HOKKAIDO UNIVERSITY

Title	Two Stabiliser loci suppress Tam3 transposition without compromising transposase production in Antirrhinum
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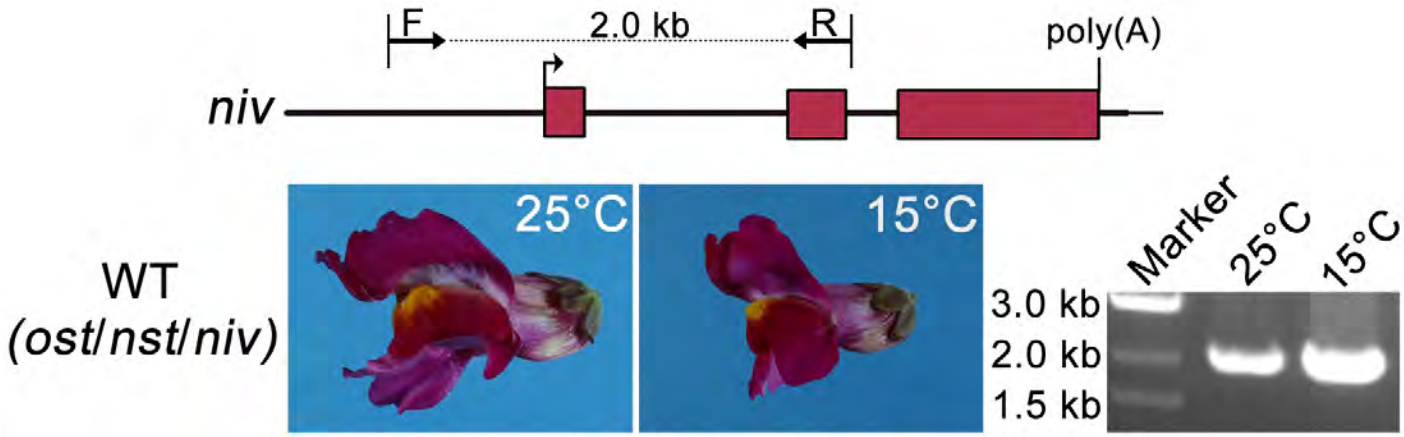
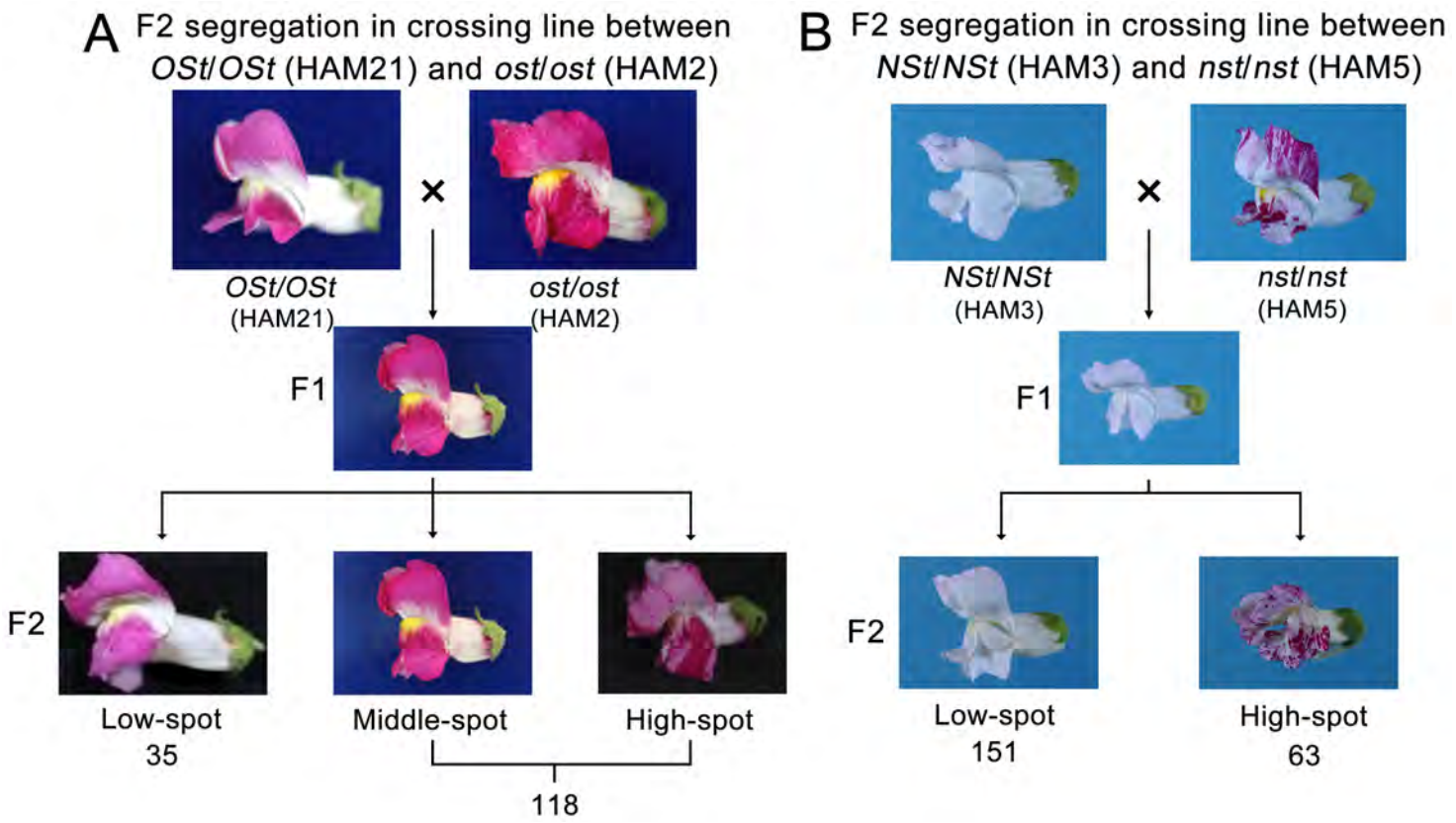


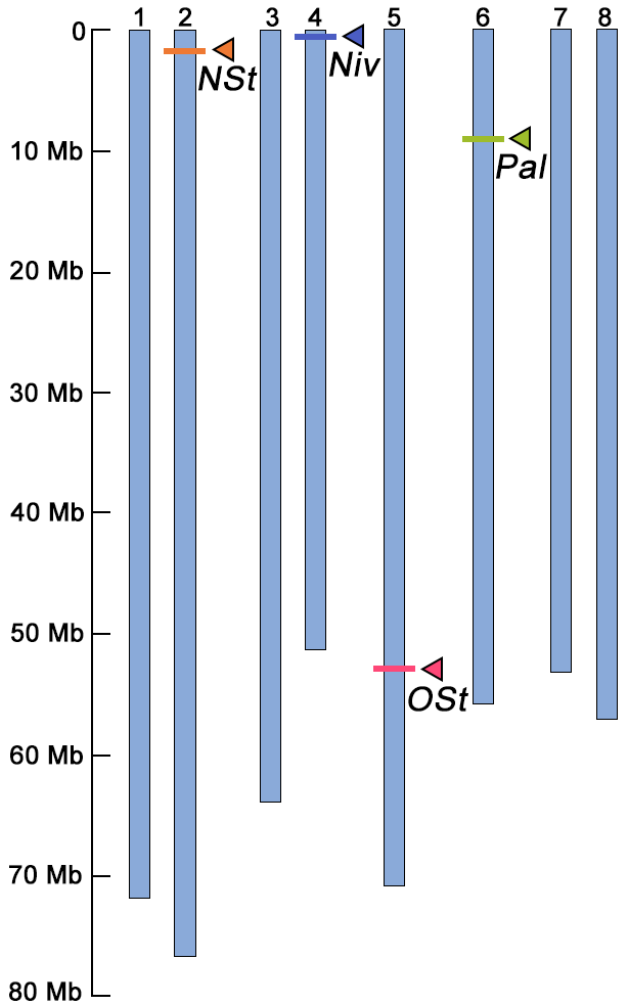
Supplementary Fig. 1
Profile of wild type (WT) *A. majus* line.
WT contains the *Niv* allele at *Niv* locus, where no Tam3 is inserted, and thus the flower petals exhibit red color at 25℃ and 15℃. Additionally, the observation of 2.0 kb bands at 25℃ and 15℃ indicates no Tam3 insertion in the *Niv* allele.



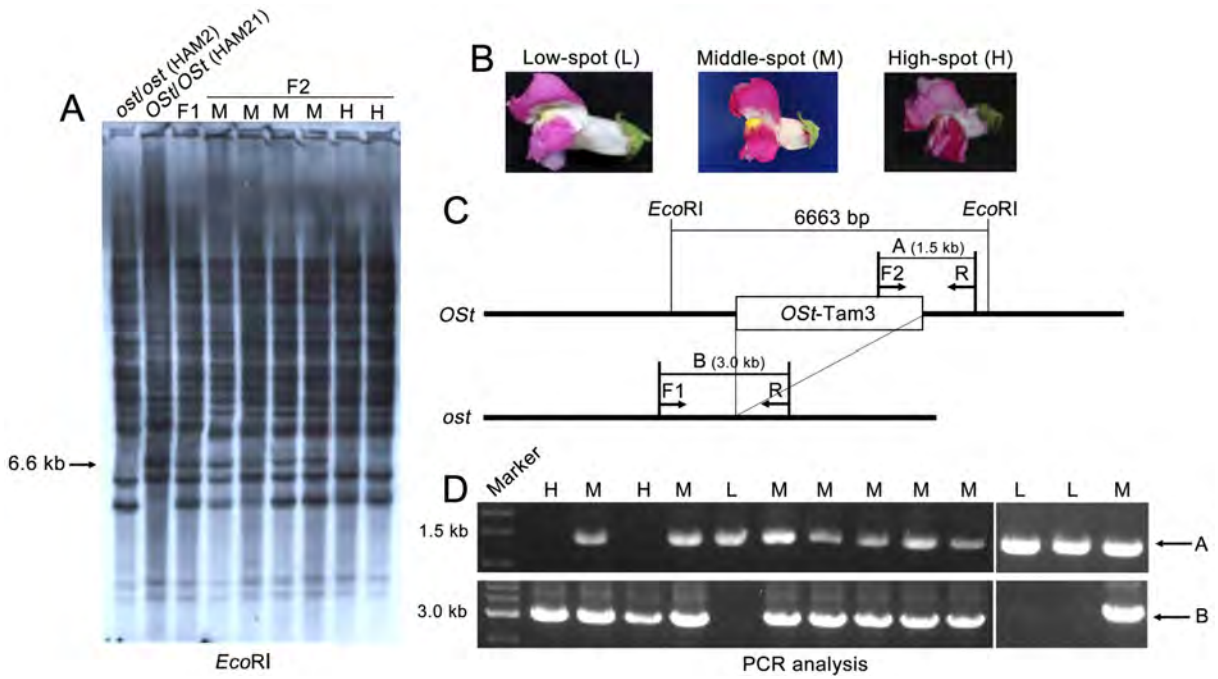
Supplementary Fig. 2

F2 segregation profiles in the *OSt*/*niv*^{rec} × *ost*/*niv*^{rec} (HAM21×HAM2) and *NSt*/*niv*$^{rec:\ beni}$ × *nst*/*niv*$^{rec:\ beni}$ (HAM3×HAM5) *Antirrhinum* crosses.

