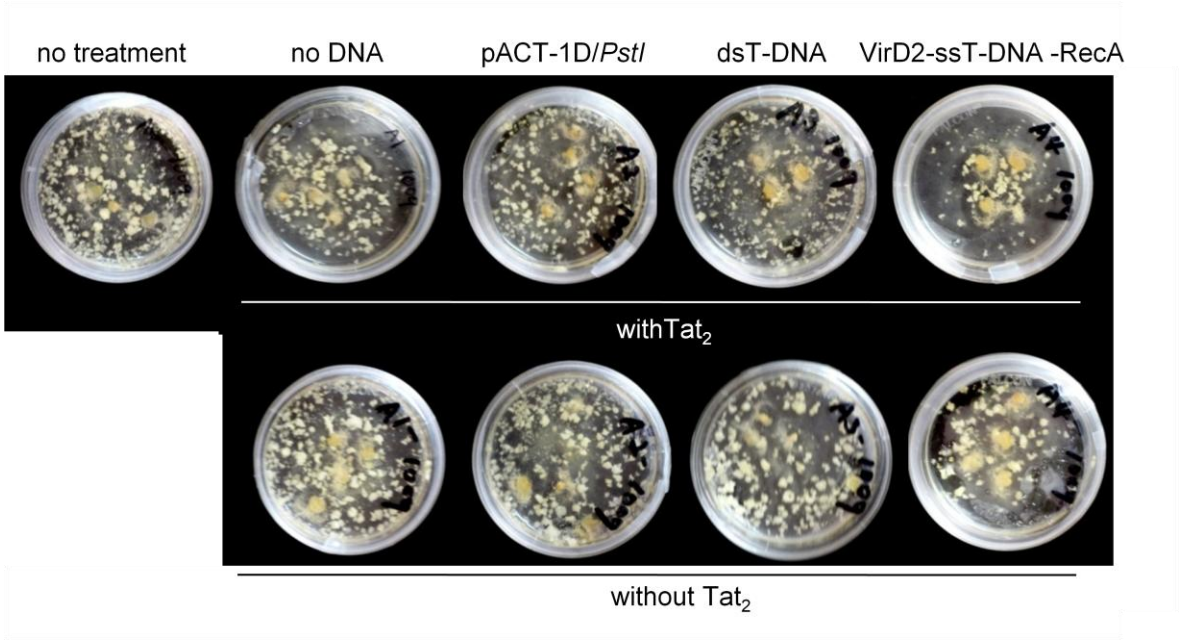
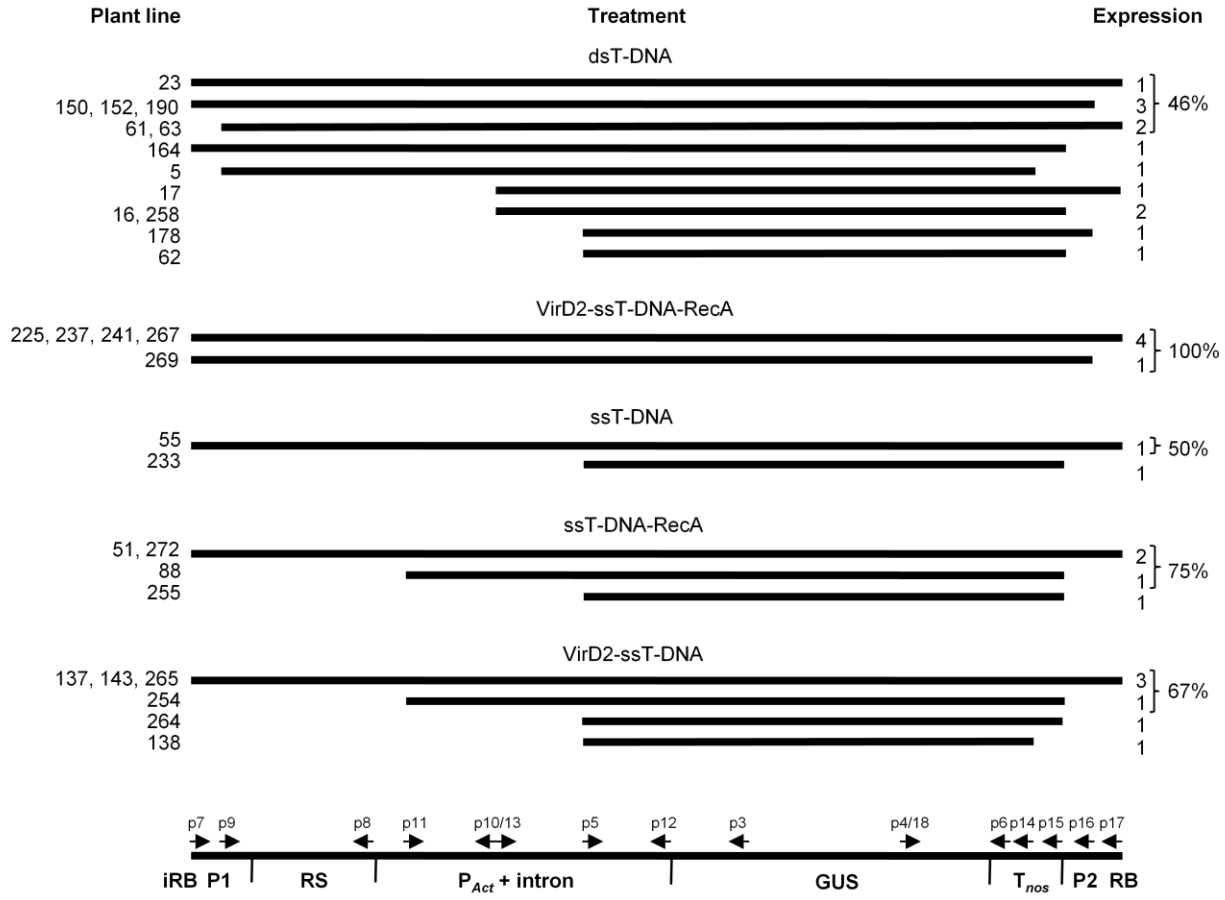
Ziemienowicz et al., Nano-complex for transformation of monocots

