Miniature Inverted-Repeat Transposable Elements of *Stowaway* Are Active in Potato

Masaki Momose,*1 Yutaka Abe†,2 and Yoshihiro Ozeki†

*Central Laboratories for Frontier Technology, Kirin Holdings, Sotome 3377, Sakura, Tochigi 329-1414, Japan and †Department of Biotechnology and Life Science, Faculty of Engineering, Tokyo University of Agriculture and Technology, Naka 2-24-16, Koganei, Tokyo 184-8588, Japan

Manuscript received April 9, 2010
Accepted for publication June 19, 2010

ABSTRACT

Miniature inverted-repeat transposable elements (MITEs) are dispersed in large numbers within the genomes of eukaryotes although almost all are thought to be inactive. Plants have two major groups of such MITEs: *Tourist* and *Stowaway*. Mobile MITEs have been reported previously in rice but no active MITEs have been found in dicotyledons. Here, we provide evidence that *Stowaway* MITEs can be mobilized in the potato and that one of them causes a change of tuber skin color as an obvious phenotypic variation. In an original red-skinned potato clone, the gene encoding for a flavonoid 3',5'-hydroxylase, which is involved in purple anthocyanin synthesis, has been inactivated by the insertion of a *Stowaway* MITE named *dTstu1* within the first exon. However, *dTstu1* is absent from this gene in a purple somaclonal variant that was obtained as a regenerated plant from a protoplast culture of the red-skinned potato. The color change was attributed to reversion of flavonoid 3',5'-hydroxylase function by removal of *dTstu1* from the gene. In this purple variant another specific transposition event has occurred involving a MITE closely related to *dTstu1*. Instead of being fossil elements, *Stowaway* MITEs, therefore, still have the ability to become active under particular conditions as represented by tissue culturing.

COLOR mutation or variegation of grain, flower petals, or fruit skin represents a suitable visual marker for the identification of genes for both pigment production and transposable elements (Clegg and Durbin 2000; Winkel-Shirley 2001; Kobayashi *et al.* 2004). Recent large-scale genome analyses have uncovered numerous transposable elements occupying large portions of eukaryotic genomes. Approximately 45% of the human genome is composed of sequences originating from >3 million copies of transposable elements (International Human Genome Sequencing Consortium 2001). Even in rice, a plant with a relatively small genome, 20% of the genomic sequence can be derived from transposable elements (Turcotte *et al.* 2001; Goff *et al.* 2002; Yu *et al.* 2002). Although almost all of these insertions are thought to be inactive, these elements are suggested to have influenced the evolution of genomes and individual genes. They can rearrange a genome through transposition, insertion, excision, chromosome breakage, or ectopic recombination (Bennewitz 2000). Moreover, some can contribute to the emergence of a novel gene by conveying a poly(A) signal, a transcription start site, a TATA box, a splicing site, or an intron (Ort *et al.* 2008).

Bioinformatic analyses using data of genome projects found a miniature inverted-repeat transposable element (MITE) (Bureau and Wessler 1992, 1994), the copy number of which reaches over thousands in a genome (Feschotte *et al.* 2002). Characteristically, a MITE is not >600 bp, does not contain any coding sequences, and has imperfect terminal inverted repeats (TIRs) at the end of the element and its target site is duplicated upon insertion. The majority of MITEs in plants are divided into two groups, *Tourist* and *Stowaway*, on the basis of the sequences of TIRs and their target sites, TAA and TA, respectively. *Tourist* MITEs are found in grasses while *Stowaway* is present not only in monocotyledonous but also in dicotyledonous plants (Bureau and Wessler 1992, 1994; Feschotte *et al.* 2002). Although huge numbers of MITEs of each family have been found since their discovery in *silico*, their dynamic features remain largely unknown. The first mobile MITE, *mPing*, was identified in rice and belongs to the *Tourist* family. Its movement was activated during long-term cell culture (Jiang *et al.* 2003) and by another culture (Kruchi *et al.* 2003). When *mPing* was inserted into the gene for *rice ubiquitin-related modifier-1* (*Rum1*), its excision resulted in reversion of the mutable slender
glume phenotype to wild type (Nakazaki et al. 2003). The identification of an active element made it possible to discover that the transposable elements Ping and Pong supplied the transposase acting on mPing (Yang et al. 2007). Movement of Stowaway MITEs in rice was also reported recently. These were mobilized in yeast cells by transposases of Mariner-like elements (MLEs) (Yang et al. 2009). Active copies of MITEs have been found only in rice. In dicotyledons the only indication that they can be mobilized has come from insertional polymorphisms between accessions or cultivars (Macas et al. 2005; Menzel et al. 2006).