A. F2 segregation in the *OSt*/*niv*^{rec} × *ost*/*niv*^{rec} (HAM21×HAM2) cross. B. F2 segregation in the *NSt*/*niv*$^{rec:\ beni}$ × *nst*/*niv*$^{rec:\ beni}$ (HAM3×HAM5) cross. The number of plants showing different spotted phenotypes in F2 is shown in Supplementary Table 1.



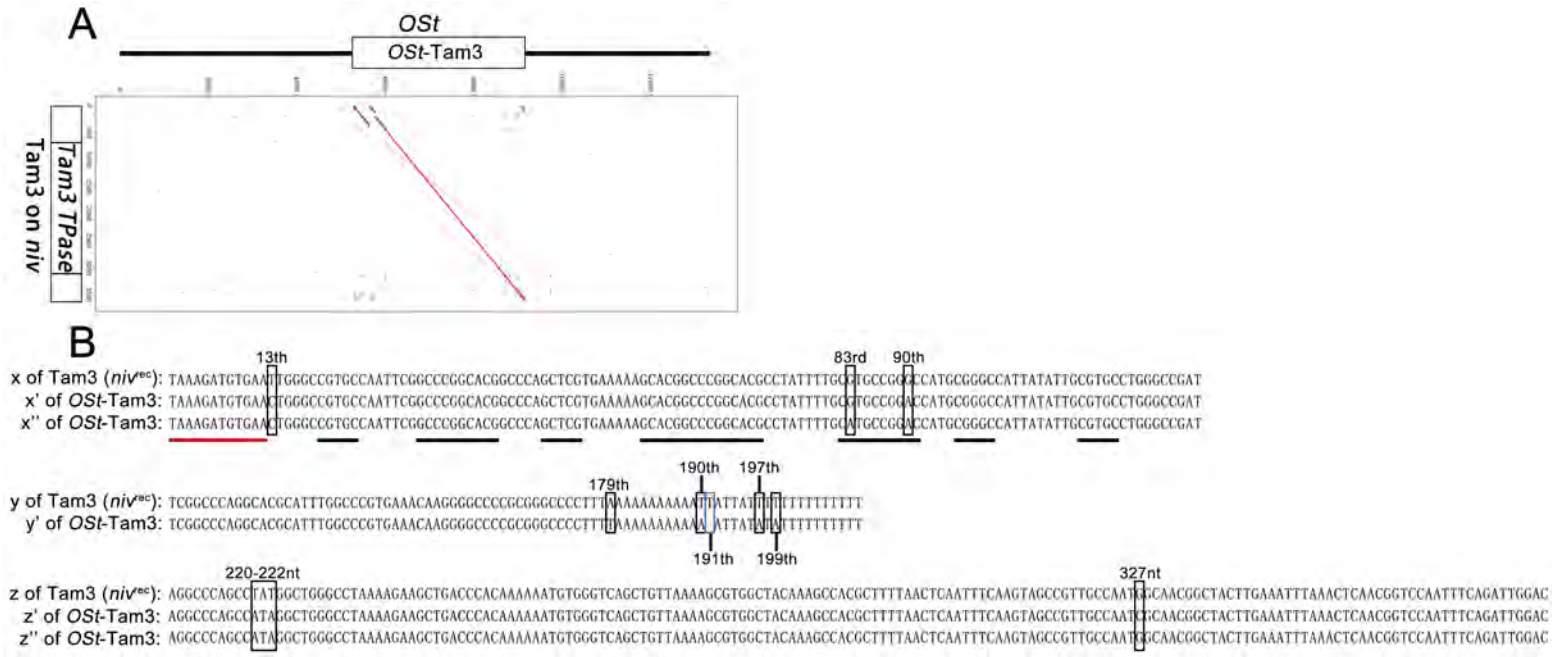
Supplementary Fig. 3
Chromosomal positions of *OSt*, *NSt*, *Niv* and *Pal* within the *Antirrhinum majus* genome.



Supplementary Fig. 4

Identification of *OSt*.

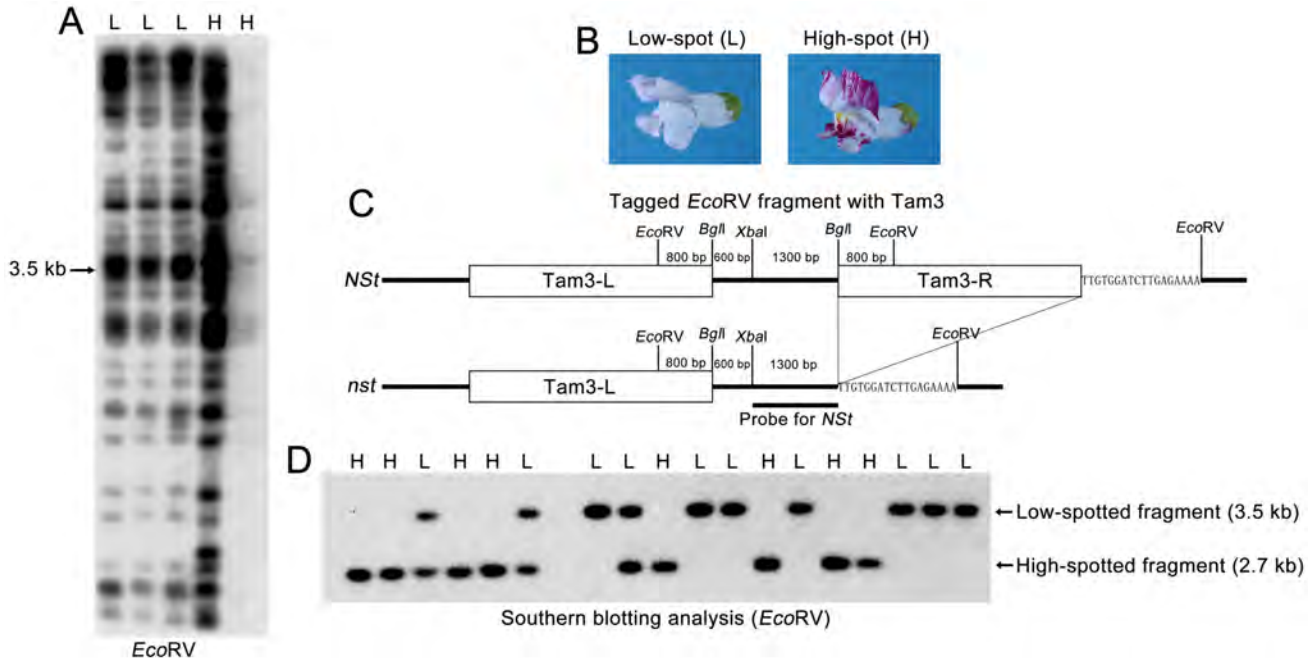
A. Transposon tagging analysis in *ost*/*niv*^{rec} (HAM2), *OSt*/*niv*^{rec} (HAM21) and their progeny plants. Each DNA was extracted and digested with *Eco*RI. The ORF region of Tam3 was used as a probe, and black arrow indicates the 6.6 kb "tag" bands in *OSt*/*niv*^{rec} (HAM21), F1 and middle-spotted F2 plants. B. Different flower variegation phenotypes of plants used for the identification of *OSt*. C. Structures of *OSt* and *ost* alleles. The two thin vertical bars in *OSt* represent *Eco*RI sites. Arrows represent the positions of primers present in *OSt* and *ost* alleles. D. PCR analysis of the allelic constitution at *OSt* locus in plants with different flower petal variegation phenotypes. The A (1.5 kb) fragment amplified using the F2 and R primers indicates the *OSt* allele, while the B (3.0 kb) fragment amplified using the F1 and R primers indicates the *ost* allele.



Supplementary Fig. 5

Dot plot analysis and sequence alignment of the *OSt* sequence.

