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Note

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)

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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

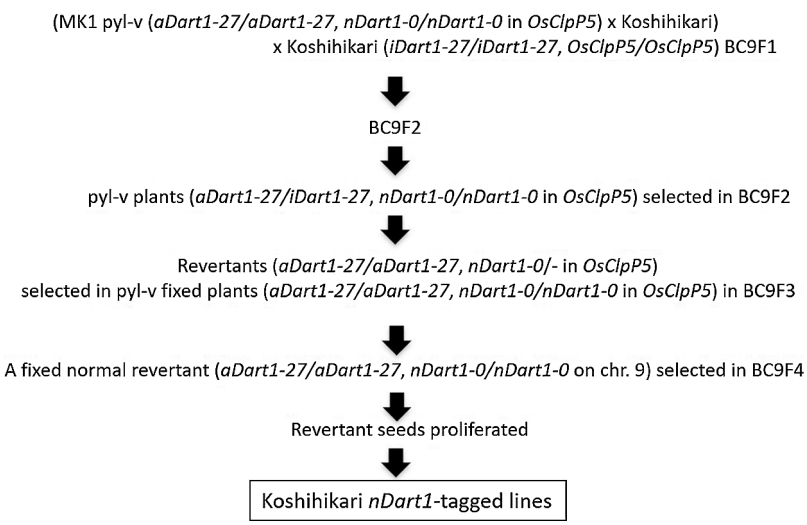
The breeding process of *nDart1*-tagged lines of Koshihikari was shown in **Fig. 1**. The donor of *nDart1-0* and *aDart1-27* was MK1 pyl-v (variegated pale-yellow leaf) and heterozygous BCnF1 plants for *nDart1-0* and *aDart1-27* successively backcrossed with Koshihikari as a recurrent parent were selected 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



Note; *aDart1-27* and *iDart1-27* indicate active *Dart1-27* and inactive *Dart1-27*, respectively, of autonomous *DNA active rice transposon1* (*Dart1*) group (Tsugane et al. 2006). pyl-v and pyl-stb show variegated and stable pale-yellow leaf phenotypes, respectively (Tsugane et al. 2006).

Fig. 1. Development of *nDart1*-tagged lines of Koshihikari.

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*.

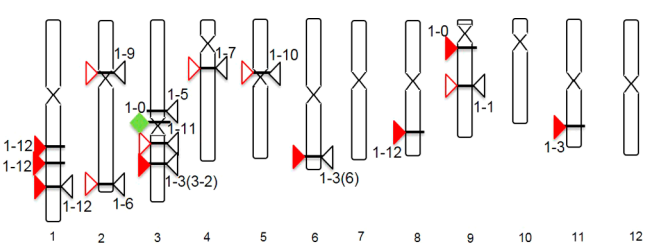


Fig. 2. Distribution of *nDart1*s in the founder line carrying *nDart1-0* and *aDart1-27* in Koshihikari. Red and white arrow heads at left side show transposed and stayed *nDart1s* in the *nDart1*-tagged lines, respectively and white arrow head at right side depicts authentic insertion site of *nDart1s*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

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 *Cvia* 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.

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

Seedling stage		Mature stage	
Phenotype	No. of lines	Phenotype	No. of lines
Abnormal	333	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
			43.2% /1696

(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*-

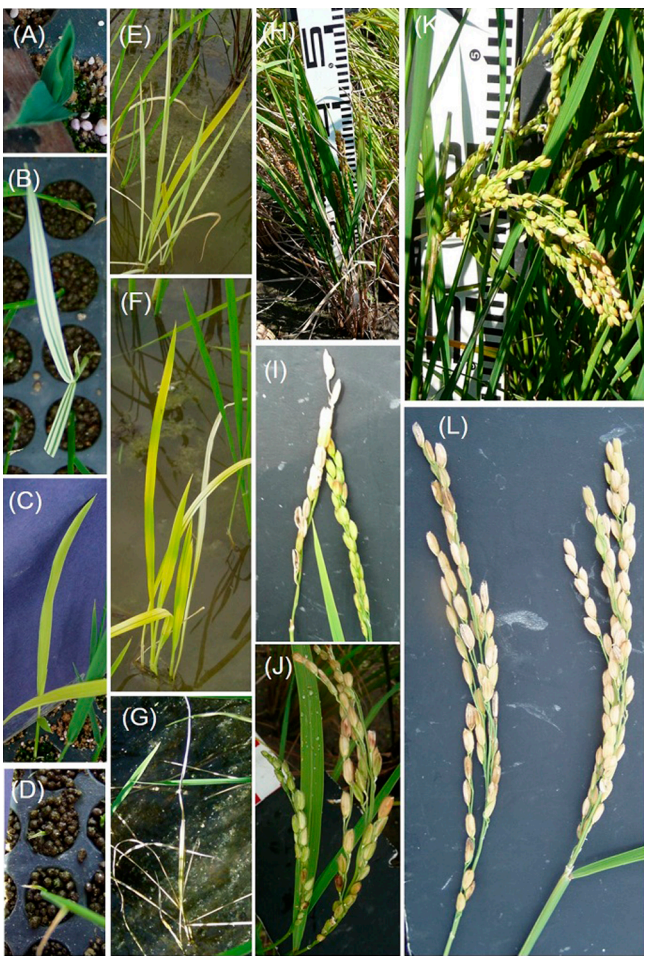


Fig. 3. Mutant phenotype observed in *nDar1*-tagged lines of Koshihikari. (A) dwarf at seedling stage. (B) variegated albino. (C) light green leaf. (D) abnormal phenotype at seedling stage. (E) stripe. (F) zebra leaves. (G) lethal. (H) round grain dwarf. (I) abnormal panicle (left). (J) stripe hull. (K) small grain. (L) large grain (left) and wild type (right).

