A Screening of phenotypes

Parents and normal F₁s

B Validation of recombinants

Parents and normal F₁s

C Haplotyping heterozygous genotypes

1. Design of specific primers

SD1

primer4 primer5

PA64s(P)

93-11(N)

2. PCR amplification results

M₁ P N NF₁ RF₁ primer4 primer5 primerP primerN

3. Genotypes of M₀

Sample primer5 primerN

P Can’t be amplified

N

NF₁ C/G

RF₁ C/G

D Identification of recombination type

CO:

SD1

NCO-GC:

SD1

CO-GC:
**S3 Fig.** Strategies and processes of recombination detection at the *SD1* locus.

**(A)** With benefits of application of sterile line PA64s, it is easy to obtain massive numbers of hybrid progeny in cross of two semi-dwarf varieties, PA64s and 93-11. After large-scale screening of F1 progeny, tall individuals with plant height > 130 cm were collected, together with parental lines (height: 90-110 cm) and normal F1s (height: 90-110 cm) as controls.

**(B)** To validate that the changes of plant height derive from recombination events of *SD1* gene, two markers (M8 and M9) were genotyped and assigned to genotype P (PA64s homozygosity), N (93-11 homozygosity) or H (heterozygosity of PA64s and 93-11). primer4 and primer5 are used to genotype M8 and M9, respectively. As we described in the introduction, two semi-dwarf varieties, PA64s and 93-11, harbor two different defective alleles, here respectively denoted as P8-P9 and N8-N9. If a recombination event occurred between these two alleles, progeny with wild-type *SD1* gene will be generated and present tall stature, namely harboring three switched genotypes of M8 and M9 (N8-P9, N8-H9, H8-P9).

**(C)** While the genotype of H8-H9 could be phased as P8-P9/N8-N9 or P8-N9/N8-P9, given that normal F1 harbors the P8-P9/N8-N9 haplotypes, but P8-N9/N8-P9 comes from a crossover event, and offspring with these two different haplotypes are respectively named as NF1 (non-recombinant F1) and RF1 (recombinant F1) here. To distinguish these two types, we employed two haplotype-specific primers primerP and primerN, which could specifically amplify the PA64s haplotype (P) and 93-11 haplotype (N), respectively. In detail, the forward primer of the primerP pair covered the break site of 383 bp deletion on PA64 haplotype, whereas the forward primer of primerN pair is located in the 383 bp deletion and the amplified sequence of primerN covers the M9 site. Expected PCR amplification results are shown in the middle, specifically, individuals with both 93-11 and PA64 haplotypes could present the combined electrophoretic bands of parental lines. After sequencing the amplification products of primer5 and primerN, NF1 and RF1 individuals show the heterozygous pattern at the M9 site (C/G nucleotide base, PA64/93-11 type) from amplification products of primer5, but respectively present homozygous G nucleotide base (93-11 type) and a homozygous C nucleotide base (PA64 type) at M9 site from amplification products of N-haplotype-specific primer primerN.

**(D)** To identify the recombination types of recombinant lines, additional 13 polymorphic loci were selected as markers covering approximately 445 kb of the flanking regions around the *SD1* gene. According to broadly accepted patterns of mitotic recombination (S1 Fig.), outcomes of CO events present heterozygous genotypes on one side and homozygous genotypes on the other side separated by breakpoint, whereas outcomes of NCO-GC events show heterozygous genotypes on both sides of the tract but homozygous genotypes on tract. In addition, outcomes with homozygous genotypes on both sides of the tract but homozygous genotypes on tract may come from two adjacent CO events or a CO event out of the *SD1* locus with a conversion event in the *SD1* gene. More complex combinations of multiple genotype switches may be caused by long-tract conversion, CO-associated conversion or repetitive CO events. Finally, according to the different outcomes listed in right, we could identify the recombination types for tall individuals.