Supplemental Fig. S1



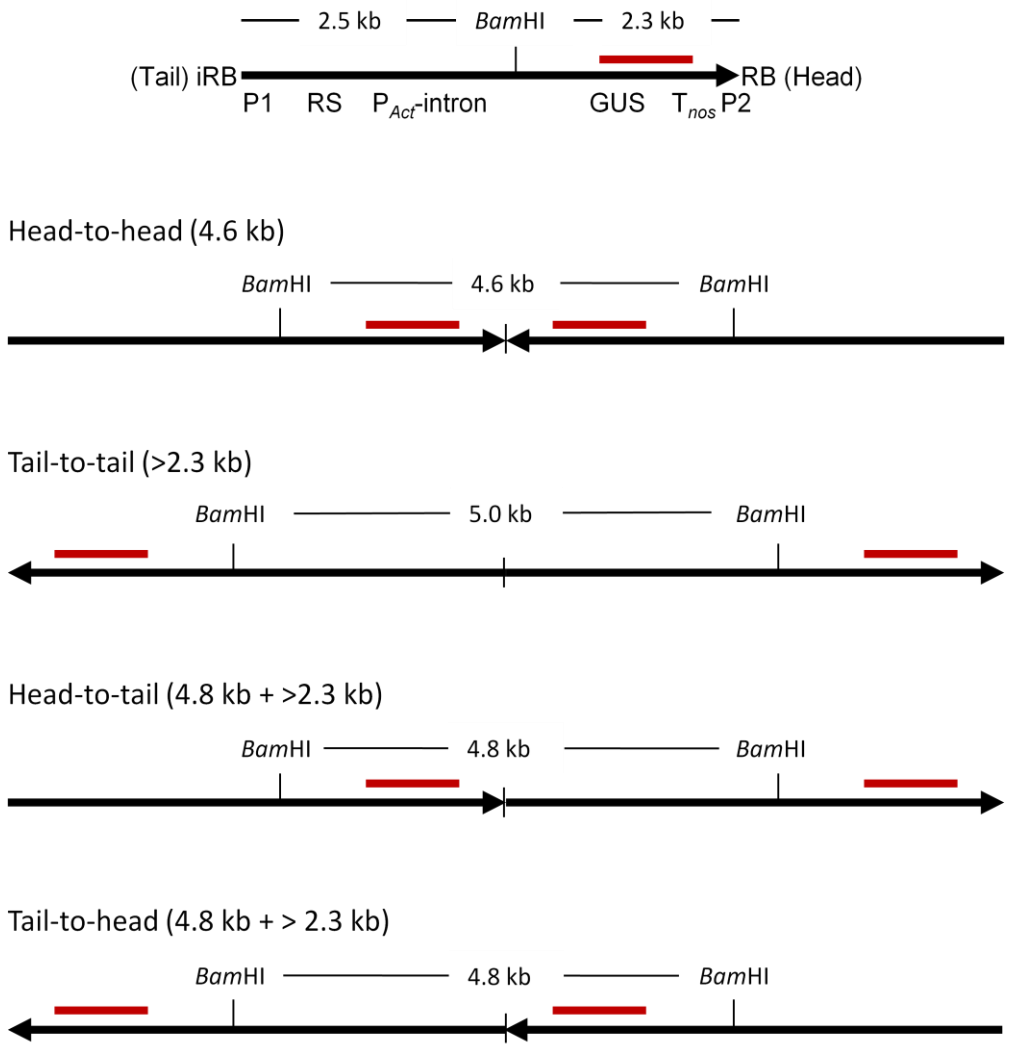
Triticale embryogenesis: the development of embryos from triticale microspores treated with various DNAs (linearized plasmid, dsT-DNA) or DNA/protein complex (VirD2-ssT-DNA-RecA) in the presence or absence of Tat_2 peptide. Mircospores were cultured for 4 to 6 weeks on embryogenesis liquid medium containing Ficoll.

