Establishment of nDart1-tagged lines of Koshihikari, an elite variety of rice in Japan

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To utilize a transposon-tagged mutant as a breeding material in rice, an endogenous DNA transposon, nDart1-0, was introduced into Koshihikari by successive backcrossing together with aDart1-27, an active autonomous element. The founder line for nDart1-tagged lines of Koshihikari carried nDart1-0 on chromosome 9 and transposed nDart1-12s on chromosomes 1 and 8 and nDart1-3 on chromosome 11. In nDart1-tagged lines, there were the most abnormal phenotypic mutants and many aberrant chlorophyll mutants at seedling stage. At mature stage, many semi-sterile mutants were observed. Dwarf, reduced culm number and lesion mimic mutants were also found. In total, 43.2% of the lines segregated some phenotypic mutants. Thus, the nDart1-tagged lines of Koshihikari are expected to be potentially useful for screening stress-tolerant mutants under abiotic or biotic stress conditions.

Key Words: DNA transposon, insertional mutagenesis, nDart1-tagged lines, Koshihikari, rice.

Introduction

Advanced gene modification techniques such as gene editing could lead to a drastic improvement of crop productivity to meet the growing population or environmental fluctuation due to climate change (Zhang et al. 2018). To apply these techniques, the function of the target gene must be elucidated. Insertional mutagenesis using T-DNA or a transposon is one of the powerful methods for functional analysis of the gene (Jiang and Ramachandran 2010). Because gene discovery in insertional mutants using PCR such as TAIL-PCR (Liu and Whittier 1995), inverse PCR (Ochman et al. 1988) and suppression PCR (Rand et al. 2005) is easier than that in physical or chemical mutagen-induced mutants applying Map-based cloning or NGS. Thus, T-DNA tagged lines in rice were created by Lee et al. (2003) and Li et al. (2006) and large mutant pools induced by the maize transposons, Ac/Ds (Chin et al. 1999, Greco et al. 2003) and En/Spm (Greco et al. 2004) have been utilized for the functional analysis of rice genes (Howard et al. 2014, Jiang and Ramachandran 2010, Wang et al. 2013). Meanwhile, an endogenous retrotransposon, the Tos17-tagged mutant pool, has been developed in Nipponbare, a japonica rice, as Hirochika (2010) reviewed. Active endogenous DNA transposons in rice, mPing (Jiang et al. 2003, Kikuchi et al. 2003, Nakazaki et al. 2003), dTok (Moon et al. 2006), nDart (Tsugane et al. 2006) and nDaiZ (Huang et al. 2009) were found. The originally identified nDart1-0 (nonautonomous DNA-based active rice transposon1-0) was discovered in the F2 of the cross between indica and japonica lines (Tsugane et al. 2006). There are thirteen nDart1 elements that showed over 98% identity with nDart1-0 in Nipponbare genome. The transpositions of nDart1 were controlled by transposase supplied by an active autonomous element, aDart1 (Nishimura et al. 2008). nDart1 was more advantageous for insertional mutagenesis than the other DNA transposons in rice. Because Tsugane et al. (2006) identified aDart1-27 on chromosome 6 as an active autonomous element derived from H-126 together with nDart1 and a large-scale nDart1-tagged mutant pool can be easily developed using an active nDart1/aDart1-27 system introduced in a given variety by crossing. In nDart1-tagged lines, so far, pale-yellow leaf (pyl), thumbelina (thl) (Tsugane et al. 2006) and snow-white leaf1 (swl1) (Hayashi-Tsugane et al. 2014) have been analyzed. In particular, nDart1-tagged mutagenesis shows the characteristics for segregating gain-of-function mutants, e.g., aberrant panicle organization1-D1 (apo1-D1) (Ikeda-Kawakatsu et al. 2009), tawawa1-D (taw1-D) (Yoshida et al. 2013) and Bushy dwarf tiller1 (Bdt1) (Hayashi-Tsugane et al. 2015). Further, the indeterminate growth (ing) mutant carrying a 100 kb deletion was found in nDart1-tagged lines, although it was unclear whether the deletion was caused by nDart1 insertion (Hayashi-Tsugane et al. 2011). Recently, Chiou et al. (2019)
revealed *Large grain (LGG)* showing incomplete dominance in *nDart1*-tagged lines of Koshihikari. These genes, except for *LGG* and *swl1*, were revealed to have been derived from mutants found in the tagged lines of MK1 (Ikeda-Kawakatsu *et al.* 2009) which was bred true from the crossing between Matsumoto-mochi, an old variety in Hokkaido, and *nDart1-0/aDart1-27* NIL of Shiokari. The *swl1* mutant was discovered in the *nDart1*-tagged lines of T-65. In particular, *taw1-D2* (Yoshida *et al.* 2013) found in MK1 was introduced into Koshihikari to demonstrate its agronomic characteristics. *apo1-D1* (Ikeda-Kawakatsu *et al.* 2009), *taw1-D2* (Yoshida *et al.* 2013), and *LGG* (Chiou *et al.* 2019) were responsible for spikelet number and grain weight, respectively, of yield components in rice. Then, it was necessary to breed *nDart1*-tagged lines in an elite variety because it could be directly used as a breeding material if a useful mutant would be found. Thus, this study reports that *nDart1*-tagged lines of Koshihikari were bred through successive backcrossing and selection of homozygous normal phenotype plants. Some mutants in the lines were observed, and a variegated albino mutant was subjected to TAIL-PCR to detect the causal gene.

