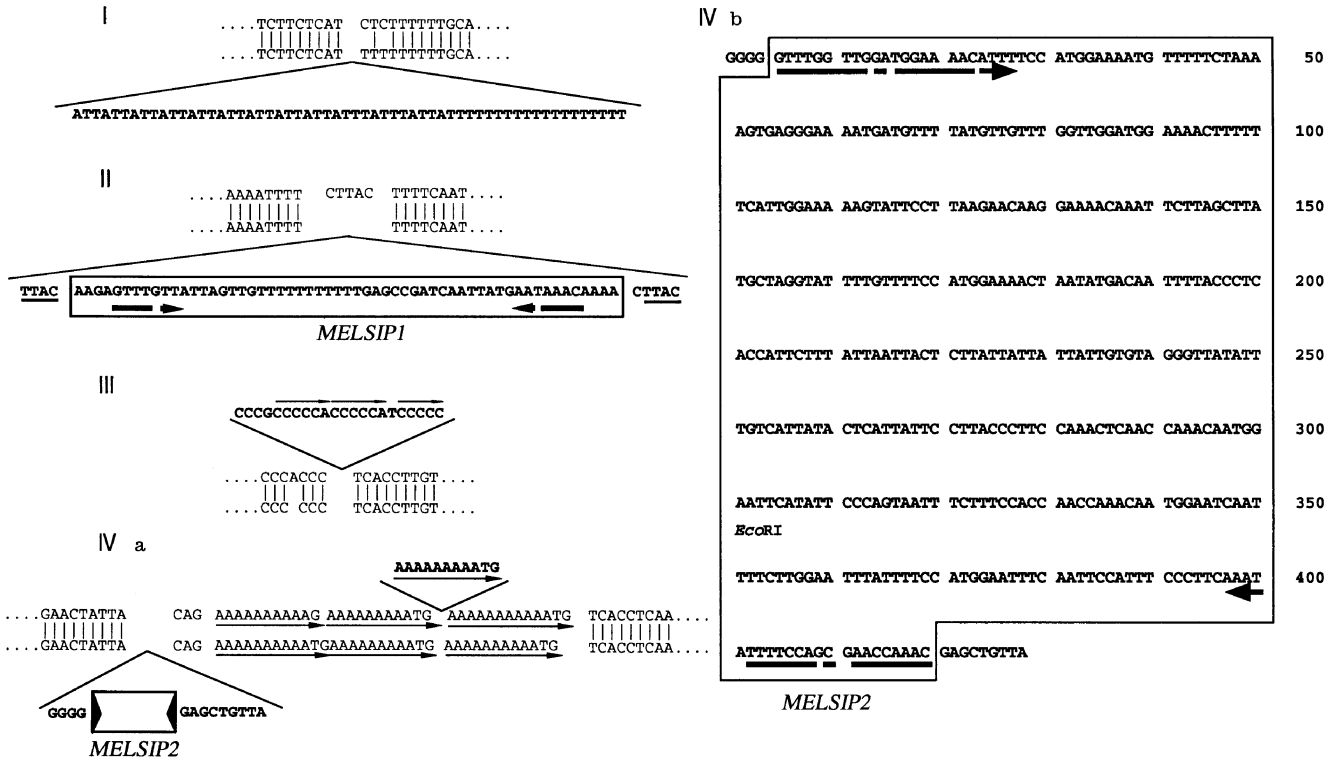

Fig. 4 DNA rearrangements in pDRA2001. **I–IV(a)** Comparison of the sequences in regions *I–IV* where DNA rearrangements were detected (**Fig. 3**). The *upper lines* indicate the sequences found in pDRA1001, and the *lower lines* represent the corresponding sequences in pDRA2001. The *horizontal arrows* in **III** and **IV(a)** indicate the motif sequences of the direct repeats. Inserted sequences are indicated by *boldface letters*, and the mobile element-like sequences, *MELSIP1* and *MELSIP2*, by *rectangles*. **IV(b)** Inserted sequences in region *IV*. The *broken horizontal arrows* within *MELSIP1* and *MELSIP2* indicate inverted repeats near their termini. Direct duplications flanking *MELSIP1* are indicated by underlining