Supplemental Fig. S2



The analysis of the intactness of the GUS transgene in the genome of GUS-positive plants. Transgene intactness analysis was performed by PCR using 10 sets of primers (supplemental Table S1) on genomic DNA isolated from plants that were regenerated from microspores treated with various DNAs (dsT-DNA, ssT-DNA) or DNA/protein complexes (ssT-DNA-RecA, VirD2-ssT-DNA, VirD2-ssT-DNA-RecA) in the presence of Tat_2 peptide. The numbers of the left indicate plant lines, whereas the numbers on the right indicate the number of plant lines showing a particular structure of the integrated transgene expression cassette. The numbers at the clamps represent the predicted percentage of GUS-positive plants that should express the transgene.

Supplemental Fig. S3



Scheme of predictions for multi-copy single-locus integration patterns based on the insertion of two transgene copies into one genomic locus. These predictions were made for the lower strand of the P_{Act}-GUS-T_{nos} expression cassette with RB representing the head of the ssT-DNA molecule and iRB representing its tail. Analogous predictions made for the upper strand (iRB as a head and RB as a tail) are: "head-to-head" (>2.3 kb), "tail-to-tail" (4.6 kb), "head-to-tail" and "tail-to-head" (4.8 kb + >2.3 kb). The red line represents 0.6 kb long GUS probe used for the Southern blotting analysis.

Supplemental Table S1 *Sequences of primers used in PCR reactions*

Primer	Sequence
p1	AG*CCATGG*TATATATCCTG/CCACTCTTCGCTATTACGCCAGC
p2	GT*CCATGG*TATATATCCTG/CCAGCGGGCAGTGAGCGCAACGC
p3	TCTGCCAGTTCAGTTCGTTG
p4	TGCTGTCGGCTTTAACCTC
p5	GTCTCGGTCTCGATCTTTGG
p6	AGACCGGCAACAGGATTCAATC
p7	GCGGGCAGTGAGCGCAACGC
p8	GACCTCGAGTATGCTAGCTAC
p9	ATAACAATTTCACACAGGAAACAGCTATGAC
p10	ATCGTGGATAGCACTTTGGG
p11	TAAAAGGTGGCCCAAAGTGA
p12	CAAAAAAGCTCCGCACGAGGC
p13	CCCAAAGTGCTATCCACGAT
p14	TGCGCGCTATATTTTGTTTTC
p15	AGGGATCTAGTAACATAGATGACACCG
p16	CCAGTGAGCGCGCGTAATACG
p17	CTCTTCGCTATTACGCCAGC
p18	TGCTGTCGGCTTTAACCTCT
p19	GATTGGTGGCATTGGAAC
p20	GATGACACCAACAGCCACAG

Right border (RB) core sequence is underlined; VirD2 cleavage site is indicated by / symbol; *Nco*I recognition sequence is indicated in italic font.