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Identification of a Novel SINE-like Sequence in Retrotransposon Fragments of Sweet Potato

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Abstract

A novel short interspersed nuclear element (SINE)-like sequence, named *IPSE1*, was identified in the retrotransposon fragments of sweet potato (*Ipomoea batatas* (L.) Lam). Sequences highly homologous to *IPSE1* were identified in three previously reported genes of genus *Ipomoea*, suggesting that *IPSE1* is conserved in genomes of *Ipomoea*. Structural characteristics of *IPSE1* were discussed.

Accession numbers: AF223309-AF223326, AF223337-AF223345

Spontaneous mutations are commonly observed during sweet potato cultivation (Hernandez *et al.*, 1964). In addition, clonal plants propagated via sprouts from storage roots and tissue culture-based methods are known to have genetic variations (Villordon and LaBonte, 1996). Although mutations have been traditionally used to improve the agricultural traits of sweet potatoes, they also cause a degradation of cultivars. Despite their importance, the molecular basis of mutations remains elusive.

Retroelements are a kind of transposable element, found in the genomes of eukaryotes. Among the plant retroelements, retrotransposons have been well characterized. They are widely distributed in plant genomes (Voytas *et al.*, 1992; Hirochika and Hirochika, 1993) and are activated by stress and tissue culture (Grandbastien, 1998). Retrotransposons are also involved in the spontaneous mutations of maize (Varagona *et al.*, 1992). Short interspersed nuclear elements (SINEs) are another group of retroelements having a quite simple structure compared to other retroelements (Deniger, 1989). In contrast to the wide distribution of retrotransposons in plant genomes, SINEs have been reported only from *Oryza* (Mochizuki *et al.*, 1992), *Solanaceae* (Yoshioka *et al.*, 1993; Pozueta-Romero *et al.*, 1998), and *Cruciferae* (Deragon *et al.*, 1994, 1996). SINEs have been shown to be actively involved in the modification of gene structures in humans (Wallace *et al.*, 1991; Makalowski *et al.*, 1994). In addition, SINEs have recently been found to change gene expression via induction of *de novo* methylation in flanking genome regions (Arnaud *et al.*, 2000).

Here, we describe the cloning of retrotransposon fragments from the sweet potato. A novel SINE-like sequence, *IPSE1*, was identified in these retrotransposon fragments.

Storage roots (>5 mm in diameter) of sweet potatoes (cv. Kokei 14 and cv. Ayamuraskai) were harvested 6 weeks after planting for RNA extraction. Embryogenic callus of Kokei 14 was induced and maintained on LS media (Linsmaier and Skoog, 1965) containing 0.5 mg l^{-1} picloram (Aldrich, WI), 3% sucrose, and 0.32% gellan gum, as previously described by Otani and Shimada (1996). Cells were harvested 2 weeks after subculture and subjected to RNA extraction. The extraction procedure for total RNA has been described previously (Yoshinaga *et al.*, 2000). Five micrograms of total RNA was treated with DNase I at 37 ℃ for 30 min in a mixture of 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 20 U RNase inhibitor, and 5 U DNase I. After extraction with a phenol:chloroform (1:1) solution and ethanol precipitation, cDNA synthesis was carried out using 2.5 μg of RNA in a mixture containing 1 × RNA PCR buffer (Takara, Japan), 5 mM $MgCl_2$, 1 mM dNTPs, 2.5 μM random 6mers, 20 U RNase inhibitor, and 5 U AMV reverse transcriptase XL (Takara, Japan) at 42 ℃ for 1 h.

