Supplemental data

Mapping analysis of *rev3-1* mutant

To map the *rev3-1* mutation on the chromosome 1, twelve CAPS markers were designed based on 12 BAC sequences. The sequences and the restriction enzymes to analyze the polymorphism between Col and L*er* are shown in the below table.

New CAPS markers to detect-the polymorphism on the bottom of chromosome

BAC	Nucleotide sequence	Restriction enzymes used to analyse polymorphism
F24J13	5'-TTAATAGTCTTCCTTCCACATC-3' 5'-ATAGCTGGAGAAAGATGAGAA -3'	*Xba* I
F42J5	5'-TCACCAAACTCCATTACCACTG -3' 5-TCGGAGTGAGTGAGTGAGAGG -3'	*Dra* I
T23K23	5'- TGAAATAGGGACAGAGTAGGTA-3' 5'-GTTGTTGTAGGCTCATAAGAAT -3'	*Sau*3A I
F12A21	5'-TTGCCCACTTTGTTCGGTTAT -3' 5'-TCTACACGAACCAGCGAAAGCT -3'	*Bam*H I
F1N21	5'-CATTTGCTTTTTGGTTGGTTAT -3' 5'-TGTTTTGAAGAATGAGAAGTTGG -3'	*Sty* I
F1O19	5'-GACCTTTACCGCAACCATACT -3' 5'-ATTATATTGGCGATTTGTGTCC -3'	*Sca* I
T1F9	5'-TTGTCAATCTTCTTCACTTTCCC -3' 5'-ACGATACGGAAATGATGAAAAAC -3'	*Spe* I
T13D8	5'-CTTCACTAAAACCAACCCAATA -3' 5'-TCTTTTGTCCTTCTGCTTCTAA -3'	*Acc* I
F23H11	5'-AGAAGAAAATAAGAGAAAAGCA -3' 5'- TGATAAGAAGACTCCATTGACT-3'	*Rsa* I
T30E16 (1)	5'-TATCTTTGTATTTGCGTGCTT -3' 5'-GGTAGAACCAAGAAGTGAGGA -3'	*Afl* III
T30E16 (2)	5'-GGCTCGAAATAACACATAAGG -3' 5'-AATACTTATCGTGTCGTGGTTG -3'	*Hin*d III
T18I24	5'-ATGTTTCTTTTCTTTGGATTGT -3' 5'-TAAAATGATTACCTGTTGAAAG -3'	*Fok* I

Detection of the chromosome rearrangement in *rev3-1* mutant

To identify the mutation responsible for the *rev3-1* phenotype, we searched for the ends of the inverted region. The sequences of seven BACs (F23H11, T2K10 and T13D8 on the left side, and F1N21, T1F15, F12B7 and F12A21 on the right side) located around the boundaries of the recombining and non-recombining region were used as probes to detect the restriction enzyme-digested patterns of *rev3-1* DNA. Both wild-type and *rev3-1* DNA showed the same digestion pattern when using F23H11, T13D8, F1N21 or F12A21. However, the BAC probes for T2K10, F12B7 and T1F15 allowed the altered band patterns to be detected by hybridization against *rev3-1* DNA. A 9.2 kb fragment, corresponding to both F12B7 and T1F15, was lost in *rev3-1* DNA digested with *Sac* I (Figure S1A). Similarly, using the T2K10 probe, a fragment was found to be missing from the pattern of *Xho* I-digested *rev3-1* DNA (Figure S1B), that seemed to correspond to the 7.9 or 6.6 kb fragment.

To narrow down the possible mutated DNA region, 11 pairs of PCR primers covering the missing 9.2-kb (right), 7.9- and 6.6-kb (left) regions were designed. The position of each target fragments was shown in Figure S1 with a rectangle. The subfragment 5 was amplified by using T2K10-4F and T2K10-6R. The sequences of primers are as follows:

F12B7-1F, 5'-AGGATGGTTTAGATTAGTTTGA-3';
F12B7-1R, 5'-GGTGACACTGACACTGATAGGTAAACAT-3'
F12B7-2F, 5'-TCTTTTACAGTTCGTCCTTTTA-3'
F12B7-2R, 5'-ATTTGTGCTTTTATTTAGTCCA-3'
F12B7-3F, 5'-TCTGGACTAAATAAAAGCACAA-3'
F12B7-3R, 5'-TTTGTCATCTACCACTGTTCC-3'
F12B7-4F, 5'-GGAACAGTGGTAGATGACAAAT-3'
F12B7-4R, 5'-AGATTTGATTTGGATGATTGTC-3'
F12B7-5F, 5'-GACAATCATCCAAATCAAATCT-3'
F12B7-5R, 5'-GGTTCCAATGTTTTCAGTTTAC-3'.
T2K10-1F, 5'-AAATTCCAACTTACACGATACC-3'
T2K10-1R, 5'-CTAGTGCATTGTCCTCCTCA-3'
T2K10-2F, 5'-AAAGAAAACTGGCATTGTGT-3'
T2K10-2R, 5'-TGGATTTTAAGTCAGCAAGTT-3'
T2K10-3F, 5'-CCTTACTGCCTTTCTTACACTT-3'
T2K10-3R, 5'-AATAATAAATGCTCCTCACCTC-3'
T2K10-4F, 5'-TGGTACCGTGTGATGATAGTG-3'
T2K10-4R, 5'-GTCTAAGGTTGTTTGGCTGTC-3'
T2K10-6F, 5'- GACAGCCAAACAACCTTAGA-3'
T2K10-6R, 5'-CATTCTTCCTACCTTCTCTCC-3'
T2K10-7F, 5'-AAAAACACTCAAACTAAATGGTA-3'