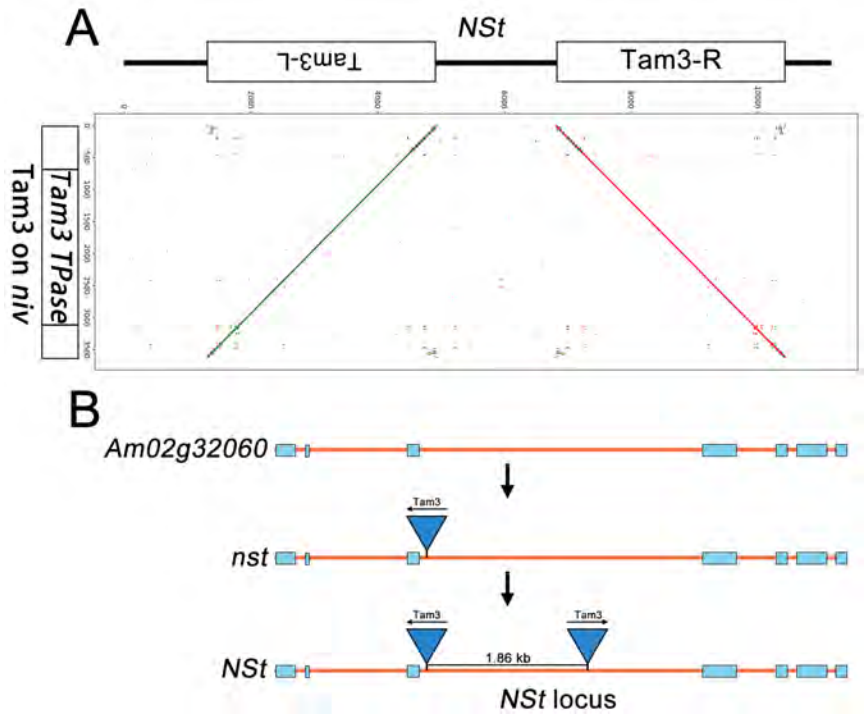
A. *OSt* sequence was aligned to the intact Tam3 sequence from *niv*^{rec}. In the dot plot, the upper horizontal axis represents *OSt* sequence, and left vertical axis represents the Tam3 sequence. Red and green dots represent the alignment of the sequence of positive and negative strands of *OSt* to the sequence of the positive strand of Tam3, respectively. The window size and mismatch limit settings in dot plot analysis are 10 and 0, respectively. B. Sequence alignment between x-y-z region of intact Tam3 and x'-y'-z'-x''-z'' region of *OSt*-Tam3. Black and blue squares indicate SNP and deletion positions, respectively. Red and black underlines indicate Terminal Inverted Repeat (TIR) and GCHCG motifs, respectively.



Supplementary Fig. 6

Identification of *NSt*.

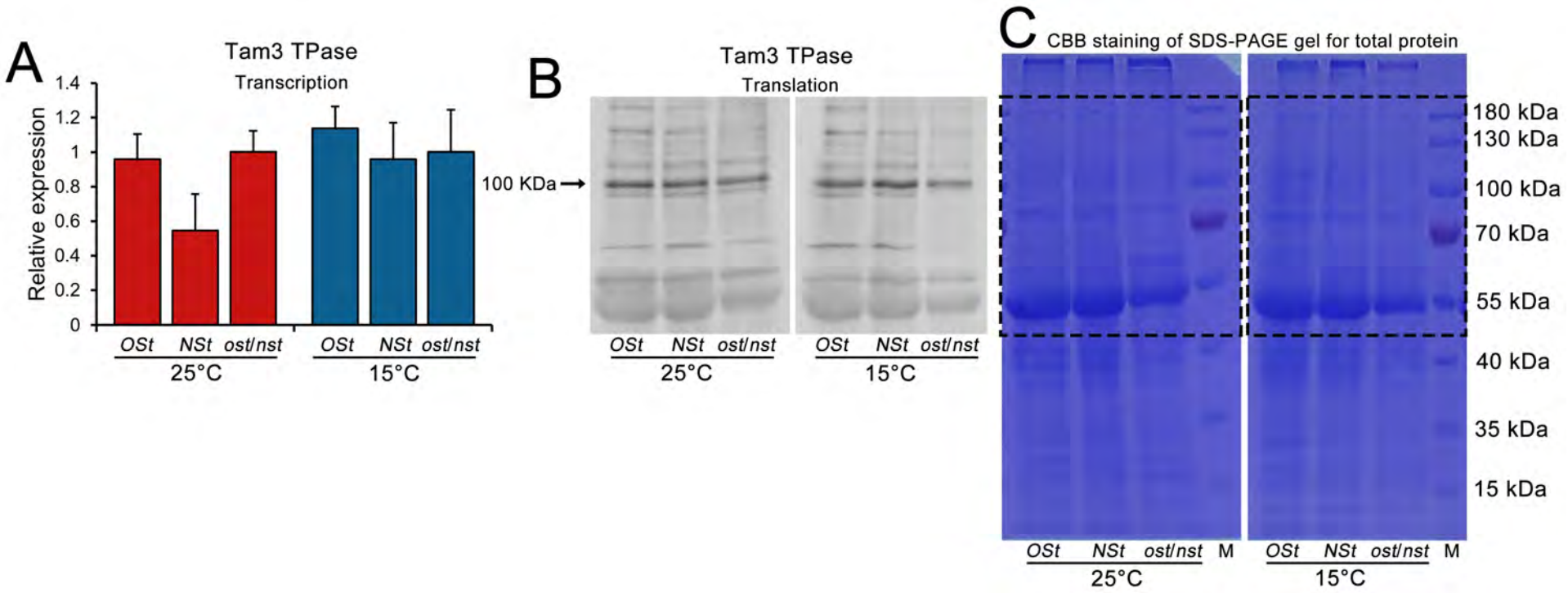
A. Transposon tagging analysis in low- and high-spotted plants. Each DNA was extracted and digested with *Eco*RV. Full-length Tam3 sequence was used as a probe, and black arrow indicated 3.5 kb tagging bands in low-spotted plants. B. Different flower variegation phenotypes of plants used for the identification of *NSt*. C. Structures of *NSt* and *nst* alleles. Vertical bars indicate the nucleotide positions of restriction enzymes including *Eco*RV, *Bgl*I and *Xba*I in *NSt* and *nst* alleles. The bold black line below *nst* in the diagram represents the probe used for Southern blotting analysis. D. Southern blotting analysis of allelic constitution at *NSt* locus in plants with different flower petal variegation phenotypes. Each DNA was extracted and digested with *Eco*RV. The probe used is shown in the C diagram. The 3.5 kb fragment was detected in low-spotted plants and indicates the *NSt* allele, while the 2.7 kb fragment was detected in high-spotted plants and indicates the *nst* allele.



Supplementary Fig. 7

Dot plot analysis of the *NSt* sequence and the process of *NSt* formation.

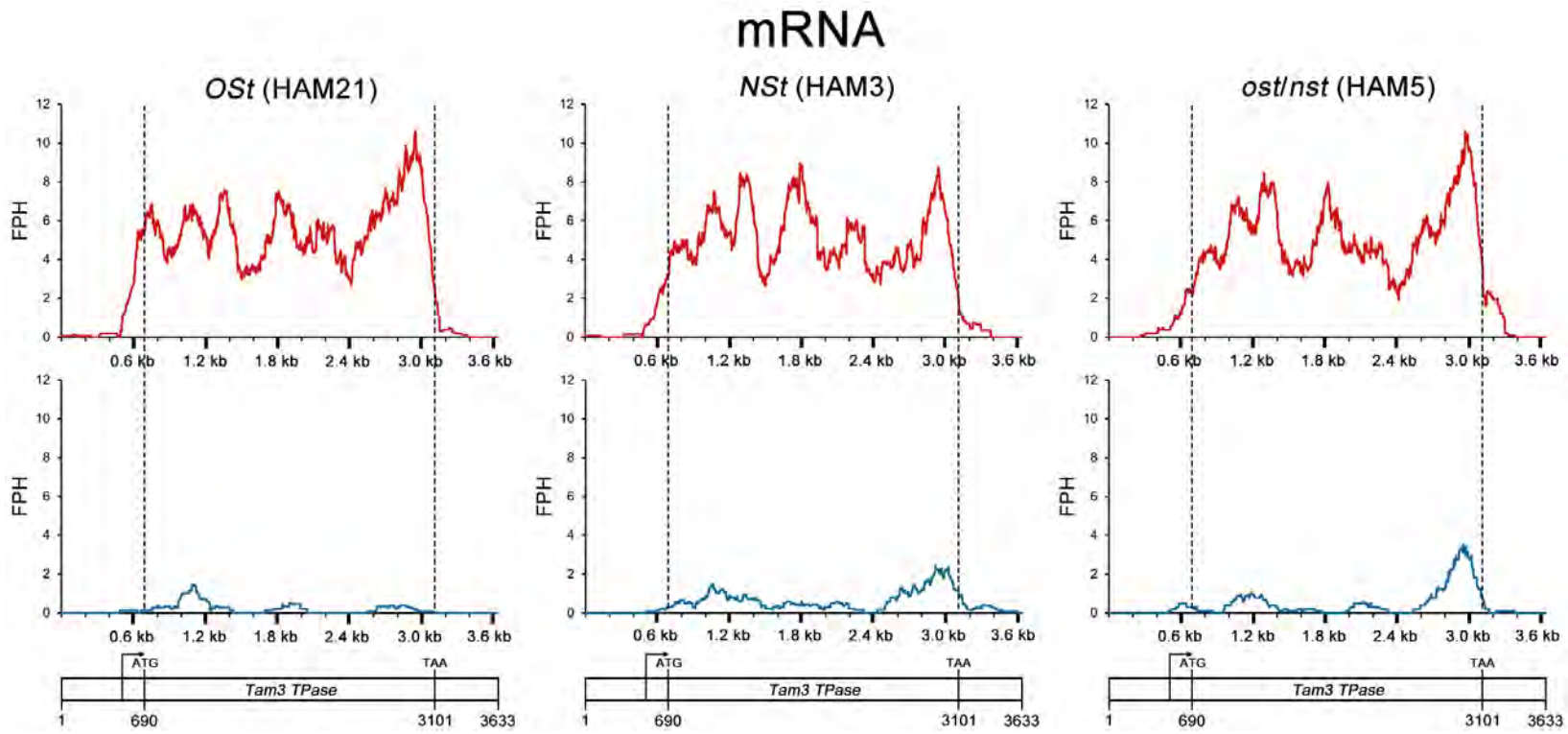
A. *NSt* sequence was aligned to the intact Tam3 sequence from *niv*^{rec}. In the dot plot, the upper horizontal axis represents *NSt* sequence, and the left vertical axis represents the Tam3 sequence. Red and green dots represent the alignment of the sequence of positive and negative strands of *OSt* to the sequence of positive strand of Tam3, respectively. The window size and mismatch limit settings for the dot plot analysis were 10 and 0, respectively. B. Proposed process of *NSt* formation. One Tam3 element was inserted into the third intron of *Am02g32060* gene, which results in *nst* formation. *NSt* formation was due to the insertion of second Tam3 element on the right side of first Tam3 element in the same intron.