How a transposable element becomes active is an interesting question since it is potentially an endogenous mutagen and could represent a force for evolution through rearrangement of a genome or production of novel genes. Cell culture is known to activate transposable elements. For example, Ac and Spm/En of class II (DNA) elements were mobilized under such conditions (Peschke et al. 1987; Peschke and Phillips 1991) and tissue culturing resulted in a vast increase of copy number of transposable elements belonging to class I (RNA) elements (Hirochika 1993). The activation of transposable elements by culture can cause genetic and phenotypic variation in clonal plants, which is one of the reasons for somaclonal variation (Lee and Phillips 1988; Keppler et al. 2000).

The active Stowaway MITEs reported here induced somaclonal variation and provide a tool to investigate how MITEs have propagated to become a major component of the plant genome and under which conditions they become active.

MATERIALS AND METHODS

Plant materials: Commercial triploid potato cultivars named “Jaga kids purple” (JKP) and “Jaga kids red” (JKR) were developed by Kirin Brewery Co. (Japan) from leaf protoplasts of a red-skinned, triploid clone “72218,” which was obtained by a cross between a tetraploid cultivar “Early rose” (S. phureja (Tuberosum) and S. phureja (S. phureja) for the first and nos. 3 and 6 for the second amplification] to amplify the full-length cDNAs for the gene by RT–PCR using primers for F3 5′-CCGCCAACATATATATTTTTTTTTT-3′, 5′-CCGCCAACATATATATTTTTTTTTT-3′ and the sequence determination of the genomic DNA for F3 5′H genes: Genomic DNA was isolated from ~100 mg of tuber skin by using an RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). To obtain the sequence of the cDNA for the flavonoid 3′,5′-hydroxylase (F3 5′H) gene of JKP, a 5′-RACE experiment was performed using a GeneRacer kit (Invitrogen, Carlsbad, CA) with supplied genes and primer-specific primers [no. 1 (5′-AACATTTTGGTGCAAAKACCATCAA-3′) and no. 2 (5′-CCTTGGCAATCTACGCGACGTA-3′) for the first and the second amplifications, respectively] that anneal to two highly conserved regions among P450 or F3 5′H genes of S. melongena (GenBank accession no. X70824 (Toguri et al. 1993b) and Petunia hybrida (GenBank accession nos. Z22544, Z22545, and X71130) (Holton et al. 1993; Toguri et al. 1993a).

Gene-specific primers for H2 and H3 (5′-CCGCCAACATATATATTTTTTTTTT-3′) and the sequence determination of the genomic DNA for F3 5′H genes: Genomic DNA was isolated from ~100 mg of leaves as described previously (Walbot and Warren 1988). Genomic DNA of the F3 5′H gene was amplified (using a 5-min extension time) with primer nos. 5 and 6. The methods for the isolation of the other F3 5′H pseudogenes, f3 5′h2 and f3 5′h3, are described in File S1.

Isolation of dTis1-2 and the sequence determination proximal to the insertion site in JKP: PCR with a primer specific for the internal sequence of dTis1 [no. 25 (5′-ATTCA TTTTGACCAAGCTTTA-3′)] yielded a JKP-specific product.
of 2.5 kb that enabled the design of two new primers [no. 26 (5’-TGTGTTTGGAGCTTACGTAATTTCAGTA-3’) and no. 27 (5’-CAAGGGGACAGAATTAGTCAGGTAGTA-3’)]. Inverse PCR on MboI-digested JKP genomic DNA followed by self-ligation [primer nos. 26 and 27 for the first and nos. 26 and 28 (5’-AGACATTCCCATTACGGGAAATGTTA-3’) for the second PCR] produced a JKP-specific ~1-kb fragment containing the flanking sequences of the dTstu1-2 insertion. Here, primer no. 28 was designed from dTstu1 internal sequence. PCR with primer nos. 29 (5’-AGCTGGAAATGAGTTCCATTTCAGTA-3’) and 30 (5’-ATGAGTTCCATTTCAGTA-3’) annealing to these flanking regions amplified the dTstu1-2 insertion locus from genomic DNAs of 72218 and JKP. The extension time of all PCR reactions was 5 min.

**MITE display:** Transposon display was carried out using primers designed from the sequence of dTstu1 and dTstu1-2 according to the procedure of CASA et al. (2000). Approximately 250 ng of genomic DNA was digested with MboI and ligated to an adapter. Aliquots of the reactions were diluted 4-fold with 0.1 × TE. Preselective amplification was performed with a primer complementary to the adapter [Mse + 0 (5’-GACGATGAGTCCTGAGTAA-3’)] and another primer complementary to an internal dTstu1-2 sequence [no. 31 (5’-CATTTCCTTCTGAGTACTA-3’)]. PCR consisted of 25 cycles at 94°C for 30 sec, 56°C for 30 sec, and extension at 72°C for 1 min with a final 5-min extension at 72°C. Aliquots of the reactions were diluted 20-fold with 0.1 × TE. Selective amplification was carried out with a selective primer [Mse + N (5’-GACGATGAGTCCTGAGTAA-N3’)] and another primer specific for the TIR and target site duplication (TSD) sequence of dTstu1 and dTstu1-2 [no. 32 (5’-ATAAATGGAAGCTCTAATC-3’)]. The latter primer was labeled at the 5’ end with 6-FAM. Temperature cycling conditions were 94°C for 5 min; 10 touchdown cycles of 94°C for 30 sec, 66°C for 30 sec (−1°C each cycle), and extension at 72°C for 1 min; followed by 25 cycles of 94°C for 30 sec, 56°C for 30 sec, and extension at 72°C for 1 min with a final 5-min extension at 72°C. The products were analyzed on an ABI PRISM 510 genetic analyzer (Applied Biosystems, Foster City, CA).