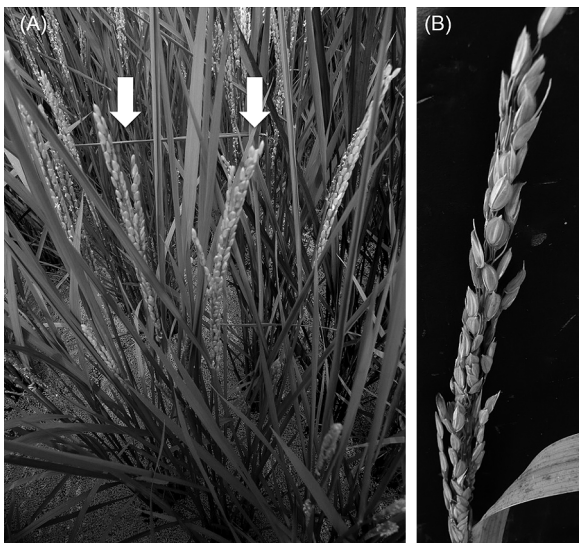


Fig. 4. Malformed glume mutant. (A) cleistogamous glumes shown by white arrow. (B) long empty glume.

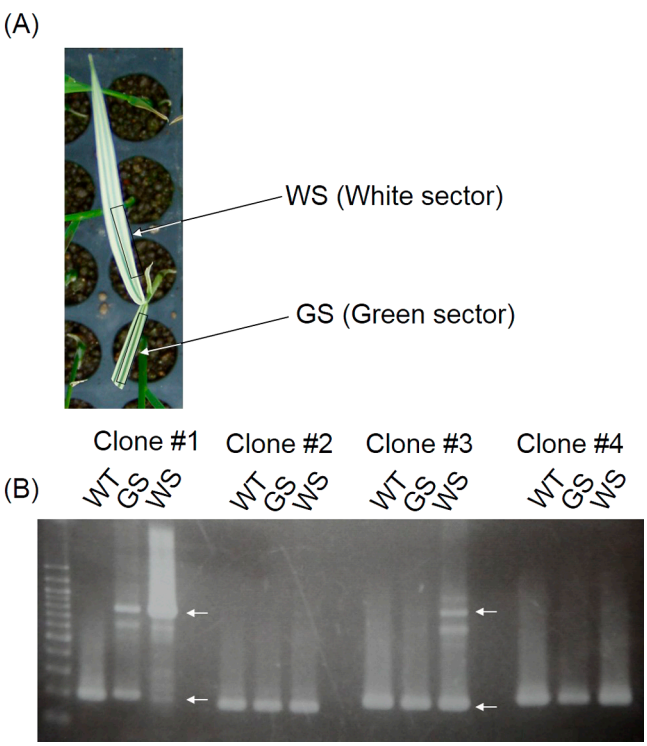


Fig. 5. Confirmation of *nDart1-0* insertion revealed by TAIL-PCR. (A) GS (Green sector) and WS (White sector) in albino of **Fig. 2B**. (B) Banding pattern of WT, GS and WS using specific primers for each clone. White arrow and arrowhead indicate amplicons with *nDart1* insertion and without *nDart1*, respectively.

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 transposon, *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 (Ochman *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

Chin, 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 (2019) *LARGE GRAIN* encodes a putative RNA-binding protein that regulates spikelet hull length in rice. Plant Cell Physiol. 60: 503–515.

Gichuhi, 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. Ouwerkerk, R.J. De Kam, C. Sallaud, C. Favalli, L. Colombo, E. Guiderdoni, A.H. Meijer, J.H. Hoge Dagger and A. Pereira (2003) Transpositional behaviour of an *Ac/Ds* system for reverse genetics in rice. Theor. Appl. Genet. 108: 10–24.

Greco, R., P.B. Ouwerkerk, 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: 3–15.

Hayashi-Tsugane, M., M. Maekawa and K. Tsugane (2015) A gain-of-function *Bushy dwarf tiller 1* mutation in rice microRNA gene *miR156d* 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. Nature 421: 163–167.

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 *dTok*, a member of the *hAT* family, in rice. Plant Cell Physiol. 47: 1473–1483.

Nakazaki, T., Y. Okumoto, A. Horibata, S. Yamahira, M. Teraishi, H. Nishida, 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. Genet. Genomics 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, A. Horigome, 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, R. Yamazaki, H. Tokunaga, Y. Kitaguchi, Y. Sato *et al.* (2013) *TAWAWA1*, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition. Proc. Natl. Acad. Sci. USA 110: 767–772.

Zhang, Y., K. Massel, I. Godwin and C. Gao (2018) Applications and potential of genome editing in crop improvement. Genome Biol. 19: 210.