**Materials and Methods**

**Plant materials**

The breeding process of *nDart1*-tagged lines of Koshihikari was shown in Fig. 1. The donor of *nDart1-0* and *aDart1-27* was MK1 pyl-v (variegated pale-yellow leaf) and heterozygous BCnF1 plants for *nDart1-0* and *aDart1-27* successively backcrossed with Koshihikari as a recurrent parent were selected based on the segregation of pyl-v and pyl-stb (stable pale-yellow leaf) in BCnF2. Because heterozygous *aDart1-27* was not distinguished from homozygote with PCR product. In BC9F2 population, pyl-v plants were selected and were confirmed to be homozygous for *aDart1-27* through segregation check of pyl-stb in BC9F3. In the homozygous BC9F3 for *aDart1-27*, revertants carrying heterozygous *nDart1-0*-inserted OsClpP5 were also segregated. Then, a revertant fixed to normal was confirmed in BC9F4 and was subjected to transposon display to detect *nDart1* insertions. Koshihikari *nDart1*-tagged lines were developed from this revertant as a founder line. In 2011, twelve seeds per line in 1696 lines were planted in seeding beds with commercial soil (Kumiai Ube Ryujo Baido, Ube Industries, Japan) and twelve plants per line were grown with a spacing of 40 cm between rows and 15 cm between plants in a paddy field at the Institute of Plant Science and Resources, Okayama University, Kurashiki, Japan. The phenotypes in the lines were observed at seedling, tillering, heading and mature stages.

**DNA extraction and transposon display**

Genomic DNA was extracted from third leaf tissue at the seedling stage as described by Gichuhi *et al.* (2016). Transposon display to identify the insertion site of *nDart1* was performed according to the method reported by Takagi *et al.* (2007).

**TAIL-PCR and PCR**

The primers for TAIL-PCR were listed in Supplemental Table 1. The sequences of ARB1 to ARB6 and ARB7 of arbitrary primers were according to Liu and Whittier (1995) and Tanaka *et al.* (2003), respectively. For TAIL-PCR, a

![Fig. 1. Development of *nDart1*-tagged lines of Koshihikari.](image-url)
total PCR reaction volume of 15 μL was prepared containing 0.5 U of LA Taq (TAKARA Japan), 1X GC buffer I, 0.67 pmol of nDart1-0 primer, 2.7 pmol of arbitrary primer and 10 ng of template DNA. PCR conditions are shown in Supplemental Table 2. The PCR product was diluted 100-fold and used as the template for the second/third PCR. PCR products were electrophoresed on 2% agarose gels and visualized after ethidium bromide staining. The PCR products were ligated with T-vector easy (Promega) and the ligated clones were sequenced with ABI3100 (Applied Biosystems). Obtained sequences were analyzed with GENETYX and insertion sites were checked with RAP-DB (The Rice Annotation Project Database; https://rapdb.dna.affrc.go.jp/). The conserved domain was searched using MOTIF Search (https://www.genome.jp/tools/motif/) and CD-Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Conserved domains were aligned with the SALAD database (https://salad.dna.affrc.go.jp/salad/).

The PCR for checking the insertion site was conducted using LA-Taq and specifically designed primers (Supplemental Table 1) as mentioned for TAIL-PCR.