Genomic DNA was extracted from young leaves

of Kokei 14 grown in a greenhouse as described below. Tissue was ground in liquid nitrogen, and added with three volumes of 2% CTAB solution (100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, 0.1 M β-mercaptoethanol, pH 8.0). After incubation at 65 ℃ for 30 min, an equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed gently for 10 min. After centrifugation at 15000 × g for 5 min, the aqueous phase was recovered and extracted once with chloroform:isoamyl alcohol (24:1). An equal volume of 1% CTAB solution (50 mM Tris-HCl, 10 mM EDTA, 1% CTAB, pH 8.0) was added and incubated for 1 hour. The supernatant was removed after centrifugation at 10,000 × g for 10 min, and the pellet was dissolved in 1 M CsCl containing 10 μ g/m*l* RNaseA. After incubation at 37 ℃ for 60 min, total DNA was recovered by means of ethanol precipitation and dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The reverse transcriptase domains of retrotransposons were amplified according to the method of Hirochika *et al.* (1992) with a minor modification. The temperatures of the PCR reaction were modified as follows: initial denaturation for 4 min at 94 ℃, followed by 30 cycles of 1 min at 94 ℃, 2 min at 42 ℃, 2 min at 72 ℃, followed by a final extension of 5 min at 72 ℃. Combinations of two 5' primers (RT-1: CA(A/G)ATGGA(C/T)GTNAA(A/G)AC and RT-7: GA(C/T)GT(A/G/C/T)AA(A/G)AC(A/G/C/T)GC(A/G/C/T)TT(T/C)(C/T)T) and one 3' primer (RT-3: TA(T/C)GT(A/G/C/T)GA(C/T)GA(C/T)ATG) were tested. For the amplification from cDNA, a second amplification was performed under the same conditions using the product of the first amplification as a template.

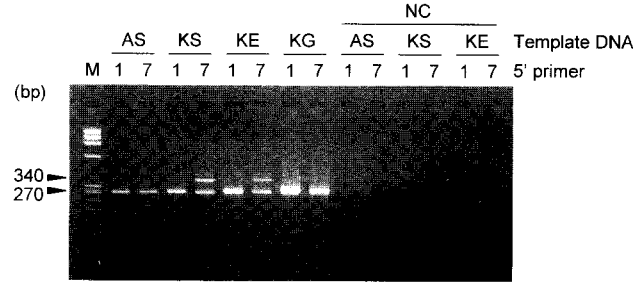

Fig. 1 PCR amplification of a reverse transcriptase fragment of retrotransposons from sweet potato cDNA and genomic DNA. AS, cDNA from Ayamurasaki storage root; KS, cDNA from Kokei 14 storage root; KE, cDNA of Kokei 14 embryogenic callus; KG, genomic DNA of Kokei 14. NC, control reactions without reverse transcription. M, marker DNA (ϕX174/*Hae* III Digest).