**RESULTS**

**Key enzyme of the color variation:** JKP is a potato cultivar with purple tubers that was obtained as a somaclonal variant of skin color after selection from plants regenerated from leaf protoplasts of clone 72218 with red tubers (Figure 1A) (OKAMURA 1991, 1994). Analysis of the anthocyanin aglycones revealed that the crucial difference between these purple and red potatoes was the presence of petunidin in the tuber skin of JKP as one of the major anthocyanidins, whereas in 72218 this was pelargonidin. The difference between petunidin and pelargonidin is the number of hydroxyl and methoxyl groups at the B-ring of these molecules. Addition of two hydroxyl groups to dihydrokaempferol, which is the precursor of pelargonidin, produces dihydrodricetin, a precursor of petunidin. This reaction is catalyzed by flavonoid 3’,5’-hydroxylase (F3’5’H) (Figure 1B). Therefore, the cause of the color variation from red (72218) to purple (JKP) was attributed to gain of F3’5’H function in the tuber skin of JKP. Recovery of the F3’5’H gene itself would most likely explain the restoration of enzyme activity since genetic analysis had revealed that the dominant allele for F3’5’H in the P locus is solely responsible for determination of the purple color phenotype (JUNG et al. 2005).

**Analysis of F3’5’H genes:** The possibility that disruption of the F3’5’H gene of 72218 was involved in the coloration of its tuber skin was assessed by RT–PCR analysis of the F3’5’H transcript. Sequencing of the obtained cDNA product revealed the presence of a MITE belonging to Stowaway, named dTstu1. This element was absent from the F3’5’H transcript in JKP, which was analyzed in parallel (Figure 2). In support of this, Southern blot analysis with F3’5’H cDNA from JKP as a probe demonstrated a reduction in size in JKP of a 5-kb EcoRV fragment present in 72218 and JKR, which is a somaclonal cultivar with red tubers simultaneously obtained from the leaf protoplast culture of 72218 that yielded JKP (OKAMURA 1991, 1994). Genomic sequence analysis of F3’5’H genes from 72218 and JKP revealed that the only difference between the full-length genes is the insertion of dTstu1 into the first exon of F3’5’H in
72218 (designated \(f^3\)5\(^h\)::dTstu1, DDBJ accession no. AB496977). This element was not present in \(f^3\)5\(^H\) of JKP (named \(f^3\)5\(^H\)::rev, DDBJ accession no. AB496976), which explained the size difference observed in Southern blot analysis (Figure 3, A and B). As the result of a stop codon within dTstu1, \(f^3\)5\(^h\)::dTstu1 should produce a truncated protein of only 24 amino acid residues in 72218, whereas \(f^3\)5\(^H\)::rev codes for a functional full-length protein of 510 amino acid residues, one residue longer than predicted for the wild type that was reported as a functional \(f^3\)5\(^H\) gene of diploid potato clone W5281.2 (GenBank accession no. AY675558) (Jung et al. 2005).

At most, three copies of \(f^3\)5\(^H\) were deduced to exist in 72218 and JKP on the basis of the results of Southern blot and genomic sequence analyses. Apart from the full-length \(f^3\)5\(^H\), the triploid 72218 and JKP possess two truncated copies of this gene (\(f^3\)5\(^h\)::dTstu1 and \(f^3\)5\(^h\)::dTstu1-2, DDBJ accession nos. AB496978 and AB496979) (Figure 3B). The sequences of each pseudogene were completely identical between 72218 and JKP. Both \(f^3\)5\(^h\)::dTstu1 and \(f^3\)5\(^H\)::rev have an EcoRV recognition site at the middle of the gene, which is absent in 7.8 kb of determined \(f^3\)5\(^h\)::rev sequence. Therefore, the largest band in Figure 3A represents \(f^3\)5\(^h\), while the 6.3-kb fragment is derived from the third allele, \(f^3\)5\(^h\)\(^3\), which contains only the latter half of the third exon, encoding the P450 signature motif conserved among all known plant \(f^3\)5\(^H\) genes. This motif is lacking in \(f^3\)5\(^h\), which strongly suggests that transcripts of this copy do not function properly. Triploid red 72218 has only pseudocopies of the gene, \(f^3\)5\(^h\)::dTstu1, \(f^3\)5\(^h\)::dTstu1-2, and \(f^3\)5\(^h\)\(^3\). Its purple somaclonal variant, JKP, has three copies of the gene, \(f^3\)5\(^H\)::rev, \(f^3\)5\(^h\)\(^2\), and \(f^3\)5\(^h\)\(^3\).