**Results**

**nDart1-insertion sites in the founder line**

The nDart1 insertion sites in the normal-fixed revertant selected as a founder line in BC9F4 were exploited through transposon display. Koshihikari has ten sites authentically; chromosomes 1, 2, 3, 4, 5, 6 and 9 located 1, 2, 3, 1, 1, 1 and 1 sites, respectively (Fig. 2). Since Nipponbare possesses thirteen sites, Koshihikari does not have nDart1-2, 1-3 (8) and 1-3 (3-1) on chromosomes 12, 8 and 3, respectively. nDart1-0 inserted in OsClpP5 was located originally on chromosome 3. The founder line possessed nDart1-0 on chromosome 9. In addition, nDart1-3 and nDart1-12 were found to be transposed on chromosomes 11 and 1 and on chromosome 8, respectively (Fig. 2). Especially, 3 new insertion sites of nDart1-12 were detected, suggesting that nDart1-12 might have better transposability than another nDart1s.

![Fig. 2. Distribution of nDart1s in the founder line carrying nDart1-0 and aDart1-27 in Koshihikari. Red and white arrow heads at left side show transposed and stayed nDart1s in the nDart1-tagged lines, respectively and white arrow head at right side depicts authentic insertion site of nDart1s in Koshihikari. Green diamond represents original insertion site of nDart1-0 in the tagged lines.](image-url)

**Mutant phenotypes observed in nDart1-tagged lines**

Mutant phenotypes observed at seedling stage and mature stage were summarized in Table 1. The abnormal seedlings were mostly observed as given in Fig. 3D and the chlorophyll mutants were also found frequently. At mature stage, many semi-sterile plants were observed. There were some dwarf, lesion mimic and reduced culm number mutants. Further, chlorophyll, glume and heading mutants were also found. Aberrant phenotype plants were present in 395 and 337 lines at seedling and mature stages, respectively. In total, 43.2% of 1696 lines segregated mutant phenotypes. The difference of copy numbers of nDart1 were analyzed in Mutants #1 (Mut#1) and #2 (Mut#2) through TD using Cvi II. Mut#1 and Mut#2 showed distinctive phenotypic characteristics, respectively. As shown in Supplemental Fig. 1, TD demonstrated 8 and 4 insertions of nDart1 in Mut#1 and Mut#2, respectively, only in the range of ca. 200 bp and ca. 400 bp. In these insertions, 4 were common in both Mut#1 and Mut#2. However, Mut#1 and #2 showed 3 and 1 mutant-specific insertions, respectively. This result suggested that nDart1 transposed frequently under the control of aDart1-27. The characteristic phenotypes at seedling, tillering and mature stages were given in Fig. 3.

(A) This extreme dwarf was characterized with dark green and broad leaves at seedling stage.

(B) This albino showed green sectors on leaves.

(C) The third light green leaf of the seedling was observed.

(D) Deteriorated second and third leaves were shown at seedling stage.

(E) Striped leaves were observed at tillering stage.

(F) Yellow-banded zebra leaf was clearly represented at tillering stage.

| Seedling stage | Mature stage |
|----------------|-------------|
| **Phenotype** | **No. of lines** | **Phenotype** | **No. of lines** |
| Abnormal       | 335         | Abnormal       | 4             |
| Aberrant chlorophyll | 32 | Lethal         | 7             |
| Lesion mimic   | 1          | Dwarf          | 38            |
| Dwarf          | 9          | Semi-dwarf     | 3             |
| Aberrant leaf  | 5          | Tillering dwarf| 7             |
| Aberrant culm  | 14         | Tetraploid     | 3             |
| Tillering      | 1          | Aberrant chlorophyll | 15 |
|                |            | Long culm      | 3             |
|                |            | Reduced culm number | 34 |
|                |            | Tiller number  | 7             |
|                |            | Lesion mimic   | 37            |
|                |            | Leaf character | 7             |
|                |            | Culm character | 6             |
|                |            | Aberrant glume | 13            |
|                |            | Aberrant panicle | 6 |
|                |            | Heading        | 10            |
|                |            | Semi-sterile   | 131           |
|                |            | Complete sterile| 6             |

| Total          | 395         | Total          | 337           |

Table 1. Mutant phenotype observed in nDart1-tagged lines in Koshihikari

![image-url]
(G) This plant with few tillers was lethal at heading stage. 
(H) Small round hulls were observed in the dwarf with dark green leaves.  
(I) A Defective panicle was shown.  
(J) Striped glumes and branch were observed.  
(K) This plant had small grains. 
(L) Grains in the left panicle were clearly larger than those of WT panicle (right).

Further, Fig. 4A showed cleistogamous hulls (white arrows). It was found that panicles possessing cleistogamous hulls did not have extruded anthers compared to another panicles. Long empty glumes were given in Fig. 4B.

