Efficient Agrobacterium-mediated transformation using immature embryo of Korean wheats

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Objectives.

The purpose of this study was to develop an efficient method of Agrobacterium-mediated transformation from immature embryos and to determine effect of Agrobacterium strains, treatments on transformation using immature embryos of Korean wheat cultivars.

Materials and Methods.

Materials:

- Wheat cultivars: Alchanmil, Geurumil, Gobunmil, Keunkangmil, Tapdongmil, Urimil.
- Agrobacterium strain / plasmids : KYTR1, EHA105 / pCAMBIA1305.1

Methods

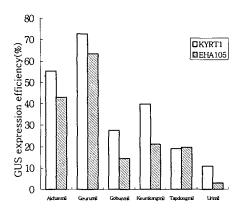
- Treatments: sonication, vacuum infiltration, and the combination of sonication and vacuum infiltration.
- Agrobacterium density : $OD_{600} = 1.0 \sim 1.5$
- Transformation: Co-cultivation with Agrobacterium for 3days at 25°C under the dark conditions.
- Analysis : GUS assay (Histochemical assay)

Results and Discussion.

Efficiency of Agrobacterium-mediated transformation was determined by GUS gene expression in immature embryos of wheat. Transient GUS expression was confirmed on 3 days after co-cultivation. In comparison of Agrobacterium strains, KYTR1 showed a higher in most cultivars (Fig 1.). The combination of sonication and vacuum infiltration showed high GUS expression efficiency compared with other treatments (sonication, vacuum infiltration) (Fig 2.). The GUS expression showed clearly difference among the cultivars.

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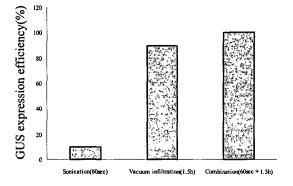


Fig. 1. The effect of Agrobacterium strains on GUS expression in immature embryos

Fig. 2. The effect of treatments on GUS expression in immature embryo of wheat (cv. Geurumil).

Efficiency(%) = Number of GUS expressed immature embryos / Number of immature embryos × 100.