As \(f^3\)5\(^H\)::rev is the only allele able to produce a full-length, nondefective protein, we conclude that excision of dTstu1 from \(f^3\)5\(^h\)::dTstu1 during the establishment of JKP is the major reason for the color change from red to purple.

**An active Stowaway MITE, dTstu1:** The sequence of dTstu1 is short (239 bp), A/T rich (67%), and marked by TIRs corresponding to the consensus CTCCCTCYGTC and a duplication of the TA target sequence at the insertion site, all characteristics of Stowaway MITEs (Bureau and Wessler 1994). The formation of DNA secondary structure is predicted for this element as well (Figure 3C). Database searches retrieved sequences similar to dTstu1 not only in genomes of Solanum but also in the other Solanaceae plants, for example, Capsicum, Petunia, or Nicotiana (GenBank accession nos. DQ309518, AY136628, and AF277455).

Comparison of the wild-type \(f^3\)5\(^H\) gene with that of JKP confirmed the addition of one amino acid residue (valine) generated by a three-nucleotide insertion, GTA, in \(f^3\)5\(^H\)::rev (Figure 3C). These nucleotides could be traced to consist of one base (G) derived from dTstu1 and two (TA) from the duplicated target site. This duplication was also present in the disrupted \(f^3\)5\(^h\)::dTstu1 of 72218 and leading to the observed size difference of 238 bp between the transcripts derived from these genes. Therefore, the presence of these three nucleotides in \(f^3\)5\(^H\)::rev of JKP strongly supports that the 239-bp dTstu1 was excised from \(f^3\)5\(^h\)::dTstu1 in 72218 as a transposable element leaving a footprint that is normally associated with transposase-mediated excision. We conclude that the \(f^3\)5\(^H\) gene in 72218 (red) had become functionless as a result of dTstu1 insertion and then reverted in JKP (purple), presumably by transposition of dTstu1 during culturing.

**Another active dTstu1-like Stowaway MITE, dTstu1-2:** Excision of dTstu1 from the \(f^3\)5\(^H\) gene during culturing of leaf protoplasts derived from 72218 raised the possibility that other dTstu1-like Stowaway MITEs had undergone transposition under these conditions. In support of this, we isolated an extra dTstu1-like element specific for JKP by use of a DNA-fingerprinting technique adapted from a method with which inter-MITE polymorphisms were detected. With this method, multiple regions between MITEs had been amplified by PCR using a primer annealing to TIRs in the outer direction (Chang et al. 2001). By using primers specific for the dTstu1 internal sequence (instead of the TIR sequences), we obtained a product for JKP not observed for 72218 that contained an element almost identical to dTstu1, named dTstu1-2 (DDBJ accession no. AB496980). After identification of the flanking regions, PCR amplification of the region containing the site of integration of dTstu1-2 in JKP produced in 72218 a fragment of one size, not containing the transposable element. In JKP, however, two fragments, one with and the other without dTstu1-2, were detected (Figure 4A), suggesting that no alleles of the locus carried the transposable element in 72218 and that dTstu1-2 had been newly inserted in an allele.
Comparison of the sequence surrounding the insertion site confirmed the presence of a duplicated TA dinucleotide, which is the target sequence of Stowaway MITEs (Figure 4B). Compared to dTstu1, dTstu1-2 had a similar length, 239 bp, but contained four base changes, two of which were in the TIRs (Figure 5). These changes made the TIRs of dTstu1-2 more complementary to each other than in the case of dTstu1. Therefore, in view of a comparable propensity for transposition, this Stowaway MITE conceivably was mobilized under the same conditions that caused dTstu1 to be excised from the F3’5’H gene. If this is the case, activation of transposition of these MITEs was induced by culturing.

To survey the active MITE copies related to dTstu1, we carried out MITE display using primers designed from the sequences of dTstu1 and dTstu1-2. More than 50 peaks were detected but slight differences existed among 72218, JKR, and JKP. JKR revealed 3 new peaks and JKP exhibited 3 new peaks and a missing peak as compared with 72218 when using a primer with selective nucleotide T (Figure S2). The insertion of dTstu1-2 in JKP was visualized as a new peak at the expected position of 315 bases in size but the excision of dTstu1 in JKP was not detected at the expected position of 50 bases due to the signal of the other putative insertion at the same position. Although most of the peaks were identical, a few polymorphisms were detected among the three clones.