**The estimated causal gene for albino mutant**

The variegated albino shown in Fig. 3B was subjected to TAIL-PCR using nDart1-specific primers (Supplemental Table 1) to exploit the nDart1 insertion site. Four sites were detected using ARB3 and 7 primers in chromosomes 3, 6 and 9 (Supplemental Table 1). Then, green sectors and white parts in the albino leaves were collected separately as shown in Fig. 5A and were subjected to PCR to amplify the putative nDart1 insertion site. Green sector and white sector must be heterozygous and homozygous for nDart1 insertion, respectively. Thus, the normal size band and nDart1-
inserted size band could be observed in PCR products for green sector. Meanwhile, only nDart1-inserted size band was produced from PCR for white sector. As shown in Fig. 5B, only the PCR result for clone #1 showed three bands for green sector and a single large band for white sector. The other clones’ PCR products showed only normal band or two bands from white sector, suggesting that false positive amplification occurred due to somatic mutation or that another nDart1-insertion site was detected for clone #3. As a result, clone #1 was presumed to be the candidate insertion site. It was revealed that nDart1 was inserted into intron1 of Os09g0560100 encoding mitochondrial transcription termination factor (Supplemental Table 3). To validate nDart1 insertion, variegated albino and stable albino plants segregated in F2 of the cross between the variegated albino and Koshihikari needed to be subjected to PCR using Koshi nD5-11-1 primers.

**Discussion**

It has been reported that transposon characteristically has insertion region preference in a genome. For example, P element of Drosophila tended to be inserted in 5' untranslated regions and 100–200 bp upstream from the transcription initiation site (Spradling et al. 1995). Takagi et al. (2010) reported that rice DNA transponson, nDart1-3 subgroup elements, also preferred to be inserted into the regions within 500 bp upstream of the translation start codon. This result suggested that many promoters or 5' UTR-disrupted mutants could be discovered in nDart1-tagged lines. In fact, there were several apo1 mutants including apo1-D1 (Ikeda-Kawakatsu et al. 2009) induced by nDart1-0 insertion into around 3 kb upstream regions from first exon of APO1 gene. In apo1-D1, a revertant carrying 8 bp footprint was found (Ikeda-Kawakatsu et al. 2009), suggesting that insertion of more than 9 bp nucleotides might be needed to induce apo1-D1 phenotype. Further, nDart1-0 insertion into 3' UTR caused tawawa1-D mutants (Yoshida et al. 2013). Thus, transposon-insertional mutagenesis could be suitable for function analysis of promoter or UTR regions.

Jiang and Ramachandran (2010) found a higher yield mutant and abiotic stress tolerant mutants in maize Ds-inserted lines of rice, suggesting that transposon insertion might potentially induce not only biotic or abiotic stress tolerance but also improvement of an agronomic trait. Chiou et al. (2019) reported that the large grain mutant found in this study (Fig. 3L) was revealed to be caused by insertion of truncated nDart1-3 followed by genomic deletion at 5' UTR of the putative RNA-binding protein gene on chromosome 11. Further, a cleistogamous-like mutant was found in the lines as shown in Fig. 4A. It was reported that the cleistogamous mutant (Yoshida et al. 2007) showed effective tolerance to high temperature at flowering due to about 1.8°C reduction in closed spikelet inside compared to outside temperature (Koike et al. 2015). Recently, hotter summer which is caused by global warming raises concern about the induction of high temperature sterility at flowering in rice that was reported by Satake and Yoshida (1978). Thus, this mutant could be useful for coping with high temperature at flowering in rice. Further, cleistogamous hull may let pollen-collecting insects such as honey bee not to visit hull with non-extruded anthers.

For detecting a causal gene, the PCR-based method is simple and easy. In particular, DNA transposon-tagged mutant frequently shows a variegated phenotype. A variegated phenotypic mutant carried both transposon-tagged homozygote and heterozygote in mutant phenotype and revertant, respectively. Thus, using an appropriate PCR-based method such as TAIL-PCR (Liu and Whittier 1995) and inverse PCR (Ohchman et al. 1988) for mutant phenotypic tissue and revertant tissue separately, a target candidate should be easily detected. Then, TAIL-PCR as one of the simple detection methods for insertion site was applied for exploiting the causal gene for variegated albino. As a result, the ease of exploiting candidate gene for the mutant through PCR product comparison between mutated tissue and revertant tissue was shown in Fig. 5A and 5B.

