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Cloning and characterization of novel promoter using thermal asymmetric interlaced (TAIL)-PCR based method from T-DNA tagged *Brassica napus cv westar*

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Objectives

To obtain unknown sequence (novel promoter) of 5′-and 3′ ends of T-DNA from *Brassica napus cv westar*, Thermal Asymmetric Interlaced PCR (TAIL-PCR) were performed. To identify β-glucuronidase (GUS) expression of novel promoter acquiredfrom TAIL-PCR procedure, several target cells, were bombarded by paticles coated with bombarding vector which include novel promoter and gus genes, and then transformed *Nicotiana tabacum* (Xanthi) plants were developed from embryogenic callus following *Agrobacterium tumefaciens* mediated transformation.

Materials and Methods

1. Material : Plant - *Brassica napus cv westar*, *Nicotiana tabacum* (Xanthi), *Nicotiana benthamiana, Cucumis melo var. makuwa.* And *Agrobacterium* strain - GV3101/pRD420
2. Methods : TAIL-PCR (Liu *et al.* 1995), Callus were obtained from leaf cuttings of Brassica napus cv westar. Culture was maintained by MES-α media containing phytohormone (0.1mg/L α-Naphthaleneacetic acid and 1.0mg/L 6-benzyladenine), 15g/L Sucrose, 0.5g/L Carbenicillin, 0.1g/L Kanamycin

Results and Discussion

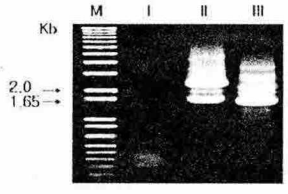

Fig. 1. Agarose gel analysis of TAIL-PCR product amplified from T-DNA insertion lines. The product specificity was confirmed by the size shift between lanes II and III. Multiple product bands observed in some samples were nested-fragment derived from annealing of the AD primer at more than one site along the target sequence molecules, as described previously (Liu and Whittier, 1995).

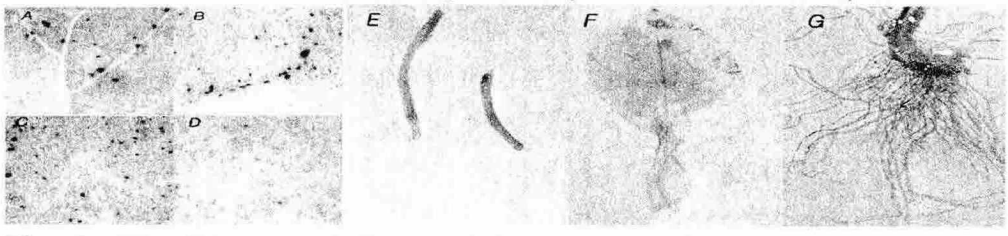

Fig. 2. Identification of Gus activity by histochemical staining in target cells. Expressing the novel promoter - gus chimeric gene : A : *Cucumis melo var. makuwa*, B : *Nicotiana tabacum* (Xanthi), C : *Nicotiana benthamiana* , D : Brassica napus cv westar. GUS activity in various tissues of the primary promoter tagged transgenic plant (*Nicotiana tabacum*(Xanthi)): E: GUS activity is exhibited in stems. F: GUS activity is exhibited leaves. G: GUS activity is exhibited predominantly in the root meristem and root hairs.

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