Seiwa SU2001 with the mutable *a-3*^{flecked}[J] line KK/SSB-4 of Japanese morning glory. Since the variegated *a-3*^{flecked}[J] line carries the *DFR-B* gene having *Tpn1* inserted, the flower phenotype of the F_1 hybrids will bear variegated flowers in a white background if the mutable *a*^{flaked}[M] allele resides within the *DFR* gene. If the *a*^{flaked}[M] allele has nothing to do with the *DFR* gene, on the other hand, the defect in the *DFR-B* gene due to the *a-3*^{flecked}[J] mutation of Japanese morning glory must be complemented by the active *DFR* gene of morning glory.

Most of the flowers in the three F_1 hybrids obtained showed similar phenotypes (Fig. 1F and G). The flowers were pale blue with variegated deep-blue flakes. The results clearly showed that the defect in the *DFR-B* gene of Japanese morning glory was complemented by the active *DFR* gene of morning glory. Surprisingly, the mutable *a*^{flaked}[M] allele of morning glory exhibited incomplete or partial dominance to the wild-type *A*[J] allele of Japanese morning glory. Moreover, white sectors were also observed in the pale-blue background (Fig. 1G). It should also be noted that the mutable *a*^{flaked}[M] alleles carried by the lines Seiwa SU2001 and KK/VR-37 were indeed allelic because we demonstrated that the F_1 hybrids between Seiwa SU2001 and KK/VR-37 bear white flowers with variegated flakes as expected (Fig. 1H). Based on these functional studies, we were able to conclude that the mutable *a*^{flaked}[M] allele has little functional association with the *DFR* gene activity.

Discussion

From over 20 mutable loci, several resulting in variegated flower phenotypes in Japanese morning glory (Imai 1934), we have demonstrated that the *a-3*^{flecked}[J] mutation bearing white flowers with colored flecks and sectors (Fig. 1A) is caused by the insertion of an *En/Spm*-like transposable element, *Tpn1*, within the *DFR-B* gene (Inagaki et al. 1994, 1996; Hoshino et al. 1995). Like the *a-3*^{flecked}[J] mutation, the variegation in the *a*^{flaked}[M] allele of morning glory (Fig. 1C) is also regarded to be caused by recurrent somatic mutations and to be involved in a mobile genetic element in a gene controlling anthocyanin biosynthesis.

In the *DFR* region of the mutable *a*^{flaked}[M] line, we have found at least two short mobile element-like sequences, *MELSIP1* and *MELSIP2* (Figs. 3 and 4). Both *MELSIP1* (57 bp) and *MELSIP2* (415 bp) are AT-rich elements, and *MELSIP2* and its relatives are present in multiple copies in the genomes of morning

glory, KK/FP-36 and KK/VR-37 (Y. Hisatomi and S. Iida, unpublished). Although no element homologous to *MELSIP2* has been reported, its structural features are similar to MITEs (miniature inverted-repeat transposable elements) recently identified in the rice genome (Bureau et al. 1996). MITEs in rice comprise nine families of elements including *Castaway*, *Ditto*, *Explorer*, *Gaijin*, *Stowaway* and *Tourist*, and some of them are also found in the genomes of other plants (Bureau and Wessler 1994a, b; Bureau et al. 1996). Most of these elements are relatively AT-rich sequences of around 0.4 kb and carry short terminal inverted repeats. It is likely that *MELSIP2* belongs to a new family of the MITEs elements. Nonetheless, both *MELSIP1* and *MELSIP2* in the *DFR* region of morning glory are highly unlikely to have anything to do with the mutable a^{flaked} allele because flower phenotypes of the F_1 hybrids between the a^{flaked}[M] line Seiwa SU2001 and the $a\text{-}3^{flecked}$[J] line KK/SSB-4 clearly indicated that the *DFR* gene in the a^{flaked}[M] line is active (Fig. 1F and G).