**DISCUSSION**

In this study we found the first active Stowaway MITEs in dicotyledons and presented the evidence of their movement. Excision of dTstu1 caused a somaclonal variation of skin color in potato tubers. Insertion of dTstu1-2 was observed at another locus in the genome of the same somaclonal variant, JKP. It became obvious that two major groups of MITEs, Stowaway and Tourist, have the potential to transpose in plants. Movement of MITEs was not proved for a long time because most of them are not inserted into genes (Okt et al. 2008) with the possibility to cause an altered phenotype and because the high copy number of MITEs in the genome precludes analysis of their individual movements. “Fingerprints” of MITE abundance, obtained by Southern hybridization with MITE DNA probes (Naito et al. 2006), showed differences among strains, which suggested movement of MITEs but did not provide direct evidence for their transposition. Previously, a case in which MITE transposition resulted in a phenotypic

**Figure 3**.—Flavonoid 3’5’-hydroxylase (F3’5’H) genes in 72218 and JKP. (A) Southern blot analysis of genomic DNA digested with EcoRV and probed with a labeled RT–PCR product of F3’5’H::rev. Approximate sizes are given on the left. The largest band represents F3’5’h2 since the EcoRV recognition site is absent in 7.8 kb of determined sequence. The 6.3-kb fragment is derived from F3’5’h3. The rest of the bands represent F3’5’h::dTstu1 or F3’5’H::rev since both F3’5’h::dTstu1 and F3’5’H::rev have an EcoRV recognition site at the middle of the gene. (B) Structure comparison of F3’5’H genes. Both F3’5’h2 and F3’5’h3 are incomplete genes, F3’5’h2 lacks the latter half of the third exon, and F3’5’h3 contains only the latter half of the third exon. Triploid red 72218 has only pseudogenes, F3’5’h::dTstu1, F3’5’h2, and F3’5’h3. Triploid purple JKP, a somaclonal variant of 72218, has F3’5’h::rev, F3’5’h2, and F3’5’h3. Coding regions (shaded boxes) are separated by introns (lines) with the dTstu1 insertion depicted by a solid bar. Arrows indicate the EcoRV recognition site in F3’5’H::dTstu1 and F3’5’H::rev. (C) Structure of dTstu1 and the nucleotide and amino acid sequences of F3’5’H genes proximal to the dTstu1 insertion site. Wild type is the previously reported functional F3’5’H gene (Jung et al. 2005). A pair of vertical sequences shows the TIRs where complementary sequences are hyphenated. An asterisk indicates a stop codon present in F3’5’h::dTstu1. The footprint remaining after dTstu1 excision (including the duplicated TA target site) is underlined.
change was reported. A MITE named mPing, belonging to Tourist, was found to be inserted in the rice Rim1 gene causing the slender glume phenotype that reverted to wild type by excision of the mobile element (Nakazaki et al. 2003). We present in this report another rare case of a MITE giving rise to an altered phenotype, namely that of dTstu1 belonging to Stowaway. We found this MITE to disrupt the F3’5’H gene of a potato clone (72218), resulting in a red tuber color. Due to the excision of dTstu1 tuber color changed to purple in the somaclonal variant. Thus, in two cases, visible phenotypes, the grain shape for mPing and the tuber color for dTstu1, provided strong evidence for the movement of MITEs belonging to Tourist and Stowaway, respectively.

As described in this report, the duplication of the target sequence TA at the insertion site of dTstu1 was observed for the F3’5’H gene of 72218. The footprint left behind in F3’5’H:rev in JKP suggests that the excision is catalyzed by a transposase. By lack of any open reading frame, the short Stowaway MITEs of both dTstu1 and dTstu1-2 are not able to code for such a transposase, which has to originate from other, unrelated transposable elements as found in the case of mPing. This Tourist MITE was mobilized by transposases derived from the Ping and Pong transposable elements (Yang et al. 2007). Mobile dTstu1 and dTstu1-2 enable us to search for transposases that control Stowaway MITEs. The Mariner-like element (MLE) is one of the most widely distributed transposable elements in eukaryotes and its transposase can interact in vitro with TIRs of a Stowaway MITE (Feschotte et al. 2005). Using yeast cells, MLE transposases of rice were proved to actually activate transposition of Stowaway MITEs of rice (Yang et al. 2009). MLE is a good candidate for a source of transposase for dTstu1 movement.

Our results show that the activation of Stowaway MITEs not only involves a transposase but also appears to occur under particular conditions. MITE displays of regenerated plants from protoplasts indicated that most of the MITE insertion sites were maintained, although a few differences emerged during tissue culture. The observed differences in sequences and in the insertion sites between the silent copies and the active ones should be investigated further as these may reveal the factors for transposition. Tissue culturing causes the activation of various transposable elements (Peschke et al. 1987; Grandbastien et al. 1989; Peschke and Phillips 1991; Hirochika 1993; Jiang et al. 2003; Kikuchi et al. 2003). It was observed that the conditions under which dTstu1 (and possibly dTstu1-2) was excised,
i.e., at some time during the culturing of leaf protoplasts isolated from 72218, caused 7% of the regenerated plants to bear purple tubers instead of the parental red potatoes (Okamura 1991). Furthermore, red tubers with small purple sectors were found in some regenerated plants that originated from cultured leaf protoplasts of 72218 (Figure S3). Such chimeric tubers or purple tubers, however, have not been found in tuber propagated 72218 plants, which are clonally reproduced as seed potatoes in the field. These facts also support the importance of cell culture conditions for the activation of dTstu1. It remains to be seen how tissue culturing confers the activation. Alteration of the epigenetic status by DNA demethylation of the element itself or of the genes encoding its transposase has been reported to activate a transposable element during tissue culture (Kaeppler et al. 2000; Cheng et al. 2006; Lisch 2009) and could therefore be part of the reason.