Supplementary Fig. 8

The transcription and translation of *Tam3 TPase*.

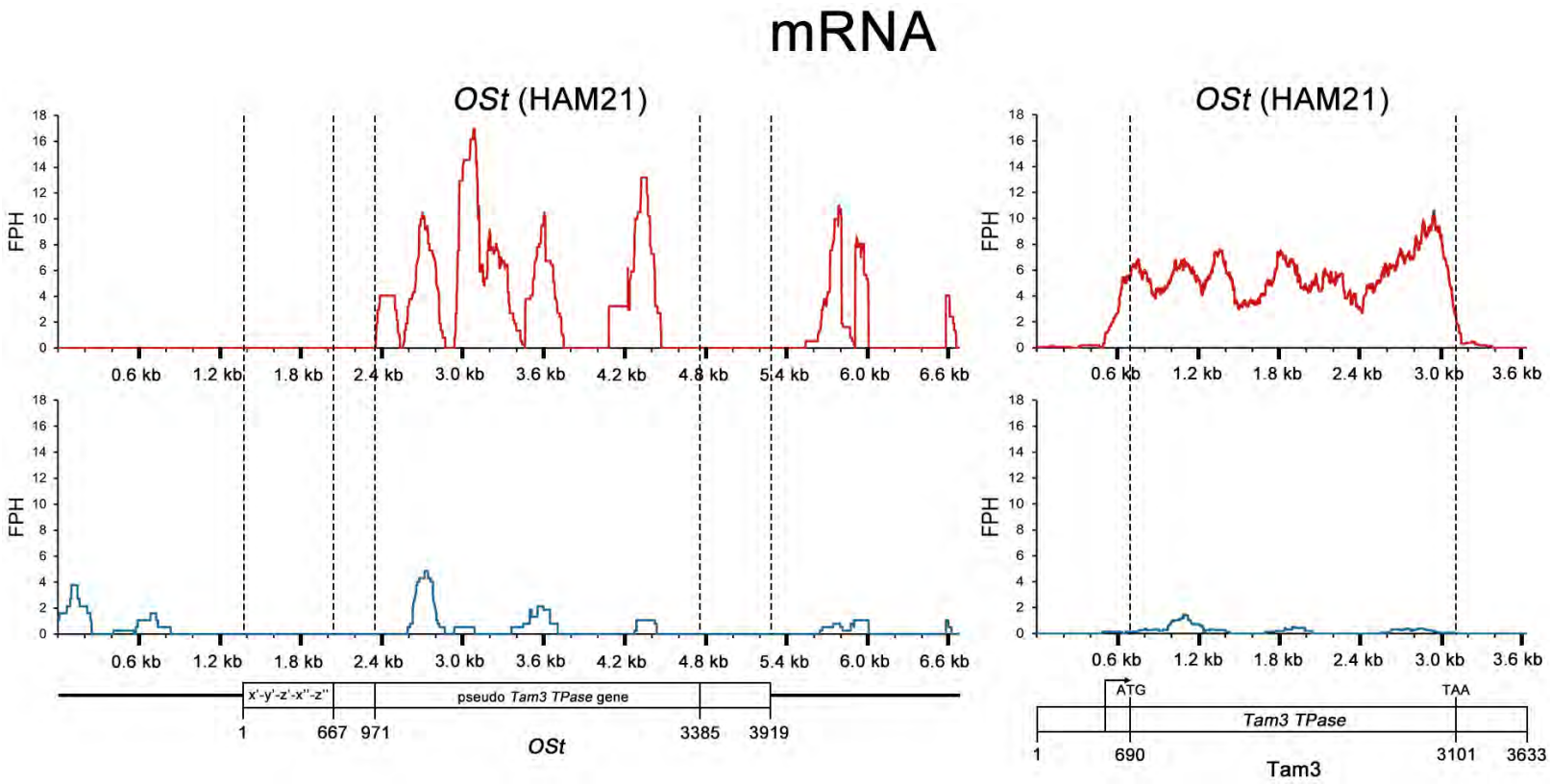
A. qRT-PCR analysis of *Tam3 TPase* expression. Expression levels were normalized to that of *UBIQ* (X67957). Data represent the means ± SD (n = 3). Significant differences were determined by Dunnett's test. The experiments were performed three times. B. Immunoblot analysis of Tam3 TPase in the same plants employed with the qRT-PCR analysis. Total protein was extracted from young leaves of *Antirrhinum* plants. The fragments indicated by arrow correspond to 100 kDa of Tam3 TPase. C. CBB staining of SDS-gel for total protein in the immunoblot analysis. Black dot squares mark the range of the B immunoblot membrane figure in the SDS-gel.



Supplementary Fig. 9

Distributions and amounts of mRNAs in Tam3 region.

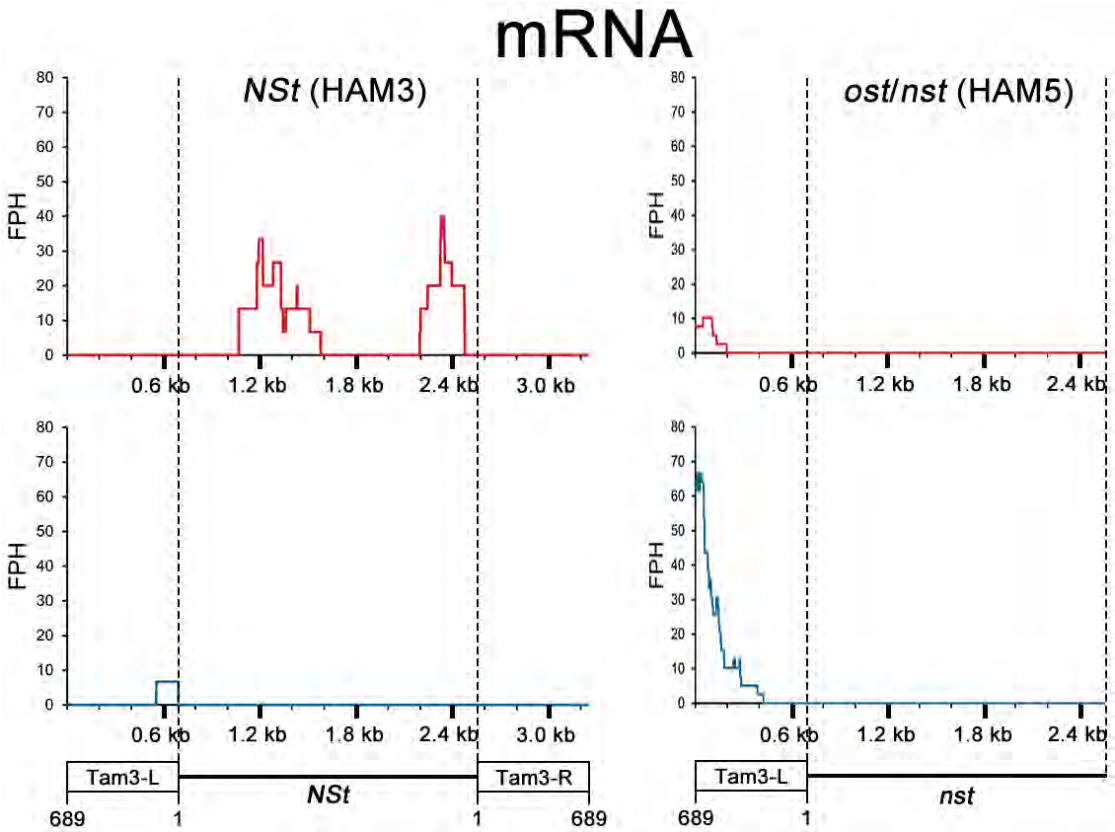
The mRNAs that were perfectly matched to the Tam3 region (1-3633 bp) were analyzed. Red and blue colors were used to distinguish sense and antisense mRNAs, respectively. The vertical fold arrow in the Tam3 structure diagram represents the transcriptional start site of *Tam3 TPase* gene. ATG and TAA are start and stop codons of Tam3 TPase, respectively. FPH, fragments per hundred mapped fragments.



Supplementary Fig. 10

Distributions and amounts of mRNAs in Tam3 and *OSt*-Tam3 regions in *OSt*/*niv*^{rec} (HAM21).