It is important to breed nDart1-tagged lines of an elite variety such as Koshihikari. If a nDart1-inserted mutant has an advantageous effect for an agronomic trait or biotic or abiotic stresses, the mutant potentially could be utilized as a breeding material or directly as a variety. In situ growing endogenous transposon-tagged lines could be simple and efficient method to screen tolerant mutants for biotic or abiotic stresses.

**Author Contribution Statement**

NH, EH, KR, KT and MM performed the experiments. HT, EH and KT analyzed the data. KT and MM designed the experiments and wrote the manuscript. NH mainly wrote the manuscript. All authors read and approved the final manuscript.

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**Literature Cited**

Chiu, H.G., M.S. Choe, S.H. Lee, S.H. Park, J.C. Koo, N.Y. Kim, J.J. Lee, B.G. Oh, G.H. Yi, S.C. Kim et al. (1999) Molecular analysis of rice plants harboring an Ac/Ds transposable element-mediated gene trapping system. Plant J. 19: 615–623.

Chiou, W.Y., T. Kawamoto, E. Himi, K. Rikiishi, M. Sugimoto, M.
Hayashi-Tsugane, K. Tsugane and M. Maekawa (2009) LARGE GRAIN encodes a putative RNA-binding protein that regulates spikelet hull length in rice. Plant Cell Physiol. 60: 503–515.

Gichuki, E., E. Himi, H. Takahashi, S. Zhu, K. Doi, K. Tsugane and M. Maekawa (2016) Identification of QTLs for yield-related traits in RILs derived from the cross between pLIA-1 carrying Oryza longistaminata chromosome segments and Norin 18 in rice. Breed. Sci. 66: 720–733.

Greco, R., P.B. Ouwwerkerk, R.J. De Kam, C. Sallaud, C. Favalli, L. Colombo, E. Guiderdoni, A.H. Meijer, J.H. Hoge Dagder and A. Pereira (2003) Transpositional behaviour of an Ac/Ds system for reverse genetics in rice. Theor. Appl. Genet. 108: 10–25.

Greco, R., P.B. Ouwwerkerk, A.J. Taal, C. Sallaud, E. Guiderdoni, A.H. Meijer, J.H. Hoge and A. Pereira (2004) Transcription and somatic transposition of the maize En/Spm transposon system in rice. Mol. Genet. Genomics 270: 514–523.

Hayashi-Tsugane, M., M. Maekawa, H. Kobayashi, S. Iida and K. Tsugane (2011) Examination of transpositional activity of nDart1 at different stages of rice development. Genes Genet. Syst. 86: 215–219.

Hayashi-Tsugane, M., H. Takahara, N. Ahmed, E. Himi, K. Takagi, S. Iida, K. Tsugane and M. Maekawa (2014) A mutable albino allele in rice reveals that formation of thylakoid membranes requires the SNOW-WHITE LEAF1 gene. Plant Cell Physiol. 55: 5–15.

Hayashi-Tsugane, M., M. Maekawa and K. Tsugane (2015) A gain-of-function Bushy dwarf tiller 1 mutation in rice microRNA gene nir156d caused by insertion of the DNA transposon nDart1. Sci. Rep. 5: 14357.

Hirochika, H. (2010) Insertional mutagenesis with Tos17 for functional analysis of rice genes. Breed. Sci. 60: 486–492.

Howard, T.P., 3rd, A.P. Hayward, A. Tordillos, C. Fragoso, M.A. Moreno, J. Tohme, A.P. Kausch, J.P. Mottinger and S.L. Dellaporta (2014) Identification of the maize gravitropism gene lazy plant1 by a transposon-tagging genome resequencing strategy. PLoS ONE 9: e87053.

Huang, J., K. Zhang, Y. Shen, Z. Huang, M. Li, D. Tang, M. Gu and Z. Cheng (2009) Identification of a high frequency transposon induced by tissue culture, nDaiZ, a member of the hAT family in rice. Genomics 93: 274–281.

Ikeda-Kawakatsu, K., N. Yasuno, T. Oikawa, S. Iida, Y. Nagato, M. Maekawa and J. Kyozuka (2009) Expression level of ABERRANT PANICLE ORGANIZATION1 determines rice inflorescence form through control of cell proliferation in the meristem. Plant Physiol. 150: 736–747.

Jiang, N., Z. Bao, X. Zhang, H. Hirochika, S.R. Eddy, S.R. McCouch and S.R. Wessler (2003) An active DNA transposon family in rice. Nat Genet. 31: 319–329.

