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Stable soybean transformation via *Agrobacterium*-mediated gene transfer using cotyledonary node

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Objectives

Soybean [*Glycine max* (L.) Merr.] is one of important crop as it is an economic source of both vegetable oil and protein. Despite the considerable improvements for transformation using the cotyledonary node (cot-node), soybean transformation systems yield low efficiencies and limited to a few cultivar. Therefore we focused on evaluating various factors that will affect development of reproducible system for transgenic soybean production.

Materials and Methods

Plant - Soybean (cv. Danbaek, Eunha), *Agrobacterium* strain - EHA105/pCAMBIA1304
The transformation was based on the protocol of Olhoft and Somers(2003)

Results and Discussion

The cot-node was wounded using blade under either the magnifier or microscope (Idea from Prof. Chung) and infected under mild vacuum 5 minutes. We found that T-DNA delivery increased under using the microscope and also formed multiple shoots more frequently. The reason can be explained that procambium was stimulated for strong division ability by cutting a precise target region. The effect of cut direction in terms of transient GUS expression was found to be important. The horizontal direction cutting of cot-node region is more effective than does vertically. The effect of *Agrobacterium* optical density was non-significant. But most of bacterial overgrowth contributed to detrimental effects on explants during co-cultivation and throughout selection period. We found a considerable level of intrinsic GUS activity in several soybean cultivars. The endogenous GUS activity often hides the activity originating from the introduced GUS gene, resulting in confusional information on GUS gene expression. When the reaction mixture contained 20% methanol, the endogenous GUS activity in the explants were almost suppressed. In previous experiments indicated that SE medium supplemented with both GA₃ and IBA has effect on shoot elongation but our result have shown that the induced-shoot did not elongate, being bleached and becoming necrotic. Further investigations are required to improve the efficiency of elongation by different combination of plant growth regulators.

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