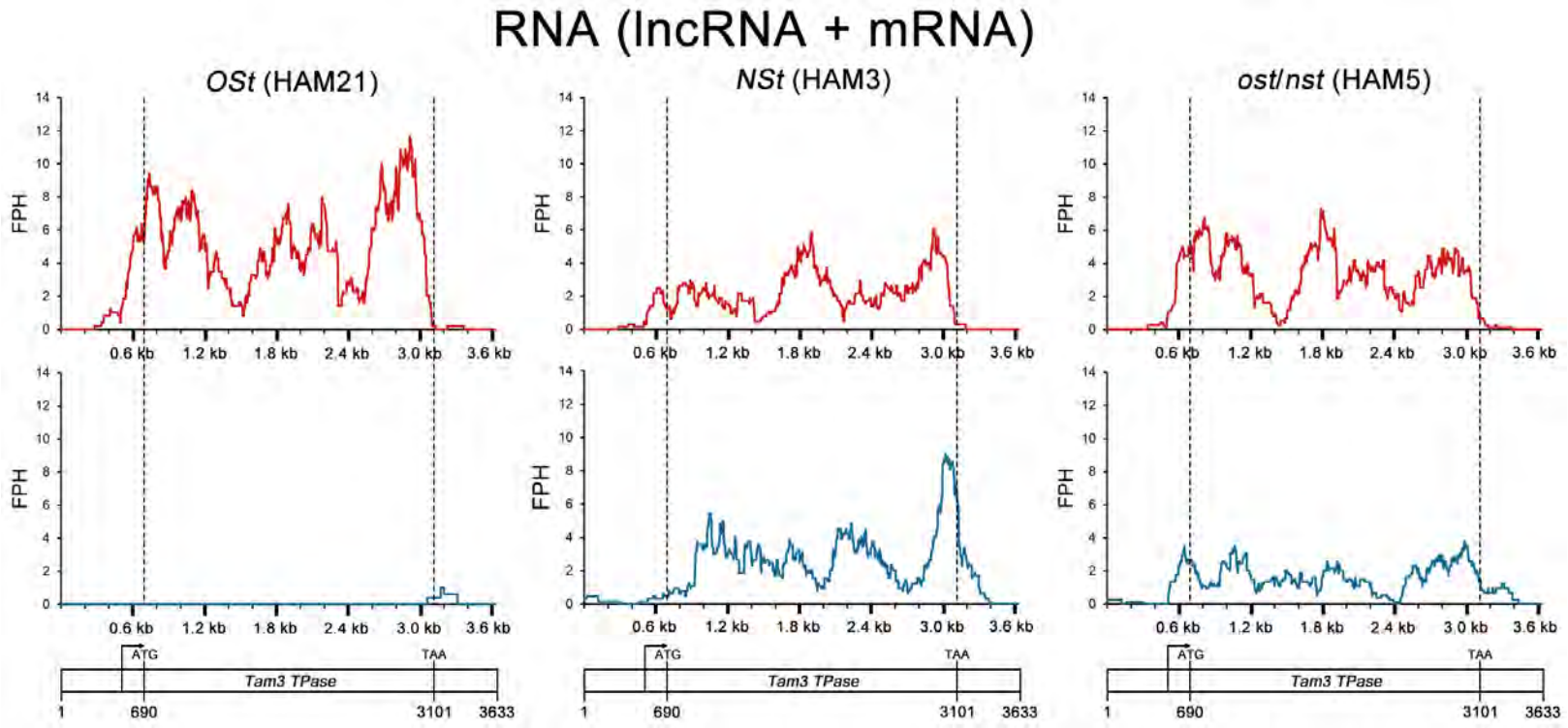
The mRNAs that perfectly matched were aligned to the Tam3 region (1-3633 bp) and the *OSt*-Tam3 region (1-3919 bp) in *OSt*/*niv*^{rec} (HAM21). Red and blue colors were used to distinguish sense and antisense mRNAs, respectively. The vertical fold arrow in the Tam3 structure diagram represents the transcriptional start site of the *Tam3 TPase* gene. ATG and TAA are start and stop codons of Tam3 TPase, respectively. FPH, fragments per hundred mapped fragments.



Supplementary Fig. 11

Distributions and amounts of mRNAs in *NSt* and *nst* alleles.

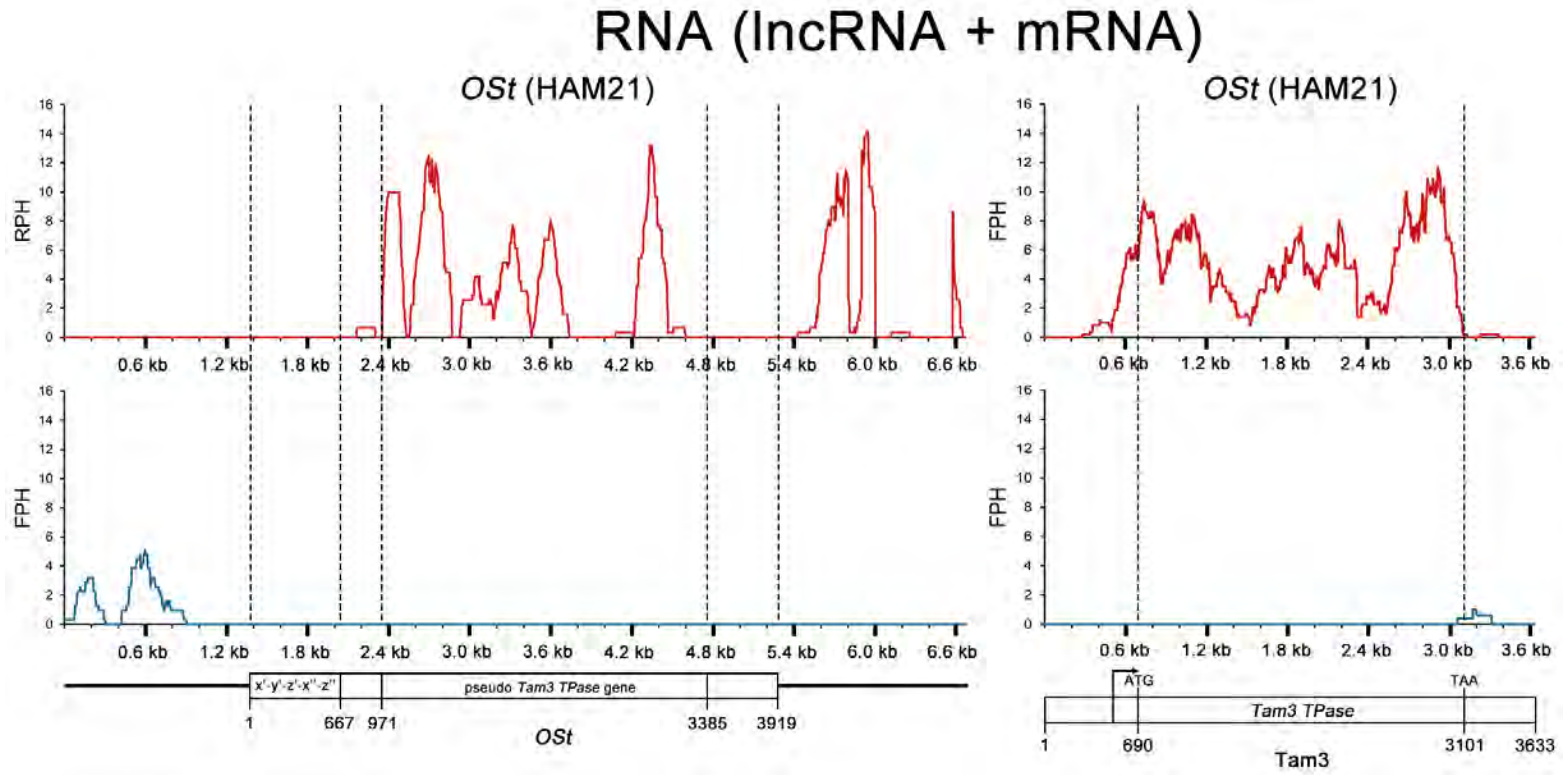
The mRNAs that perfectly matched were aligned to the 5' region of Tam3-L of *nst* (1-689 bp) and the flanking region of Tam3-L of *nst* (1867 bp) in *nst*/*niv*$^{\text{rec: beni}}$ (HAM5), and aligned to 5' regions of Tam3-L (1-689 bp) and Tam3-R (1-689 bp) of *NSt* and internal region (1867 bp) between Tam3-L and Tam3-R of *NSt* in *NSt*/*niv*$^{\text{rec: beni}}$ (HAM3). Red and blue colors were used to distinguish sense and antisense mRNAs, respectively. FPH, fragments per hundred mapped fragments.



Supplementary Fig. 12

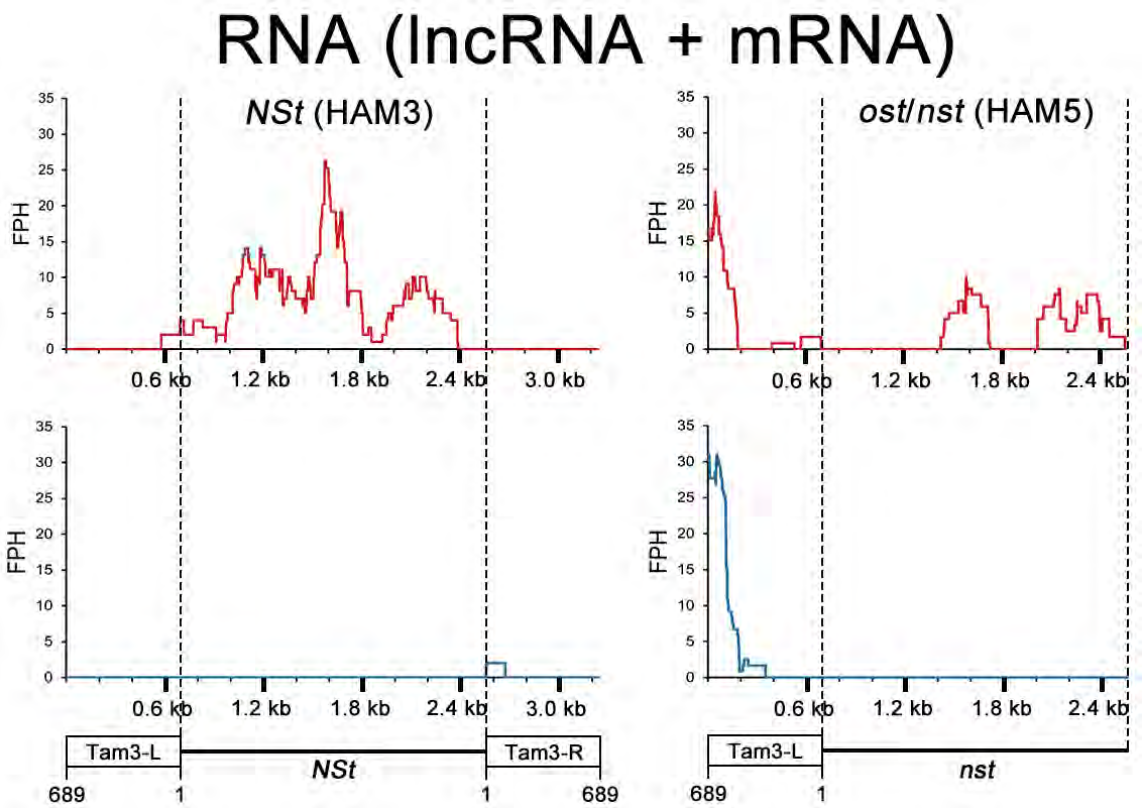
Distributions and amounts of RNAs (lncRNAs + mRNAs) in the Tam3 region.