Compared with the mutable $a\text{-}3^{flecked}$[J] system of Japanese morning glory, however, two aspects, namely incomplete dominance and the appearance of white sectors in the heterozygous A[M]/a^{flaked}[M] condition, are peculiar to the mutable a^{flaked}[M] system of morning glory (Fig. 1D). A few mutations associated with DNA rearrangements are known to exhibit a semi-dominant character in genes for pigmentation. For example, *niv-525* and *niv-571* are semi-dominant alleles of the *nivea* locus encoding CHS enzyme in *Antirrhinum* (Coen and Carpenter 1988; Bollomann et al. 1991). In Niv^+/niv^- heterozygotes, these niv^- alleles act in *trans* to reduce the steady-state level of *CHS* mRNA produced by the Niv^+ allele, resulting in very pale flowers. Although the molecular mechanism of incomplete dominance is not fully understood, it must be some sort of allelic interaction between wild-type and mutant alleles. In the case of the mutable a^{flaked}[M] allele, such allelic interactions do occur not only intraspecifically between the mutable a^{flaked}[M] allele and the wild-type A[M] allele of morning glory but also interspecifically between a^{flaked}[M] and the corresponding wild-type allele A[J] of Japanese morning glory (Fig. 1D and F).

The appearance of white sectors in the heterozygous A[M]/a^{flaked}[M] condition in the mutable a^{flaked}[M] system of morning glory (Fig. 1D) has been regarded as a somatic mutation at the wild-type A[M] allele to change it to the mutant allele a[M] (Imai and Tabuchi 1935; Kasahara 1956; Epperson and Clegg 1987; 1992). If this is the case, high-frequency somatic mutation to alter the wild-type A[M] allele into the mutant a[M] allele must be caused either by the mutant a[M] allele itself or by another modifier element carried in the morning glory genome, because the corresponding wild-type A[J] allele of Japanese morning glory can also convert into the mutant a[J] allele in the F_1 hybrids between Seiwa SU2001 and the $a\text{-}3^{flecked}$[J] line KK/SSB-4 (Fig. 1G). To identify the mutable a^{flaked}[M] allele conferring these interesting phenotypes, we are currently characterizing DNA rearrangements in the genes for flower pigmentation other than the *DFR* gene.

Acknowledgments We thank Anton G.M. Gerats for providing the petunia *DFR-A* cDNA clone, Thomas A. Bickle for reading the manuscript, and Yoshiki Habu and Atsushi Hoshino for discussion. This work was supported in part by grants from the Ministry of Education, Science, Sport and Culture of Japan. It was also supported partly by the Sasakawa Scientific Research Grant from the Japan Science Society to Y.H. and by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists to Y.H.

References

Baker EE (1917) Heredity studies in the morning-glory (*Ipomoea purpurea* [L.] Roth). Bull Cornell Univ Agric Exp Stn 392:3–38

Beld M, Martin C, Huits H, Stuitje AR, Gerats AGM (1989) Flavonoid synthesis in *Petunia hybrida*: partial characterization of dihydroflavonol 4-reductase genes. Plant Mol Biol 13: 491–502

Bilang R, Iida S, Peterhans A, Potrykus I, Paszkowski J (1991) The 3′-terminal region of the hygromycin-B-resistance gene is important for its activity in *Escherichia coli* and *Nicotiana tabacum*. Gene 100:247–250

Bollomann J, Carpenter R, Coen ES (1991) Allelic interactions at the *nivea* locus of Antirrhinum. Plant Cell 3:1327–1336

Bureau TE, Wessler SR (1994a) Mobile inverted-repeat elements of the *Tourist* family are associated with the genes of many cereal grasses. Proc Natl Acad Sci USA 91:1411–1415

Bureau TE, Wessler SR (1994b) Stowaway: a new family of inverted repeat elements associated with the genes of both monocotyledonous and dicotyledonous plants. Plant Cell 6:907–916

Bureau TE, Ronald PC, Wessler SR (1996) A computer-based systematic survey reveals the predominance of small inverted-repeat elements in wild-type rice genes. Proc Natl Acad Sci USA 93:8524–8529

