Supplementary File 1. Additional materials and methods to this study.

Materials and Methods

1. Cultivation of Sterile Seedlings

Select healthy, plump *Brassica juncea* seeds. First, immerse the seeds in 75% ethanol for 1–2 min for surface sterilization, then discard the ethanol. Next, soak the seeds in 3%–5% sodium hypochlorite (NaClO) solution for 15 min. During the disinfection process, shake the container continuously to ensure thorough disinfection of each seed. Discard the NaClO solution and rinse the seeds four times with sterile water, each rinse lasting 1 min. Sow the sterilized seeds evenly at a density of approximately 50 seeds per vessel onto 1/2 MS medium. After sowing, place the vessels in a culture room at 23°C with a 16/24 h light photoperiod and culture for 5–6 days.

2. Preparation of Bacterial Resuspension

Inoculate *Agrobacterium rhizogenes* strain K599 carrying the *RUBY* plasmid into LB medium containing kanamycin and culture overnight until OD_{600} = 0.6. Centrifuge the bacterial culture at 5000 rpm for 10 min, discard the supernatant, and resuspend the pellet in 50 mL of 1/2 MS solution supplemented with 100 μL of 100 mM acetosyringone (AS).

3. Infection

Select healthy sterile seedlings and cut off the root at approximately 1 cm above the base (hypocotyl) to obtain explants. Immerse the explants in the bacterial resuspension solution for 15 min, ensuring that the cut ends are fully submerged. After immersion, remove the explants and blot off excess bacterial liquid with sterile filter paper. Place the infected explants on sterile co-cultivation medium (CIM: MS medium 4.4 g/L, sucrose 15 g/L, phytagel 2.7 g/L) and incubate at 22°C in the dark for 48 h.

4. Hairy Root Induction

Transfer the explants to culture boxes (16 cm × 16 cm × 8 cm) containing hairy root induction medium (MS medium 4.4 g/L, sucrose 30 g/L, phytagel 2.7 g/L, timentin 300 mg/L). Place the boxes in a culture room at 23°C with a 16/24 h light photoperiod and culture for 14 days. After 14 days, red hairy roots of 2–4 cm in length can be observed emerging from the callus.

5. Callus Culture and Shoot Differentiation

Select bright red hairy roots and cut them into small segments of approximately 5–8 mm. Place the segments on callus induction medium (MS medium 4.4 g/L, sucrose 30 g/L, phytagel 2.7 g/L, 6-BA 5 mg/L, NAA 0.5 mg/L, timentin 300 mg/L) and culture at 23°C with a 16/24 h light photoperiod for 20 days. After 20 days, callus formation can be observed on the hairy root segments. Transfer the callus to shoot induction medium (6-BA 0.5 mg/L, NAA 0.5 mg/L) and continue culturing for 30 days. Well-developed callus masses with signs of shoot primordia differentiation will form. Continue culturing until small plantlets with 3–4 leaves are formed, then proceed to the next step.

6. Plantlet Regeneration

Transfer the small plantlets to rooting medium (MS medium 4.4 g/L, sucrose 30 g/L, phytagel 2.7 g/L, IBA 0.1 mg/L, NAA 0.2 mg/L, timentin 300 mg/L) and culture at 23°C with a 16/24 h light photoperiod. After approximately 14 days, red roots will develop. Then, acclimatize the plantlets and transplant them to soil.