The RNAs including lncRNAs and mRNAs that were perfectly matched to the Tam3 region (1-3633 bp) were analyzed. Red and blue colors were used to distinguish sense and antisense RNAs, respectively. The vertical fold arrow in the Tam3 structure diagram represents the transcriptional start site of the *Tam3 TPase* gene. ATG and TAA are start and stop codons of the Tam3 TPase, respectively. FPH, fragments per hundred mapped fragments.

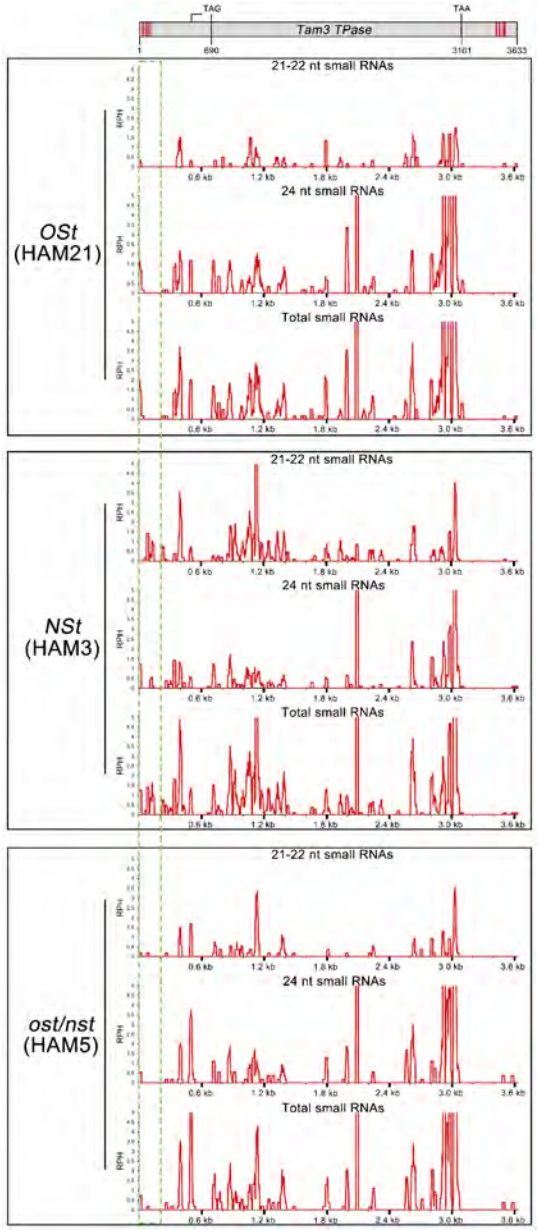


Supplementary Fig. 13

Distributions and amounts of RNAs (lncRNAs + mRNAs) in Tam3 and *OSt*-Tam3 regions in *OSt*/*niv*^{rec} (HAM21).
The RNAs including lncRNAs and mRNAs that perfectly matched were aligned to the Tam3 region (1-3633 bp) and the *OSt*-Tam3 region (1-3919 bp) in *OSt*/*niv*^{rec} (HAM21). Red and blue colors were used to distinguish sense and antisense RNAs, respectively. The vertical fold arrow in the Tam3 structure diagram represents the transcriptional start site of the *Tam3 TPase* gene. ATG and TAA are start and stop codons of the Tam3 TPase, respectively. FPH, fragments per hundred mapped fragments.



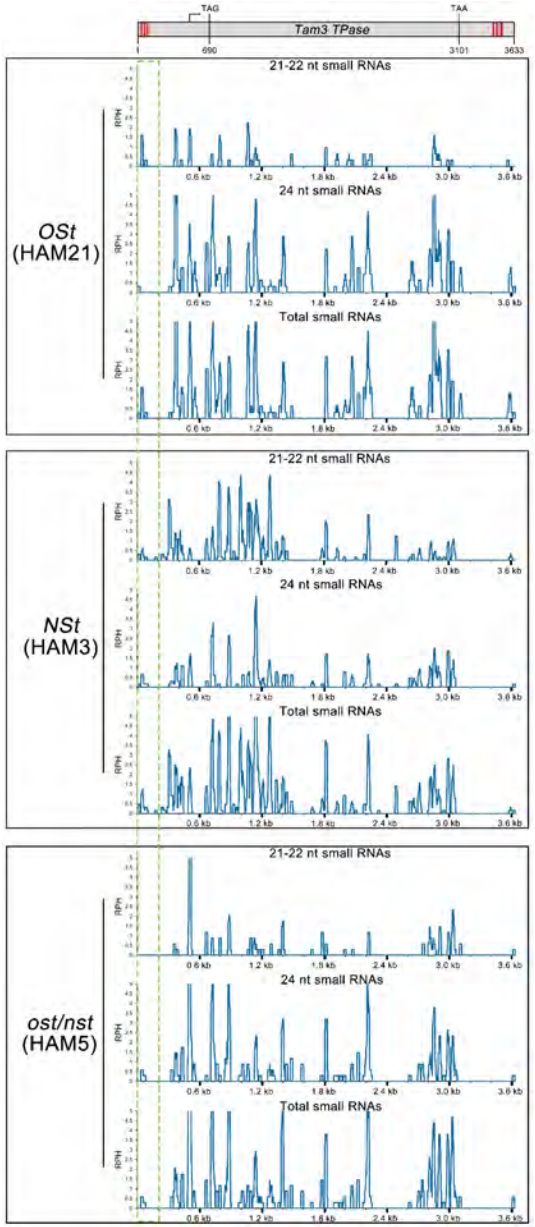
Supplementary Fig. 14
Distributions and amounts of RNAs (lncRNAs + mRNAs) in *NSt* and *nst* alleles.
The RNAs including lncRNAs and mRNAs that perfectly matched were aligned to the 5' region of Tam3-L of *nst* (1-689 bp) and the flanking region of Tam3-L of *nst* (1867 bp) in *nst*/*niv*$^{\text{rec: beni}}$ (HAM5), and aligned to the 5' regions of Tam3-L (1-689 bp) and Tam3-R (1-689 bp) of *NSt* and the internal region (1867 bp) between Tam3-L and Tam3-R of *NSt* in *NSt*/*niv*$^{\text{rec: beni}}$ (HAM3). Red and blue colors were used to distinguish sense and antisense RNAs, respectively. FPH, fragments per hundred mapped fragments.



Supplementary Fig. 15

Distributions and amounts of sense sRNAs in the Tam3 region.

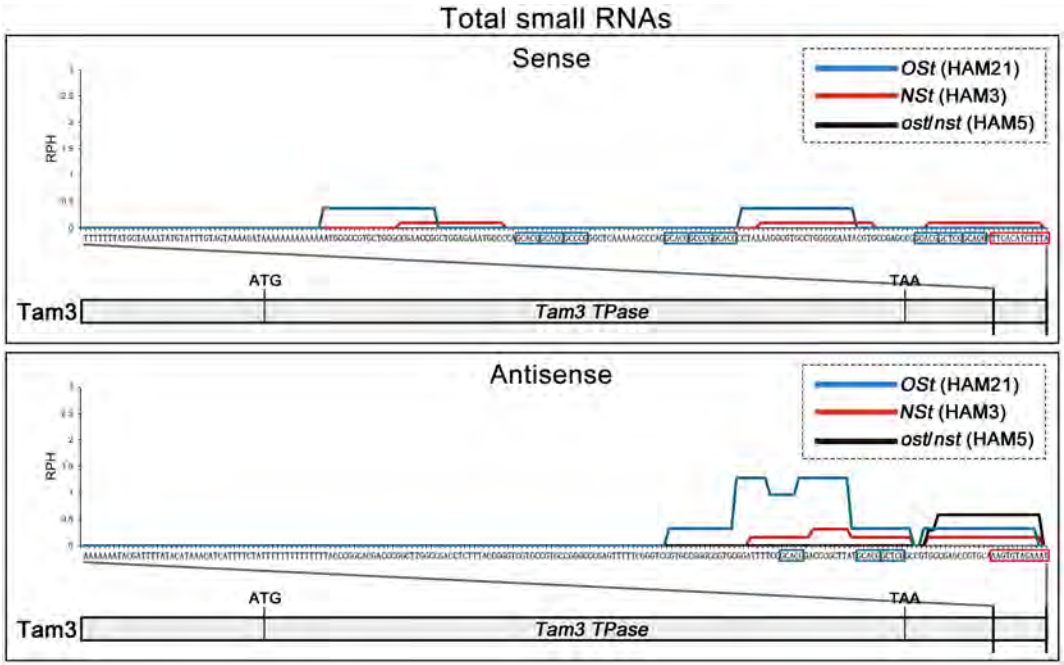
To employ the same mapping approach in the three genotypes because of several SNPs exist in the 5' rearranged region of *OSt*-Tam3, thus the mapping was carried out as follows: sRNAs firstly were perfectly matched to the sense strand of Tam3 (1-3633 bp), and remained sRNAs were then collected and finally perfectly aligned to the sense strand of *OSt*-Tam3 (1-3919 bp). sRNAs in the first step and sRNAs perfectly aligned to the sense strand in the 5' 1-201 bp region of *OSt*-Tam3 were combined together, and then 21, 22 and 24 nt sRNAs were selected respectively and simply combined together as total sRNAs. The 21, 22, 24 nt and total sRNAs were analyzed in *OSt*, *NSt* and *ost*/*nst*, respectively. The vertical fold arrow and red vertical line in the Tam3 structure diagram represent the transcriptional start site of the *Tam3 TPase* gene and the position of the GCHCG motifs, respectively. ATG and TAA are start and stop codons of the Tam3 TPase, respectively. RPH, reads per hundred mapped reads.



