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Breeding of self-compatible strains by RNA interference using SP11/SCR in Brassica rapa L.

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Self-incomparibility(SI) discriminating self and non-self pollen is regulated by S-locus genes in Brassica. In most S- haplotypes, a set of three highly polymorphic genes, SLG, SRK, and SP11/SCR, is located at the S-locus region. In this work SP11/SCR encodes a highly polymorphic Cys-rich small basic protein, which is the sole male determinant of S-specificity in Brassica. In other to disrupted SP11 gene, SP11S9 and SP11S60 promoters fused to the SP11S52 and SP11S60 RNAi cassettes, and introduced into a self-incompatible strains. Transgenic plants of B. rapa L. were produced by inoculating hypocotyls sections with Agrobacterium tumefaciens strain binary pBI101, which contains carrying a vector β-glucuronidase (GUS) hygromycin-resistance. kanamycin-resistance, and co-cultivation medium at pH5.2 with tobacco feeder cells was effective to enhance infection frequency evaluated by the number of hypocotyl sections. Transgenic plants in cv. Osome were obtained by inoculating the hypocotyl sections in the bacterial inoculum for 30 min, and co-cultivation at 25°C for 3 days with the highest transformation efficiency. We finally obtained two hundred seventy transgenic plants. After self-pollination, PCR analysis of selected NP7II and S-allele specific primers T1 plants revealed that introduced RNAi vectors were experimented for pollination amplification test by aniline blue staining method. SP11/SCR transcripts of the transgenic plants were analyzed by RT-PCR and Real-time PCR. From these results obtained twenty three strains of self-compatibility in T1 transgenic plants. Detected by the antibiotics tolerant tested in T2 generation.

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