T2K10-7R, 5'-GTCCACATAGGCATACATTGA-3'

PCR analysis revealed that a 1.6-kb subfragment (Figure S1A, subfragment 3) in the 9.2-kb region was not amplified when the *rev3-1* DNA was used as a template. All subfragments in the 7.9-kb region were amplified, indicating that this region was intact in *rev3-1*. However, one subfragment of 0.8-kb (Figure S1B, subfragment 7) in the 6.6-kb region was not amplified. The failure of amplification might be due to rearrangements taken place within these subfragments.

To elucidate the rearrangement in the *rev3-1* chromosome, TAIL-PCR was performed using arbitrary degenerated (AD) primers with 12 specific (SP) primers or 9 SP primers for T2K10 or F12B7. The AD primers are prepared as described previously (Liu et al., 1995). The sequences of the SP primers are as follows:

T2K10-SP2, 5'-AATGGTCCACATAGGCATACATTGAAG-3'
T2K10-SP3, 5'-GCCAACAACAACAAAAAGTGTACGTTGG-3'
F8A5-SP3, 5'-CTTGGTATTAAGTGTGTTTAGCTGCAAGAG-3'
F8A5-SP6, 5'-TAGCATCACACACAACACTTTTAGTTCC-3'
F12B7-SP6, 5'-TAAGAAACGTTCCTTTGGGTTTCAGATC-3'
F12B7-SP12, 5'-AATCTTTACCTACTGACCCCTGCCTTTT -3'

Sequencing of TAIL-PCR products revealed that the sequence of T1F15 containing subfragment 3 was broken and rejoined to the sequence of T2K10. Similarly, T2K10 containing subfragment 7 was also broken and rejoined to a fragment other than T1F15. By searching for homologous sequences in the *Arabidopsis* DNA database, it was found the new sequence of the fragment corresponded to F8A5, which was located about 200 kb downstream of T2K10. These results suggest that there are at least three break points in chromosome 1 (See the text and Figure 3).

Reference

Liu, Y.-G., Mitsukawa, N., Oosumi, T., and Whittier, R.F. (1995). Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J. **8**, 457-463.

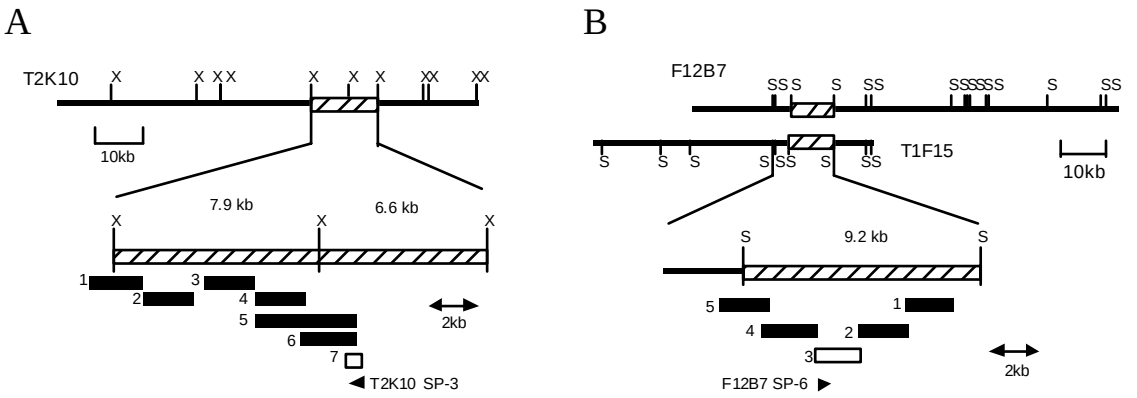

Figure S1. Detection of the *rev3-1* mutation
Restriction maps and PCR analyses on the left side (A) or right side (B) of the non-recombinating region. The probes for T2K10, T1F15 and F12B7 detected the altered band patterns against *rev3-1* DNA. The hatched boxes indicate the missing fragments in *rev3-1*. X, *Xho* I site; S, *Sac* I site. Black rectangles indicate the amplified region by PCR. White rectangles indicate the regions that were not amplified by PCR. Arrowheads indicate the positions and directions of specific primers used in TAIL-PCR.