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Tib1    AFLHGDLEEEIYMLQPEGFEDKENQ-NLVCRLNKSLYGLKQAPRCWYKRFDSFIMCLGCNRLNADPCAYFKRFGEDNFVILLL  82
Tib2    AFLNGLLEEEVYVEQPPGFLKDVGA-DKVYKLKKALYGLKQAPRAWYDTLSCFLLQCCFTKGLVDKTLFRIKDGDHILLVQI-  81
Tib3    ---NGNLSETIFMRQPPGYTDKLFP-DHVCLLKRSLYGLKQAPRAWFTRLHTFLLSIGFQASKTDVSLFYFSQGSASVYLLV-  78
Tib4    ---HGDLEEEVYMEQPPGFVAQGES-GKVCKLKKSLYGLKQSPRAWFGRFSSVITEFGMQRSVCDHTVFFKHTNTGCILLII-  78
Tib5    ---NGALAETVYMCQPPGYVDVQLP-DHVCLLKRSLYGLKQAPRAWFNRLHTFLLSVGFKASKTDVSLFYYSRGSACVYLLV-  78
Tib6    ---NGDLKEEIYMKLPPGMPMIAPH--EVCKLRRSLYGLKQAPRAWFEKFRDTLLTFSFTQSQYDSSLFFHKTTTGMVFLLV-  77
Tib7    AFLNGDLEEEIYMEQPHGCVAPGQE-EKVCRLKKSLYGLKQAPKQWYEKFHKTIIELGYTTNRSDACLYTKEIESH-VVIICL  81
Tib8    ---HGDLKEEVYMEQPPGFVAQGESSGMVCKLRRSLYGLKQSPRAWFGRFSSVVCEFGMVRSEVDHSVFYRHSSGKCIYLVV-  79
Tib9    ---NGELEEEVYMEIPPGFEKNSSG-RLVCKLEKALYGLKQSPRAWFDRFAKSIVKFGYTQCQADHTLFVKFSSTGKLAILIV  79
Tib10   ---HGDLHEEVYMEQPPGFVAQGES-GKVCRLRRSLYGLKQSPRAWFGKFSQAVESFGMMKSKSDHSMFYKWSKTGIIWLVV-  78
Tib11   AFLNGDLEEEVYMKQPEGFSSNKGE-HLVCKLKKSIYGLKQASRQWYIKFDGIISSYGFVESPIDNCIYQKVSGSKICFLVL-  81
Tib12   ---HGDLVEEVYMEQPPGFVAQGEN-GKVCRLRKSLYGLKQSPRAWFGRFSGAITEFGMRRSAYDHTLFYKHSNHGSILLIV-  78
Tib13   ---NGDLEEVVYMKQPEGFSSSEDN-HLVCKLKKSIYGLKQASRQWYIKFDGVISSYGFVENILDHCIYQKTSGSKICFLVL-  78
Tib14   ---NGYLKEDVYVAQPKGFEDPKHP-DHVYKLKKALYGLKQAPRAWYERLTEYLVNKGYSRGSVDKTLFIKRYDSDLIITQI-  78
Tib15   ---HGDLEEEVYMVLPPGFKSDSPN--QVCKLLRSLYGLKQASRQWNAKLTKALQDDGFQQSTADPSLLQKLQITHSLLYFV-  77
Tib16   ---NGELDEEIYMDQPEGFVVPGQE-KKVCKLVKSLYGLKQAPKQWHEKFDHSMLTNGFKINESDKCIYVKETVDG-YVILCL  78
Tib17   AFLNGELDEEIYMEQPEGFIVPGQE-RKVCKLVKSLYGLKQAPKQWHEKFDHAMLTNGFKINESDKCVYTKETING-FVILCL  81
Tib18   AFLNGYLKEYVYVAQPKGFEDPKHP-EHVYKLKKALYGLKQAPRAWYDRLTEYLVHKGYSRGSVDKTLFIKRIGSDLIIAQI-  81
Tib19   AFLNGLLHEEIFVAQPKGFENPQYP-DYVYKLKKALYGLKQAPRAWYERLTQYLLKNGYTRGGADKTLFVKRATGHITVAQ--  80
Tib20   AFLNGELDEEIYMKQPEGFIVLGQE-SKVCKLVKSLYGLKQTPKQWHENFDHAMLTNGLKINESDKCVYTKETIDG-YVILCL  81
Tib21   AFLNGDLEEEVFMKQPEGFSSRDGE-NLVCKLKKSIYGLKQASRQWYIKFDGVISSFGFAENPLDHCIYQKVSGSKICFLVL-  81
Tib22   AFLNGDLEEEIYMDQPPGCIEPGQE-GKVCRLKKSLYGLKQAPKQWYEKFHKTLIDLGYSANRSDACLYTKEIESK-VVIICL  81
Tib23   AFLNGELEEEVYMKQPEGFVVAGQE-HKVCKLIKSLYGLKQAPKQWHDRFDQVVLANGYRINQSDKCIYSKFDNQGQGVIICL  82
Tib24   ---NGLLHEEIFVAQPKGFENPQYP-DYVYKLKKALYGLKQAPRAWYERLTQYLIQNGYTRGGADKTLFVKKTTKHITVAQV-  78
Tib25   ---HGTLSEEVYMKQPPGFCDPEHP-KHVCRLKKALYGLKQAPRAWYMELSSFLISFGFRKSKADYSLFIYQCDDVLAYFMV-  78
Tib26   ---NGHLSETVFMKQPPGYEDPTHP-DYVCHLQRLLYGLKQAPRAWFKRLHDFLLSTGFRASKTDVSLLYYSAHHSKVFLLV-  78
Tib27   ---HGDLNEEVYMVLPPGFEGEKPG--QVCKLLRSLYGLKQASRQWNAKLSSALVEKGFTQSKSDPSLFTKGCDTSFIAVLV-  77
Tib28   ---NGTLHEDVYMEQPSGFVDSSRP-THICKLTKAIYGLKQAPRAWYLELSSFLLQFGFQKSVSDASLFVYNMSSVVVYFLV-  78
Tib29   ---HGDLSEEVYMEQPPGFVAQGES-GKVCRLKKSLYGLKQSPRAWFGRFSSAVVKFGMQRSAYDHTVFYKHTNYGCVLLIV-  78
Tib30   ---HGDLQEEVYMEQPPGFVAQGES-GLVCKLRRSLYGLKQSPRAWFGRFSSVVCEFGMTRSKVDHSVFYRHSNGKCIYLVV-  78
Tib31   ---HGDLKEEVYMEQPPGFVAQGES-SLVCKLRRSLYGLKQSPRTWIVRFSSVVCEFGMVRSEVDHSVFYRHSNGKCIYLVV-  78
Tto1    AFLHGDLEEEIYMEQPDGFQQKGKE-DYVCRLRKSLYGLKQAPRQWYKKFESVMGQHGYKKTTSDHCVFAQKFSDDDFIILLL  82
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Fig. 2 Alignment of deduced amino acid sequences for the reverse transcriptase domain of sweet potato retrotransposon families. Sequences corresponding to oligonucleotide primers are removed because they are thought to be different from actual sequences. Conserved amino acid residues are reversed. The sequence of tobacco retrotransposon *Tto1* (D83003) is included for comparison. Accession numbers for *Tib1-31* are AF223309-AF223326 (mRNA) and AF223337-AF223345 (DNA).

