

Effect of L-cysteine on *Agrobacterium* inoculation in wheat transformation

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Objective

The purpose of this study was to increase an efficiency of *Agrobacterium*-mediated transformation using thiol compound.

Materials and Methods

Material plants : Alchanmil, Geurumil, Gobunmil, Jaeraeneulmil, Keumkangmil, Topdongmil, Urimil, Bobwhite

Methods : Efficiency of GUS expression from wheat cultivars were evaluated.

- ▶ Preparation of explants
 - Immature embryos : 0.8 ~ 1.5 mm (freshly isolated), Mature seeds
- ▶ Inoculation & cocultivation
 - Pre-culture time : 1 h
 - Inoculation time : 0.5 h - *Agrobacterium* strains : KYRT1
 - *Agrobacterium* cell culture : OD_{600nm} 0.8 ~ 1.0
 - Treatment : sonication 30 sec & vacuum infiltration 0.5 h
 - Cocultivation time : 3 days
- ▶ Assay of GUS activity
 - Wheat explants were assayed for GUS 3 days after inoculation with *Agrobacterium*.

Results and Discussion

- ▶ The addition of L-cysteine (400 mg/l) to the solid cocultivation medium consistently resulted in a significantly greater frequency of GUS expression.
- ▶ Explants cocultured in the absence of L-cysteine had an average frequency [Immature embryos (IE) : 35.9% and Mature embryos (ME) : 39.6%], whereas explants cocultured in medium containing 400 mg/l L-cysteine scored significantly higher frequency (IE: 51.1% and ME : 42.2%). Alchanmil and Geurumil had a low GUS expression frequency (37.5%) from the addition of L-cysteine in the mature embryos.
- ▶ In conclusion, the addition of L-cysteine to the solid cocultivation medium increased efficiency of *Agrobacterium*-mediated gene delivery in immature embryos and mature embryos.

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Table 1. The effect of thiol compound on transient expression of the GUS gene in wheat varieties.

Variety	Embryos with GUS expression (%)			
	Immature embryos		Mature embryos	
	KYRT1	KYRT1+L-cysteine	KYRT1	KYRT1+L-cysteine
Alchanmil	28.6	38.1	56.3	37.5
Geurumil	31.8	40.9	50.0	37.5
Gobunmil	40.9	50.0	40.0	42.9
Jaeraeneulmil	27.3	51.7	12.5	31.3
Keumkangmil	42.9	61.9	43.8	56.3
Topdongmil	36.4	54.5	33.3	56.3
Urimil	38.1	57.1	31.3	50.0
Bobwhite	33.3	53.8	50.0	56.3

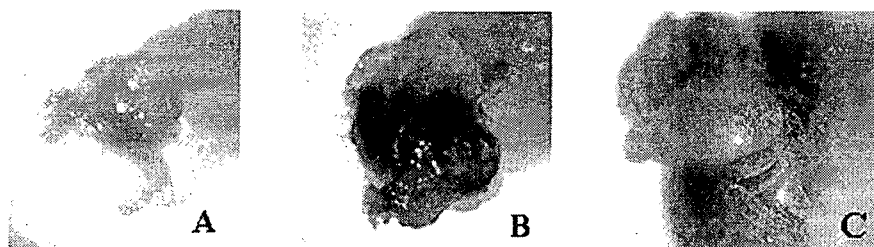


Fig.1. Transient GUS expression in *Agrobacterium*-infected explants.

A. *Agrobacterium* - mediated transient GUS expression of immature embryo.

B. Transient GUS expression on callus induction medium of immature embryo for 2 weeks.

C. *Agrobacterium* - mediated transient GUS expression of mature embryo.