How MITEs have spread over various genomes and in such high numbers is still obscure but poses one of the important questions to be tackled to comprehend the evolution of the eukaryotic genome. Active MITEs, like dTstu1, can provide a tool for this investigation.

We thank Kazuyoshi Hosaka for 72218 tubers; Yoshio Itoh, Takayasu Hiroe, Toshihiro Toguri, Noboru Onishi, Naoyuki Umemoto, and Masahiko Okamura for discussions; and Chika Aoyama for assistance with experiments. We are grateful to Atsuko Momose for critical reading of the manuscript. This work was partly supported by a grant from the “Technical Development Program for Making Agribusiness in the Form of Utilizing the Concentrated Know-how from the Private Sector” of the Ministry of Agriculture, Forestry and Fisheries, Japan.

LITERATURE CITED

Ben netzen, J. L., 2000 Transposable element contributions to plant gene and genome evolution. Plant Mol. Biol. 42: 251–269.

Bureau, T. E., and S. R. Wessler, 1992 Tourist: a large family of small inverted repeat elements frequently associated with maize genes. Plant Cell 4: 1283–1294.

Bureau, T. E., and S. R. Wessler. 1994 Stowaway: a new family of inverted repeat elements associated with the genes of both monocotyledous and dicotyledous plants. Plant Cell 6: 907–916.

Casa, A. M., C. Brouwer, A. Nagel, L. Wang, Q. Zhang et al., 2000 The MITE family Heartbreaker (Hbr): molecular markers in maize. Proc. Natl. Acad. Sci. USA 97: 10083–10089.

Chang, R.-X., L. O’Donouhue and T. E. Bureau, 2001 Inter-MITE polymorphisms (IMP): a high throughput transposon-based genome mapping and fingerprinting approach. Theor. Appl. Genet. 102: 773–781.

Cheng, C., M. Daigen and H. Hirochika, 2006 Epigenetic regulation of the rice retrotransposon Tos17. Mol. Gen. Genomics 276: 378–390.

Chegw, M. T., and M. L. Durbin, 2000 Flower color variation: a model for the experimental study of evolution. Proc. Natl. Acad. Sci. USA 97: 7016–7023.

Feschotte, C., N. Jiang and S. R. Wessler, 2002 Plant transposable elements: where genetics meets genomics. Nature Rev. Genet. 3: 329–341.

Feschotte, C., M. T. Osterlund, R. Peeler and S. R. Wessler, 2005 DNA-binding specificity of rice mariner-like transposases and interactions with Stowaway MITEs. Nucleic Acids Res. 33: 2153–2165.

Goff, S. A., D. Ricke, T.-H. Lan, G. Presting, R. Wang et al., 2002 A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296: 92–100.

Grandbastien, M. A., A. Spelmman and M. Caroche, 1989 Tnt1, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. Nature 337: 376–380.

Hirochika, H., 1993 Activation of tobacco retrotransposons during tissue culture. EMBO J. 12: 2521–2526.

Holtom, T. A., F. Brugliera, D. R. Lenter, Y. Tanaka, C. D. Hyland et al., 1993 Cloning and expression of cytochrome P450 genes controlling flower colour. Nature 366: 276–279.

International Human Genome Sequencing Consortium, 2001 Initial sequencing and analysis of the human genome. Nature 409: 860–921.

Jiang, N., Z. Bao, X. Zhang, H. Hirochika, S. R. Eddy et al., 2003 An active DNA transposon family in rice. Nature 421: 163–167.

Jung, C. S., H. M. Griffiths, D. M. De Jong, S. Cheng, M. Bodis et al., 2005 The potato P locus codes for flavonoid 3’,5’-hydroxylase. Theor. Appl. Genet. 110: 269–275.

Kaeppler, S. M., H. F. Kaeppler and Y. Rhee, 2000 Epigenetic aspects of somaclonal variation in plants. Plant Mol. Biol. 43: 179–188.

Kikuchi, K., K. Terauchi, M. Wada and H. Hiran, 2003 The plant MITE mPing is mobilized in anther culture. Nature 421: 167–170.

Kobayashi, S., N. Goto-Yamamoto and H. Hirochika, 2004 Retrotransposon-induced mutations in grape skin color. Science 304: 982.

Lee, M., and R. L. Phillips, 1988 The chromosomal basis of somaclonal variation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 413–437.

