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Calli initiated from thin mature embryo fragments are appropriate targets for wheat transformation

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Objective

This study evaluated the culture procedure to make good and sufficient materials for an efficient genetic transformation.

Materials and Methods

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

Methods

▸ Aseptic embryo isolation

· Mature seeds were imbibed for 48 h in sterile water at room temperature.

· Mature embryos were aseptically excised with two methods.

1. Through a sterile nylon mesh (approximately 600㎛ porosity) fixed over the mouth of a small flask.
2. Cut embryos to 4~5 fragments with a sterile scalpel.

▸ Culture initiation

Thin mature embryo fragments	Treatment
Material 1 Embryo fragments through nylon mesh	A. Each embryo fragments were maintained at 25℃ in darkness. B. Each embryo fragments were mixed 5 ml callus liquid medium. These were incubated on a rotary shaker (130 rpm) at 25℃ with a 16 h photoperiod.
Material 2 Cutting embryo fragments with a scalpel	

Results and Discussion

▸ **Callus and suspension culture**

Calli were induced from fragments through nylon mesh. Calli formation in suspension culture were much better than in solid culture (Table 1). While, callus induction from material 2 in solid culture was higher than in suspension culture. Calli from cutting embryo fragments are white to green-white in suspension culture (Material 2).

▸ **Suspension cultures**

Selected embryogenic calli from solid medium that retained a high competence for somatic embryogenesis. The germinating plantlets were well-developed during suspension culture (Fig. 1. C and D). This culture system may be provide an appropriate target to gene transfer method.

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Table 1. Frequency of embryogenic callus from thin mature embryo fragments of eight wheat genotypes.

Genotype	Embryogenic callus induction (%)			
	Material 1 Embryo fragments through nylon mesh		Material 2 Cutting embryo fragments with a scalpel	
	solid medium	suspension culture	solid medium	suspension culture
Alchanmil	40.1	50.0	89.1	58.8
Geurumil	35.2	65.6	85.5	50.1
Gobunmil	45.6	64.9	78.8	61.6
Jaeraeneulmil	18.2	66.0	71.5	72.5
Keumkangmil	41.1	64.9	88.1	67.7
Topdongmil	42.9	60.0	84.6	65.4
Urimil	48.7	63.8	73.3	66.9
Bobwhite	47.8	60.5	86.8	70.0

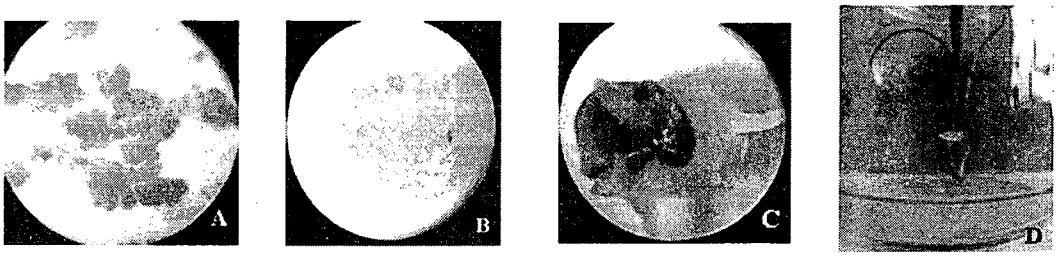

Fig. 1. Mass production of wheat from embryogenic callus culture and embryogenic cell suspension cultures.

A. Numerous calli developed from suspension culture after 6 weeks.

B. Embryogenic callus from crushed embryo fragments formed on the solid medium for 1 week.

C. Development of green shoot from suspension culture.

D. Plantlet developed from somatic embryo.