Supplementary Fig. 16

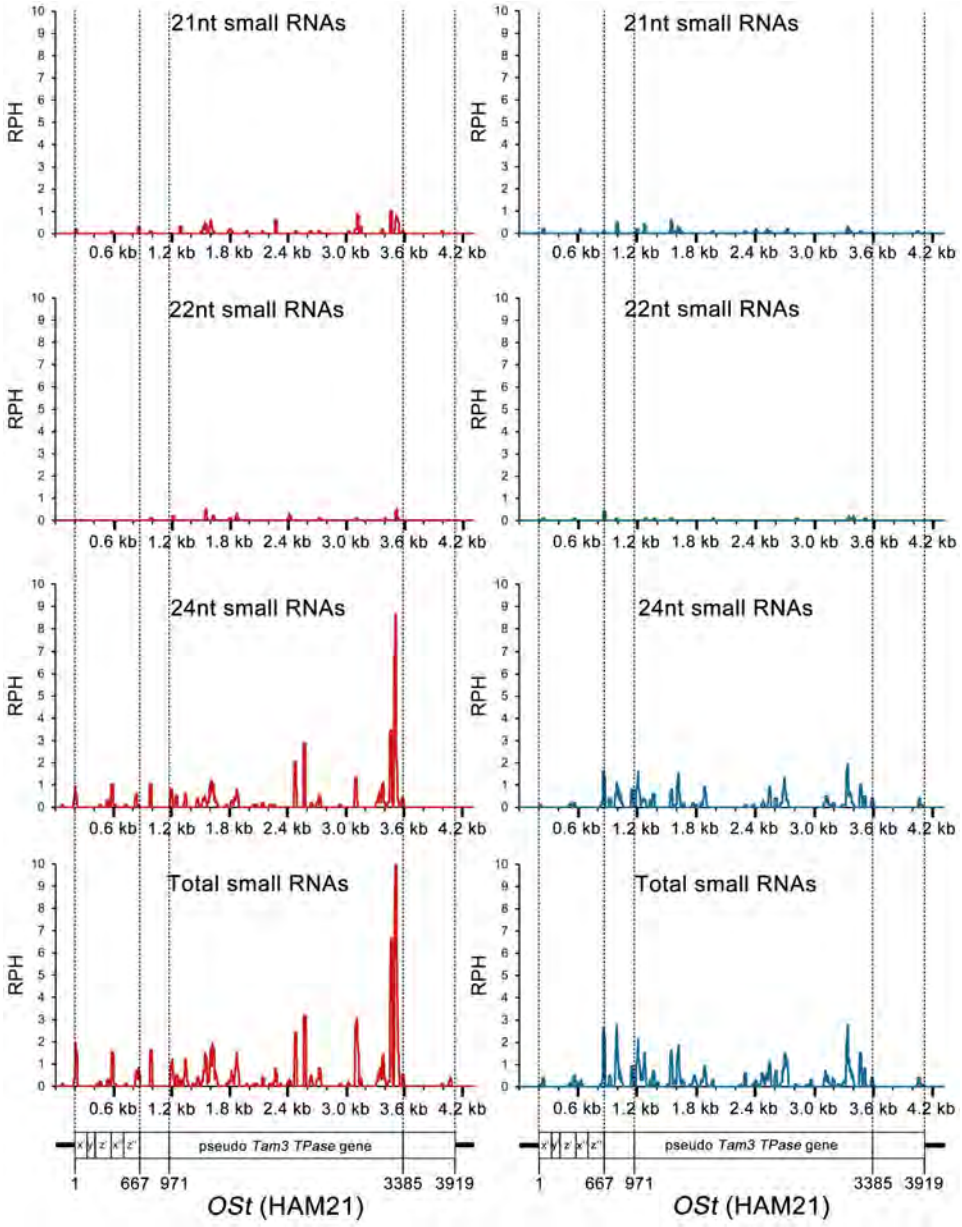
Distributions and amounts of antisense sRNAs in the Tam3 region.

To employ the same mapping approach in the three genotypes because of several SNPs exist in the 5' rearranged region of *OSt*-Tam3, thus the mapping was carried out as follows: sRNAs firstly were perfectly matched to the antisense strand of Tam3 (1-3633 bp), and remained sRNAs were then collected and finally perfectly aligned to the antisense strand of *OSt*-Tam3 (1-3919 bp). sRNAs in the first step and sRNAs perfectly aligned to the antisense strand in the 5' 1-201 bp region of *OSt*-Tam3 were combined together, and then 21, 22 and 24 nt sRNAs were selected respectively and simply combined together as total sRNAs. The 21, 22, 24 nt and total sRNAs were analyzed in *OSt*, *NSt* and *ost*/*nst*, respectively. The vertical fold arrow and red vertical line in the Tam3 structure diagram represent the transcriptional start site of the *Tam3 TPase* gene and the position of the GCHCG motifs, respectively. ATG and TAA are start and stop codons of the Tam3 TPase, respectively. RPH, reads per hundred mapped reads.



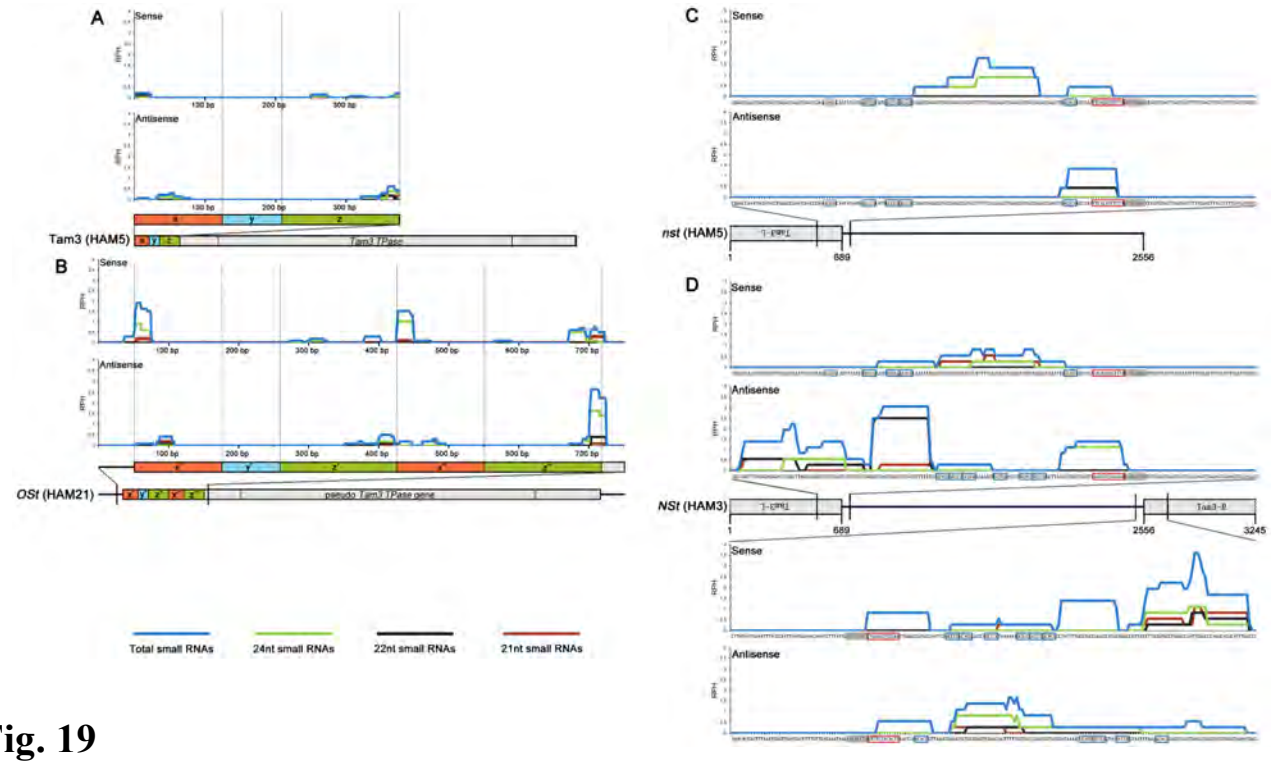
Supplementary Fig. 17
Distributions and amounts of total sRNAs in 3' region of intact Tam3.

To employ the same mapping approach in the three genotypes because of several SNPs exist in the 5' rearranged region of *OSt*-Tam3, thus the mapping was carried out as follows: sRNAs firstly were perfectly matched to Tam3 (1-3633 bp), and remained sRNAs were then collected and finally perfectly aligned to *OSt*-Tam3 (1-3919 bp). sRNAs in the first step and sRNAs perfectly aligned to the 5' 1-201 bp region of *OSt*-Tam3 were combined together, and then 21, 22 and 24 nt sRNAs were selected respectively and simply combined together as total sRNAs. The total sRNAs were divided into two strands: the sense and antisense strands of Tam3, and then these sRNAs perfectly matched to 3' region of Tam3 (3433-3633 bp) were analyzed in *OSt*, *NSt* and *ost*/*nst*. Red and blue squares on nucleotide sequences indicate Terminal Inverted Repeat (TIR) and GCHCG motifs, respectively. Lines indicate as follows; blue: *OSt* (HAM21), red: *NSt* (HAM3), black: *ost*/*nst* (HAM5). RPH, reads per hundred mapped reads.



Supplementary Fig. 18

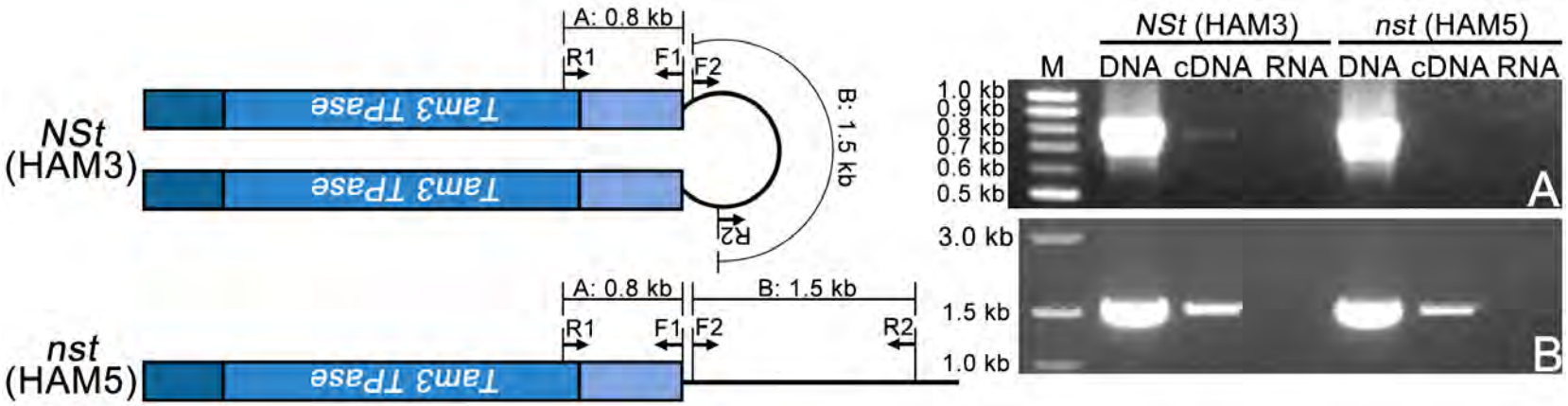
Distributions and amounts of sRNAs in *OSt*-Tam3 region. sRNAs that were perfectly matched to the *OSt*-Tam3 region (1-3919 bp) were analyzed. Red and blue colors were used to distinguish sense and antisense sRNAs, respectively. RPH, reads per hundred mapped reads.