PCR products were separated on a 2% low melting-point agarose gel and recovered from the gel. These fragments were cloned into pBluescript II KS (+). The nucleotide sequences of the clones were analyzed with a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and an ABI PRIZM™ 310 Genetic Analyzer (PE Applied Biosystems, CA). Sequence data and multiple alignment analyses were carried out using GENETYX-MAC software (Software Development, Japan). The Fasta program (Pearson and Lipman, 1988) on Genome Net (fasta@genome.ad.jp) was used to search for sequence homology.

The reverse transcriptase domain of a retrotransposon contains highly conserved amino acid sequences. Thus, PCR amplification has been successfully used to amplify retrotransposon fragments from genomic DNA or cDNA of various plants (Voytas *et al.*, 1992; Hirochika and Hirochika, 1993). In addition to genomic DNA, we amplified cDNA that were synthesized from the mRNA of storage root and embryogenic callus to obtain the sequences from highly transcribed retrotransposons. All DNA templates gave the expected amplified fragments of approximately 270 bp (**Fig. 1**).

Amplified fragments were cloned into the plasmid vector pBluescript II KS(+), and nucleotide sequences of a total of 219 clones were analyzed. All clones contained sequences highly homologous to the reverse transcriptase domain of previously identified retrotransposons, and 147 clones were found to have an open reading frame (ORF) throughout the amplified region. Based on the identity (<90%) between the deduced amino acid sequences, these clones were classified into 31 families (**Fig. 2**) and named *Tib1-Tib31* (*Tib*; Transposon of *Ipomoea batatas*). Eleven families (*Tib1, 3-5, 6, 8, 9, 12-15*) were found exclusively in cDNA, 7 families (*Tib2, 7, 10, 11,16, 19, 22*) in both cDNA and genomic DNA, and 13 families (*Tib17, 18, 20, 21, 23, 24-31*) exclusively in genomic DNA.

