Genetic transformation of hybrid parents in *Brassica napus* with bivalent genes and their resistances to *Sclerotinia sclerotiorum*

Wang Xin-fa[1], Wang Hang-zhong*[1], Hu Zan-min[2], Liu Gui-hua[1],Zheng Yuan-ben[1]

1. Institute of Oil Crops Research, Chinese Academy of Agricultural Sciences, Wuhan 430062,China

2. Institute of Genetics, Chinese Academy of Sciences, Beijing 100151, China

By screening and optimizing such factors that may affect *Agrobacterium* infection, efficiency like physiological condition of explants, *Agrobacterium* suspension medium composition, concentration of bacterium used for infection, duration of cocultivation with *Agrobacterium*, etc., a high-efficiency *Agrobacterium*-mediated genetic transformation system of *Brassica napus* was established. The plant constitutive expression vector pBLGC with β-1,3-glucanase and chitinase genes was introduced into hybrid parents (restorers and maintainers) in *B. napus* (two commercial winter cultivars Zhongyouza No. 1 and Zhongyouza No. 4, pol-CMS hybrids) via *Agrobacterium*-mediated transformation of cotyledonary petioles. The transformation efficiency of resistant-plants ranged form 7% to 11%. Fifty-eight transgenic plants of restorers and maintainers were obtained. Using isolated microspore of restorers of those cultivars as receptor, the foregoing bivalent genes were introduced by *Agrobacterium*-mediated method. Factors that affect the microspore embryogenesis were studied. Results indicated that microspores cocultivated 6-7 hours with *Agrobacterium* when the number of microspores approached that of bacterium, then centrifugated and resuspended in NLN medium containing 100-200mg/l carbenicillin, could inhibit bacterial growth and did not affect the microspore embryogenesis. Forty-seven kanamycin-resistant transgenic haploid plants were obtained. Evidence of transgene integrations were confirmed by PCR, Southern hybridization, and segregation of the kanamycin-resistant trait in the progeny. The mechanism of resistance to *Sclerotinia sclerotiorum* was studied through compared activities of β-1,3-glucanase, chitinase, polyphenoloxidase (PPO), peroxidase (PO), phenylalanine ammonia lyase (PAL) and oxalic acid oxidase of the transgenic T1 and T2 plants with those of the non-transgenic controls, and other resistant, medium resistant and susceptible cultivars.