Coen ES, Carpenter R (1988) A semi-dominant allele, *niv*-525, acts in *trans* to inhibit expression of its wild-type homologue in *Antirrhinum majus*. EMBO J 7:877–883

Coen ES, Robbins TP, Almeida J, Hudson A, Carpenter R (1989) Consequences and mechanisms of transposition in *Antirrhinum majus*. In: Berg DE, Howe MM (eds) Mobile DNA. American Society for Microbiology, Washington, D.C., pp 413–436

Ennos RA, Clegg MT (1983) Flower color variation in the morning glory, *Ipomoea purpurea*. J Hered 74:247–250

Epperson BK, Clegg MT (1987) Instability at a flower color locus in the morning glory. J Hered 78:346–352

Epperson BK, Clegg MT (1988) Genetics of flower color polymorphism in the common morning glory (*Ipomoea purpurea*). J Hered 79: 64–68

Epperson BK, Clegg MT (1992) Unstable white flower color genes and their derivatives in the morning glory. J Hered 83:405–409

Forkmann G (1994) Genetics of flavonoids. In: Harborne JB (ed) The flavonoids: advances in research since 1986. Chapman and Hall, London, pp 537–564

Hoshino A, Inagaki Y, Iida S (1995) Structural analysis of *Tpn1*, a transposable element isolated from Japanese morning glory bearing variegated flowers. Mol Gen Genet 247:114–117

Imai Y (1927) The genetics of *Pharbitis purpurea*. J Coll Agric Imp Univ Tokyo 9:199–222

Imai Y (1931) Analysis of flower colour in *Pharbitis nil*. J Genet 24:203–224

Imai Y (1934) On the mutable genes of *Pharbitis*, with special reference to their bearing on the mechanism of bud-variation. J Coll Agric Imp Univ Tokyo 12:479–523

Imai Y, Tabuchi K (1935) Recurrent mutation in the flaked alleles of *Pharbitis purpurea*. J Genet 35:433–446

Inagaki Y, Hisatomi Y, Suzuki T, Kasahara K, Iida S (1994) Isolation of a *Suppressor-mutator*/*Enhancer*-like transposable element, *Tpn1*, from Japanese morning glory bearing variegated flowers. Plant Cell 6:375–383

Inagaki Y, Hisatomi Y, Iida S (1996) Somatic mutations caused by excision of the transposable element, *Tpn1*, from the *DFR* gene for pigmentation in sub-epidermal layer of periclinally chimeric flowers of Japanese morning glory and their germinal transmission to their progeny. Theor Appl Genet 92:499–504

Kasahara K (1956) Genetic studies on mutable genes in *Ipomoea purpurea* (in Japanese) Bull Res Lib Arts Hosei Univ 1:1–31

Marinus MG (1973) Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map. Mol Gen Genet 127:47–55

Martin C, Gerats T (1993) The control of flower coloration. In: Jordan BR (ed) The molecular biology of flowering. CAB Int, Wallingford, UK, pp 219–255

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nevers P, Shepherd NS, Saedler H (1986) Plant transposable elements. Adv Bot Res 12:103–203

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Schoen DJ, Giannasi DE, Ennos RA, Clegg MT (1984) Stem color and pleiotropy of genes determining flower color in the common morning glory. J Hered 75:113–116

Yoneda Y (1976) Studies on interspecific hybrid in *Pharbitis*. I. Morphological observation on a hybrid of *Pharbitis nil* × *Pharbitis purpurea*. Rep Dept Lib Arts Shizuoka Univ (Sciences) 12:27–34

Yoneda Y (1979) Studies on interspecific hybrid in *Pharbitis*. V. *P. nil* × *P. purpurea* hybrid, with special reference to the Japanese morning glory (in Japanese). Rep Dept Lib Arts Shizuoka Univ (Sciences) 15:35–45

Yoneda Y (1990) Japanese morning glory. In: Ammirato PV, Evans DA, Sharp WR, Bajaj YPS (eds) Handbook of plant cell culture, vol. 5; ornamental species. McGraw-Hill Publ, New York, pp 509–533