In addition to the expected fragments of approximately 270 bp, cDNA from Kokei 14 gave major amplification fragments of approximately 340 bp when the RT-7 primer was used (**Fig. 1**). Nucleo-

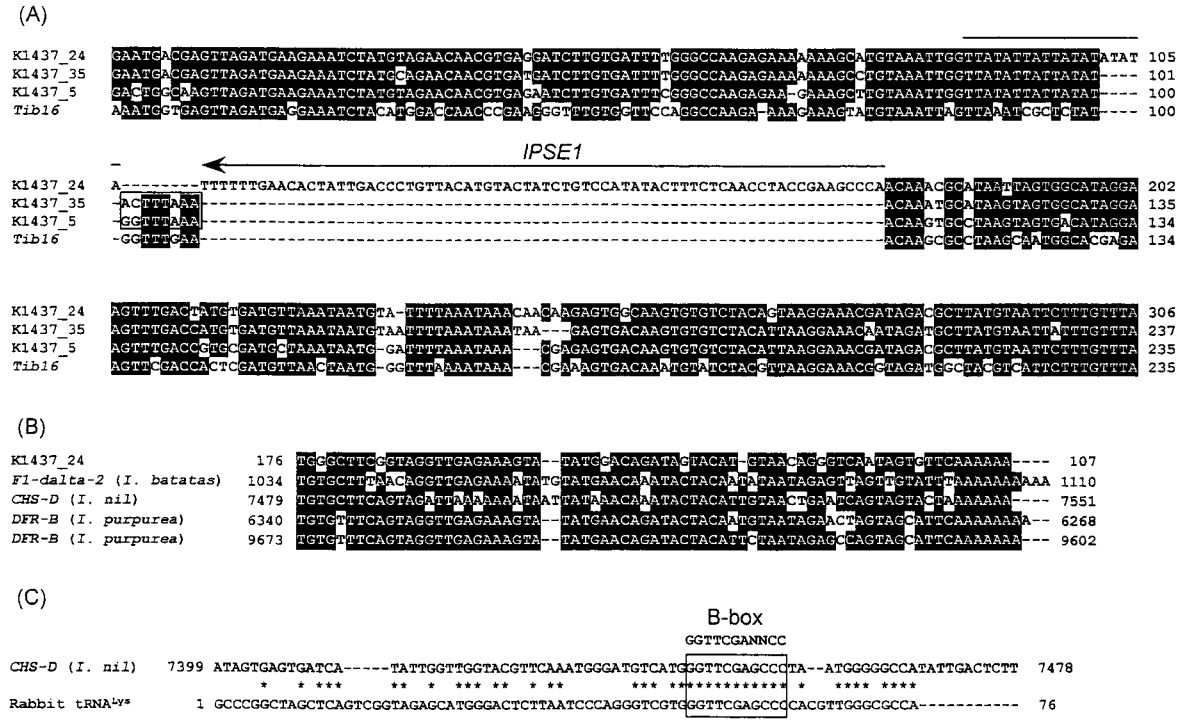

Fig. 3 **(A)** Nucleotide sequence of a reverse transcriptase-like fragment (K1437_24) having an *IPSE1* insertion. Nucleotide sequences of *Tib16* and reverse transcriptase-like fragments (K1437_5 and K1437_35) containing only flanking (TTA) and (TA) repeats (overlined) of *IPSE1* are aligned for comparison. An open box indicates a putative target site of *IPSE1*. The accession number for *IPSE1* is AF295596. **(B)** Alignment of *IPSE1* in reverse transcriptase-like fragment K1437_24 with other *IPSE1*s in *Ipomoea*. Accession numbers are as follows: F_1 δ-*2* gene (encoding δ-subunit of mitochondrial F_1-ATP synthase) of sweet potato, AB026910; *CHS-D* gene of Japanese morning glory, AB027533; *DFR-B* gene of common morning glory, AB011667. **(C)** Alignment of the 5' upstream sequence of *IPSE1* in *CHS-D* gene of Japanese morning glory with rabbit $tRNA^{Lys1}$ (Accession number, K00289).

tide sequence analysis showed that these fragments also contained reverse transcriptase-like sequences highly homologous to *Tib16*. However, they contained several stop codons and an inserted sequence of approximately 70 bp. This inserted sequence had a poly-A motif at its 3' end and flanking (TTA) and (TA) repeats (**Fig. 3A**), all of which are typical structural characteristics of SINEs. Interestingly, sequences highly homologous to this insertion were found in the *$F_1\delta$-2* gene (encoding the δ-subunit of mitochondrial F_1-ATP synthase) of the sweet potato (Maeo *et al.*, 1999), the *CHS-D* gene of the Japanese morning glory (*Ipomoea nil*) (Johzuka-Hisatomi *et al.*, 1999), and the *DFR-B* gene of the common morning glory (*Ipomoea purpurea*) (Inagaki *et al.*, 1999)(**Fig. 3B**). Therefore, this sequence is thought to be conserved in the genomes of *Ipomoea*. We named these sequence *IPSE1* (*Ipomoea* SINE-like element 1).