Lisch, D., 2009 Epigenetic regulation of transposable elements in plants. Annu. Rev. Plant Biol. 60: 43–66.

Macas, J., A. Kobléková and F. Neumann, 2005 Characterization of Stowaway MITEs in peas (Pisum sativum L.) and identification of their potential master elements. Genome 48: 831–839.

Menzel, G., D. Dechiva, H. Keller, C. Lange and H. Himmelbauer, 2006 Mobilization and evolutionary history of miniature inverted-repeat transposable elements (MITEs) in Beta vulgaris L. Chromosome Res. 14: 831–844.

Naiko, K., E. Cho, G. Yang, M. A. Campbell, K. Yano et al., 2006 Dramatic amplification of a rice transposable element during recent domestication. Proc. Natl. Acad. Sci. USA 103: 17620–17625.

Nakazaki, T., Y. Okamoto, A. Horibata, S. Yahamira, M. Terashij et al., 2003 Mobilization of a transposon in the rice genome. Nature 421: 170–172.

Okamura, M., 1991 Somaclonal variation and variety improvement: development of potato new variety “JAGA KIDS”. Tissue Cult. 17: 207–212.

Okamura, M., 1994 Pomato: potato protoplast system and somatic hybridization between potato and a wild tomato, pp. 209–223 in Biotechnology in Agriculture and Forestry 27, Somatic Hybridization in Crop Improvement I, edited by Y. P. S. Bajaj. Springer-Verlag, Heidelberg, Germany.

Oki, N., K. Yano, Y. Okamoto, T. Tsukiyama, M. Terashij et al., 2008 A genome-wide view of miniature inverted-repeat transposable elements (MITEs) in rice, Oryza sativa ssp. japonica. Genes Genet. Syst. 83: 321–329.

Peschke, V. M., and R. L. Phillips, 1991 Activation of the maize transposable element Suppressor-mutator (Spm) in tissue culture. Theor. Appl. Genet. 81: 96–97.

Peschke, V. M., R. L. Phillips and B. G. Gengenbach, 1987 Discovery of transposable element activity among progeny of tissue cultured-derived maize plants. Science 238: 804–807.

Toguri, T., M. Azuma and T. Ohtani, 1993a The cloning and characterization of a cDNA encoding a cytochrome P450 from the flowers of Petunia hybrida. Plant Sci. 94: 119–126.

Toguri, T., N. Umemoto, O. Kobayashi and T. Ohtani, 1993b Activation of anthocyanin synthesis genes by white light in eggplant hypocotyl tissues, and identification of an inducible P-450 cDNA. Plant Mol. Biol. 23: 933–946.

Tomida, Y., and K. Kawakami, 1989 On the species hybrids between Solanum tuberosum and S. phureja 253. Potato Sci. 9: 71–78.

Turcotte, K., S. Skrinivasan and T. Bureau, 2001 Survey of transposable elements from rice genomic sequences. Plant J. 25: 169–179.
Walbot, V., and C. Warren, 1988 Regulation of Mu element copy number in maize lines with an active or inactive Mutator transposable element system. Mol. Gen. Genet. 211: 27–34.

Winkel-Shirley, B., 2001 It takes a garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. Plant Physiol. 127: 1399–1404.

Yang, G., F. Zhang, C. N. Hancock and S. R. Wessler, 2007 Transposition of the rice miniature inverted repeat transposable element mPing in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 104: 10962–10967.

Yang, G., D. H. Nagel, C. Feshotte, C. N. Hancock and S. R. Wessler, 2009 Tuned for transposition: molecular determinants underlying the hyperactivity of a Stowaway MITE. Science 325: 1391–1394.

Yu, J., S. Hu; J. Wang, G. K.-S. Wong, S. Li et al., 2002 A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science 296: 79–92.

Communicating editor: A. H. Paterson
Miniature Inverted-Repeat Transposable Elements of *Stowaway* Are Active in Potato

Masaki Momose, Yutaka Abe and Yoshihiro Ozeki

Copyright © 2010 by the Genetics Society of America
DOI: 10.1534/genetics.110.117606
FIGURE S1.—Approximate positions of primers used in this study. Arrow heads show primers and their directions used for the analysis of (A) F3’5’H (pseudo) genes as described in supplementary methods, (B) the insertion locus of dTstu1-2 and (C) MITE display. Coding regions are marked by shaded boxes and the Stowaway MITE is indicated by a black bar.
FIGURE S2.—MITE display using primers designed from sequences of d'Tstul and d'Tstul-2. Mse+T was used as a selective primer. The left panels show peaks from 0 base to 120 bases, the middle from 120 to 360 bases and the right from 360 to 600 bases. Differences of 'JKR' and 'JKP' as compared with '72218' are indicated by arrows. An arrowhead shows a new peak of 315 bases in size.
FIGURE S3.—Tuber appearance of one of the plants regenerated from protoplasts of ‘72218’. Arrows indicate purple variegation (which appear as a dark line in the picture) present against the red background.
**FILE S1**