Jiang, S.-Y. and S. Ramachandran (2010) Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. Int. J. Biol. Sci. 6: 228–251.

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

Koike, S., T. Yamaguchi, S. Ohmori, T. Hayashi, O. Yatou and H. Yoshida (2015) Cleistogamy decreases the effect of high temperature stress at flowering in rice. Plant Prod. Sci. 18: 111–117.

Lee, S., J. Kim, J.S. Son, J. Nam, D.H. Jeong, K. Lee, S. Jang, J. Yoo, J. Lee, D.Y. Lee et al. (2003) Systematic reverse genetic screening of T-DNA tagged genes in rice for functional genomic analyses: MADS-box genes as a test case. Plant Cell Physiol. 44: 1403–1411.

Li, A.H., Y.F. Zhang, C.Y. Wu, W. Tang, R. Wu, Z.Y. Dai, G.Q. Liu, H.X. Zhang and X.B. Pan (2006) Screening for and genetic analysis on T-DNA-inserted mutant pool in rice. Acta genetica Sinica 33: 319–329.

Liu, Y.-G. and R.F. Whittier (1995) Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25: 674–681.

Moon, S., K.H. Jung, D.E. Lee, W.Z. Jiang, H.J. Koh, M.H. Heu, D.S. Lee, H.S. Suh and G. An (2006) Identification of active transposon dIbk, a member of the hAT family, in rice. Plant Cell Physiol. 47: 1473–1483.

Nakazaki, T., Y. Okumoto, A. Horibata, S. Yamahira, M. Teraishi, N. Hishida, H. Inoue and T. Tanisaka (2003) Mobilization of a transposon in the rice genome. Nature 421: 170–172.

Nishimura, H., N. Ahmed, K. Tsugane, S. Iida and M. Maekawa (2008) Distribution and mapping of an active autonomous aDart element responsible for mobilizing nonautonomous nDart1 transposons in cultivated rice varieties. Theor. Appl. Genet. 116: 395–406.

Ochman, H., A.S. Gerber and D.L. Hartl (1988) Genetic applications of an inverse polymerase chain reaction. Genetics 120: 621–623.

Rand, K.N., T. Ho, W. Qu, S.M. Mitchell, R. White, S.J. Clark and P.L. Molloy (2005) Headloop suppression PCR and its application to selective amplification of methylated DNA sequences. Nucleic Acids Res. 33: e127.

Satake, T. and S. Yoshida (1978) High temperature-induced sterility in indica rice at flowering. Jpn. J. Crop. Sci. 47: 6–17.

Spradling, A.C., D.M. Stern, I. Kiss, J. Roote, T. Laverty and G.M. Rubin (1995) Gene disruptions using P transposable elements: an integral component of the Drosophila genome project. Proc. Natl. Acad. Sci. USA 92: 10824–10830.

Takagi, K., N. Ishikawa, M. Maekawa, K. Tsugane and S. Iida (2007) Transposon display for active DNA transposons in rice. Genes Genet. Syst. 82: 109–122.

Takagi, K., M. Maekawa, K. Tsugane and S. Iida (2010) Transposition and target preferences of an active nonautonomous DNA transposon nDart1 and its relatives belonging to the hAT superfamily in rice. Mol. Genom. Genomes 284: 343–355.

Tanaka, K., K. Murata, M. Yamazaki, K. Onosato, A. Miyao and H. Hirochika (2003) Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. Plant Physiol. 133: 73–83.

Tsugane, K., M. Maekawa, K. Takagi, H. Takahara, Q. Qian, C.H. Eun and S. Iida (2006) An active DNA transposon nDart causing leaf variegation and mutable dwarfism and its related elements in rice. Plant J. 45: 46–57.

Wang, N., T. Long, W. Yao, L. Xiong, Q. Zhang and C. Wu (2013) Mutant resources for the functional analysis of the rice genome. Mol. Plant 6: 596–604.

Yoshida, H., J. Itoh, S. Ohmori, K. Miyoshi, E. Uchida, M. Kimizu, Y. Matsumura, M. Kusaba, H. Satoh et al. (2007) superwoman1-cleistogamy, a hopeful allele for gene containment in GM rice. Plant Biotechnol. J. 5: 835–846.

Yoshida, A., M. Sasao, N. Yasuno, K. Takagi, Y. Daimon, R. Chen, H. Tokunaga, Y. Sato and H. Yoshida (1978) High temperature-induced sterility in indica rice at flowering. Jpn. J. Crop. Sci. 47: 6–17.