*IPSE1*s are distinctly shorter than previously reported plant SINEs (normally approximately 100 to 200 bp). In addition, most of *IPSE1*s were not found to contain promoter motifs for RNA polymerase III, which are usually found in SINEs of tRNA origin. Only the *IPSE1* in the *CHS-D* gene of the Japanese morning glory had a 5' upstream region similar to tRNA (**Fig. 3C**). Although this sequence was defective, it contained the B-box of the RNA polymerase III promoter motif (**Fig. 3C**). These observations indicate that *IPSE1* is also of tRNA origin. Except for this tRNA-like sequence, *IPSE1*s did not show significant homology to known plant SINEs.

Other clones from Kokei 14 cDNA showed strong homology to the clones containing *IPSE1*, but lacked the insertion of *IPSE1* (**Fig. 3A**). These clones contained only flanking (TTA) and (TA) repeats of *IPSE1*, indicating that the formation of (TTA) and (TA) repeats took place before the integration of *IPSE1*. From the comparison of these clones with clones containing *IPSE1*, the target site of *IPSE1* was inferred to be 5'-TTTAAA(G/C)(G/A)-3' (**Fig. 3A**). This sequence is quite similar to the target site of mammalian SINEs, 5'-TTAAAA-3' (Jurka, 1997), and similar in part to the target site of SINEs from *Cruciferae*, 5'-YAAANNNG-3' (Tatout *et al.*, 1998). This observation is consistent with the previous report that plant and mammalian SINEs have similarities in target site selection (Tatout *et al.*, 1998). However, unlike previously reported SINEs, duplication of the target site caused by integration was not observed for *IPSE1*.

In this report, we have described the cloning and characterization of sweet potato retroelements. The sequences reported here may contain minor errors derived from PCR amplification. However, it is difficult to discriminate such errors from existing sequence heterogeneity of retroelements. Further characterization of these retroelements will contribute to our understanding of sweet potato mutations and evolution of the genus *Ipomoea*.

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References

Arnaud, P., Goubely, C., Pélissier, T., Deragon, J.-M., 2000. SINE retroposons can be used *in vivo* as nucleation centers for *de novo* methylation. Mol. Cell Biol., **20**: 3434-3441.

Deninger, P. L., 1989. SINES: short interspersed repeated DNA elements in higher eucaryotes. In: Berg, D. E., Howe, M. M. (Eds.): Mobile DNA, 619-636. American Society of Microbiology, Washington, DC.

Deragon, J.-M., Landry, B. S., Pélissier, T., Tutois, S., Tourmente, S., Picard, G., 1994. An analysis of retroposition in plants based on a family of SINEs from *Brassica napus*. J. Mol. Biol., **39**: 378-386.

Deragon, J.-M., Gilbert, N., Rouquet, L., Lenoir, A., Arnaud, P., Picard, G., 1996. A transcriptional analysis of the S1Bn (*Brassica napus*) family of SINE retroposons. Plant Mol. Biol., **32**: 869-878.

Grandbastien, M.-A., 1998. Activation of plant retrotransposons under stress conditions. Trens Plant Sci., **3**: 181-187.

Hernandez, T. P., Hernandez, T., Miller, J. C., 1964. Frequency of somatic mutations in several sweet potato varieties. J. Amer. Soc. Hort. Sci., **85**: 430-433.

Hirochika, H., Fukuchi, A., Kikuchi, F., 1992. Retrotransposon families in rice. Mol. Gen. Genet., **233**: 209-216.