**Supporting Methods**

Isolation and sequences determination of the genomic DNA for \( F3'5'H \) genes: For the isolation of \( F3'5'H \) pseudo-gene, \( f3'5'h2 \), inverse PCR was carried out with primers No. 1 and 7 for the 1st, and No. 2 and 8 for the 2nd amplification using as a template self-ligated genomic DNA from 'JKP' which had been digested by \( MboI \). Primers No.7 and 8 were based on the highly conserved region among P450 or \( F3'5'H \) genes. The product was cloned and its sequence enabled the design of primers No. 9, 10 and 11, which were used in inverse PCR (with primers No. 2 and 9 for the 1st and No. 10 and 11 for the 2nd amplification) on self-ligated \( XbaI \)-digested genomic DNA of 'JKP'. The sequence of the resulting 5 kb product provided information to design primers No. 12 and 13 that were used in PCR reactions on genomic DNA from 'JKP' and '72218' to compare their \( f3'5'h2 \) alleles (primers No. 5 and 12 for the 1st and No. 5 and 13 for the 2nd amplification). The third copy of \( F3'5'H \) pseudo-gene, \( f3'5'h3 \), was isolated after a series of inverse PCR reactions that yielded sequence to design primers that were used in subsequent rounds. Inverse PCRs were done on \( HincII \)-digested genomic DNA followed by self-ligation with primers No. 14 and 15 and then self-ligated \( EcoRI \)-digested DNA with a primer set of No. 16 and 17 for the 1st followed by a set of No. 17 and 18 for the 2nd amplification. The penultimate round of inverse PCR was performed on \( HindIII \)-digested and self-ligated DNA with primers No. 18 and 19 for the 1st, and No. 20 and 21 for the 2nd amplifications. Finally, primers could be designed (No. 22 and 23 for the 1st and No. 18 and 24 for the 2nd amplification) to amplify \( f3'5'h3 \) from both 'JKP' and '72218' genomic DNAs as templates (see Figure S1). The extension time in all PCRs was 5 min.
| Primer | Sequence |
|--------|----------|
| 1      | 5'-AACATTTTTTGCTCAATAAAKCATCAA-3' |
| 2      | 5'-CCGTGTAATCCATCCAAGCTA-3' |
| 3      | 5'-CCGAATTCATTAGGACATTTATGATCTGTT-3' |
| 4      | 5'-GGCATTAGTTGTAGTTTG-3' |
| 5      | 5'-CCGTGTTCTTTCATTTCATCTC-3' |
| 6      | 5'-AGCAATATGTTGGACATTT-3' |
| 7      | 5'-CCGTAGTTATGCTTATGTTTG-3' |
| 8      | 5'-GGGATTAAATCTGGAGAAGAG-3' |
| 9      | 5'-TATCCCAAGTTGACACC-3' |
| 10     | 5'-ACCGAATGGACATCCAAGAC-3' |
| 11     | 5'-TACCGAAACACTCTTTACTTAA-3' |
| 12     | 5'-GCCTCACATCAAATGCAGATCC-3' |
| 13     | 5'-TCATGAAATGCTGACAAATTTAT-3' |
| 14     | 5'-GCATATCCAAAGATCTTTCCA-3' |
| 15     | 5'-TGGCAAAAGCTGTACTCTTG-3' |
| 16     | 5'-TTACGTTACGGTCTTAAGAG-3' |
| 17     | 5'-AGAGGAGGATAAACAATCTTTAT-3' |
| 18     | 5'-AACAGGACTCCAGCTGACTA-3' |
| 19     | 5'-CTTAAATCCATCCACTAC-3' |
| 20     | 5'-GAACATGAGTTGACGACACCC-3' |
| 21     | 5'-AGGCAATCCTGCCAAATCCACA-3' |
| 22     | 5'-TTGTAAAGTGAGGACCT-3' |
| 23     | 5'-CATTCGTCCTTACGATGGACA-3' |
| 24     | 5'-ATTCAATCTCCTCAGGATGATT-3' |
| 25     | 5'-ATTCATTTGACGACTATTCTAATG-3' |
| 26     | 5'-TGTTTTTTGCAGTTATGATTCT-3' |
| 27     | 5'-CAGGGAGGACATTTAGG-3' |
| 28     | 5'-AGACATTTCATGGACAAATTGTTA-3' |
| 29     | 5'-AGCTGAAATATGAGATTGAAATTAG-3' |
| 30     | 5'-ATTGCTGTATATCAGTACGGAG-3' |
| 31     | 5'-CATTCCTTTTGAGGACATGACT-3' |
| 32     | 5'-ATAAAATGTCGGGACRGAGGAGTA-3' |
| Mse+0  | 5'-GACGTAGCTCCTGAGTAA-3' |
| Mse+N  | 5'-GACGTAGCTCCTGAGTAAAN-3' |