Supplementary Fig. 19

Distributions and amounts of sRNAs in 5' regions of Tam3, *OSt*-Tam3 and Tam3 of *nst* and *NSt*.

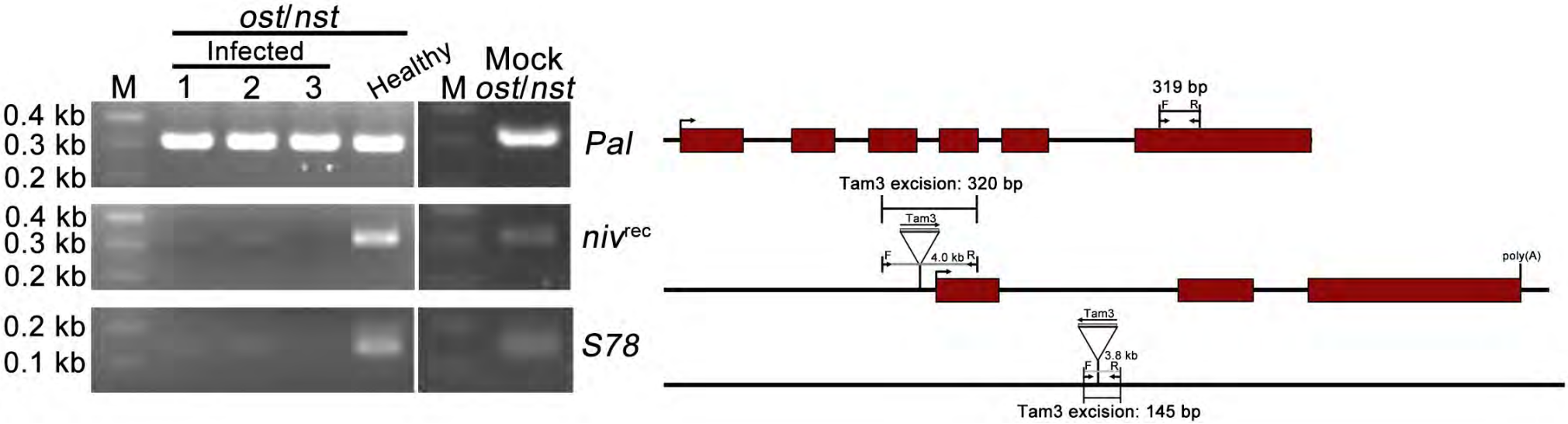
A. Distributions and amounts of sRNAs in 5' x-y-z region of Tam3 in *nst*/*niv*$^{rec:\ beni}$ (HAM5). B. Distributions and amounts of sRNAs in 5' x'-y'-z'-x''-z'' region of *OSt*-Tam3 in *OSt*/*niv*^{rec} (HAM21). C. Distributions and amounts of sRNAs in the 5' region of Tam3-L of *nst* (660-689 bp) and flanking region of Tam3-L of *nst* (690-740 bp) in *nst*/*niv*$^{rec:\ beni}$ (HAM5). D. Distributions and amounts of sRNAs in the 5' regions of Tam3-L (660-689 bp) and Tam3-R (2557-2585 bp) of *NSt* and flanking regions of Tam3-L (690-740 bp) and Tam3-R (2503-2556 bp) in *NSt*/*niv*$^{rec:\ beni}$ (HAM3). sRNAs that perfectly matched were aligned to the regions. Gray, red and blue squares on nucleotide sequences indicate Target Site Duplication (TSD), Terminal Inverted Repeat (TIR) and GCHCG motifs, respectively. Lines indicate as follows; blue: total sRNAs, green: 24nt sRNAs, black: 22nt sRNAs and red: 21nt sRNAs. RPH, reads per hundred mapped reads.



Supplementary Fig. 20

Detection of a possible hairpin transcript in the *NSt* and *nst* alleles.

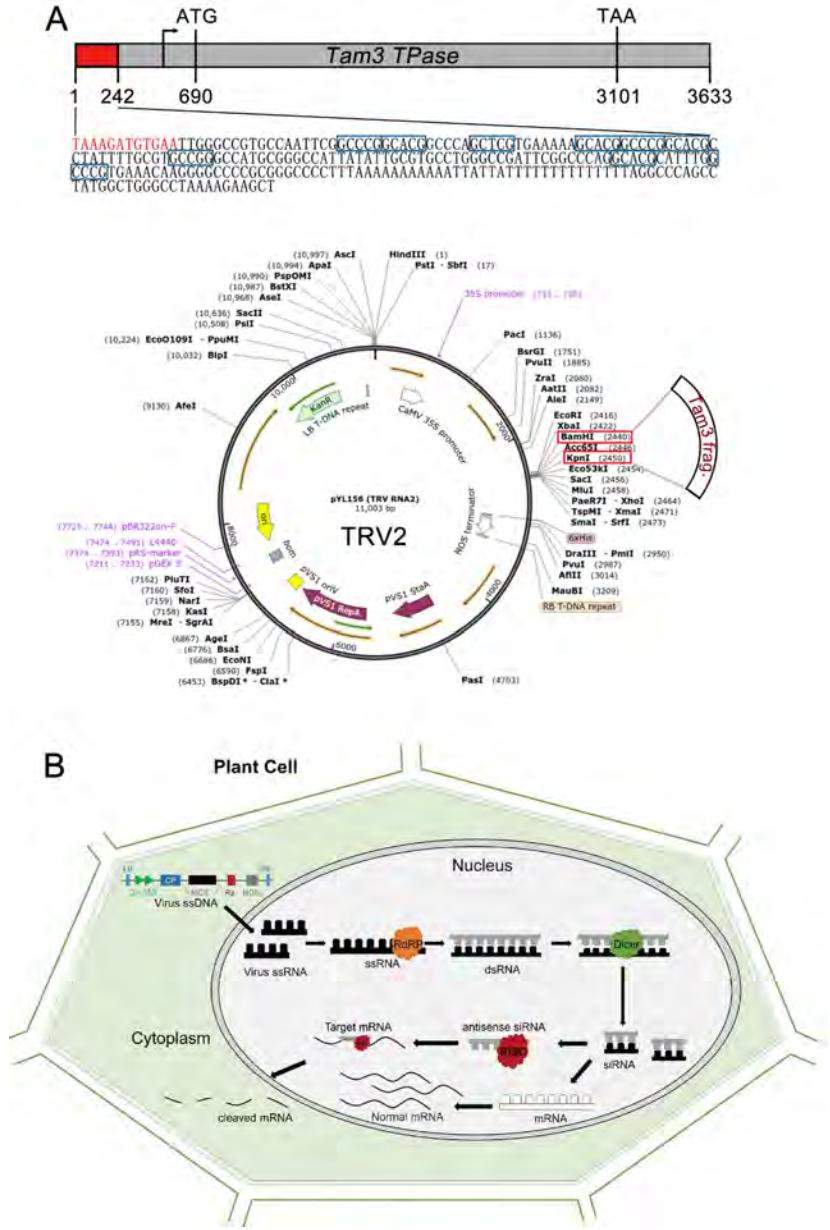
In panel A, the F1 and R1 primers amplified a 0.8 kb PCR fragment in the DNA and cDNA samples with the *NSt* allele and DNA sample with *nst* allele, but not in the RNA direct sample with the *NSt* allele and RNA samples with the *nst* allele. Panel B shows a 1.5 kb PCR fragment amplified with the DNA and cDNA of *NSt* and *nst* alleles using F2 and R2 primers. F1 and R1 are located at the 5' end of both Tam3-L and Tam3-R. F2 and R2 primers are presented in the internal region between two Tam3 elements of *NSt* (1-1867 bp). Supplementary Table 4 shows the primer sequences.



Supplementary Fig. 21

Detection of Tam3 excision events at *niv*^{rec} and *S78* loci in TRV-Tam3- and Mock-inoculated plants and plants without TRV inoculation.

The amplification of *DFR* gene at *Pallida* locus was used as a control for Tam3 excision events because of no Tam3 insertion at *Pallida* locus. The vertical fold arrows in *Pal* and *niv*^{rec} structures represent the transcriptional start sites of *DFR* and *CHS* gene, respectively. The red boxes in *Pal* and *niv*^{rec} structures represent the exons of *DFR* and *CHS* gene, respectively. Small arrows indicate the positions of primer using for Tam3 excision detection in the loci. And big arrows in *niv*^{rec} and *S78* structures show the direction of inserted Tam3 element. Supplementary Table 4 show the primer sequences.



Supplementary Fig. 22

Profiles of TRV-mediated VIGS.

A. Construction of TRV RNA2 plasmid carrying Tam3 fragment. The 242 bp of 5' terminal Tam3 fragment (1st nt to 242nd nt) was amplified and cloned into the TRV RNA2 vector between *Bam*HI and *Kpn*I restriction sites. B. Mechanism of TRV-mediated VIGS. The model figure was modified from (Shi et al., 2021).Virus ssRNA was transcribed from virus ssDNA inserted with desired fragment, and then RdRP protein syntheses the complementary ssRNA using virus ssRNA as a template to produce dsRNA. The dsRNA was cleaved into sRNAs by Dicer protein, and then RISC binds to the sRNAs as a whole to degrade target mRNAs.