Hirochika, H., Hirochika, R., 1993. *Ty1-copia* group retrotransposons as ubiquitous components of plant genomes. Jpn. J. Genet., **68**: 35-46.

Inagaki, Y., Johzuka-Hisatomi, Y., Mori, T., Takahashi, S., Hayakawa, Y., Peyachoknagul, S., Ozeki, Y., Iida, S., 1999. Genomic organization of the genes encoding dihydroflavonol 4-reductase for flower pigmentation in the Japanese and common morning glories. Gene, **226**: 181-188.

Johzuka-Hisatomi, Y., Hoshino, A., Mori, T., Habu, Y., Iida, S., 1999. Characterization of the chalcone synthase genes expressed in flowers of the common and Japanese morning glories. Genes Genet. Syst., **74**: 141-147.

Jurka, J., 1997. Sequence patterns indicate an enzymatic involvement in integration of mammalian retroposons. Proc. Natl. Acad. Sci. USA, **94**: 1872-1877.

Linsmaier, E. M., Skoog, F., 1965. Organic growth factor requirement of tobacco tissue culture. Physiol. Plant., **18**: 100-127.

Maeo, K., Morikami, A., Soga, M., Imanishi, S., Nakamura, K., 1999. Expression patterns of two genes for the delta-subunit of mitochondrial F_1-ATP synthase from sweet potato in transgenic tobacco plants and cells. Plant Cell Physiol., **40**: 866-873.

Makalowski, W., Mitchell, G. A., Labuda, D., 1994. Alu sequences in the coding regions of mRNA: a source of protein variability. Trens Genet., **10**: 188-193.

Mochizuki, K., Umeda, M., Ohtsubo, H., Ohtsubo, E., 1992. Characterization of a plant SINE, p-SINE*1*, in rice genomes. Jpn. J. Genet., **57**: 155-166.

Otani, M., Shimada, T., 1996. Efficient embryogenic callus formation in sweet potato. Breeding Science, **46**: 257-260.

Pozueta-Romero, J., Houlné, G., Schantz, R., 1998. Identification of a short interspersed repetitive element in partially spliced transcripts of the bell pepper (*Capsicum annuum*) *PAP* gene: new evolutionary and regulatory aspects on plant tRNA-related SINEs. Gene, **214**: 51-58.

Tatout, C., Lavie, L., Deragon, J. -M., 1998. Similar target site selection occurs in integration of plant and mammalian retroposons. J. Mol. Evol., **47**: 463-470.

Turker, M. S., Bestor, T. H., 1997. Formation of methylation patterns in the mammalian genome. Mutat. Res., **386**: 119-130.

Varagona, M. J., Purugganan, M., Wessler, S. R., 1992. Alternative splicing induced by insertion of retrotransposon into the Maize waxy gene. Plant Cell, **4**: 811-820.

Villordon, A. Q., LaBonte, D. R., 1996. Genetic variation among sweetpotatoes propagated through nodal and adventitious sprouts. J. Amer. Soc. Hort. Sci., **120**: 170-174.

Voytas, D. F., Cummings, M. P., Konieczny, A., Ausubel, F. M., Rodermel, S.R., 1992. *Copia*-like retrotransposons are ubiquitous among plants. Proc. Natl. Acad. Sci. USA, **89**: 7124-7128.

Wallace, M. R., Andersen, L. B., Saulino, A. M., Gregory, P. E., Glover, T. W., Collins, F. S., 1991. A de novo Alu insertion results in neurofibromatosis type 1. Nature, **353**: 864-866.

Yoshioka, Y., Matsumoto, S., Kojima, S., Ohshima, K., Okada, N., Machida, Y., 1993. Molecular characterization of a short interspersed repetitive element from tobacco that exhibits sequence homology to specific tRNAs. Proc. Natl. Acad. Sci. USA, **90**: 6562-6566.

Yoshinaga, M., Tanaka, M., Nakatani, M., 2000. Changes in anthocyanin content and composition of developing storage root of purple-fleshed sweet potato (*Ipomoea batatas* (L.) Lam). Breeding Science, **50**: 59-64.