M-106

Optimization of Agrobacterium-mediated transformation in soybean

Shuxuan Li, Nanjing Agricultural University, Jiangsu, China

High transformation efficiency is a prerequisite for study of gene function and molecular breeding. *Agrobacterium tumefaciens*-mediated transformation is a preferred method in many plants. However, the transformation efficiency in soybean is still low. The objective of this study is to optimize *Agrobacterium*-mediated transformation in soybean by improving the infection efficiency of *Agrobacterium* and regeneration efficiency of explants. Firstly, four factors affecting *Agrobacterium* infection efficiency were investigated by estimation of the rate of GUS transient expression in soybean cotyledonary explants, including *Agrobacterium* concentrations, soybean explants, *Agrobacterium* suspension medium, and co-cultivation time. The results showed that an infection efficiency of over 96% was achieved by collecting the *Agrobacterium* at a concentration of OD650 = 0.6, then using an *Agrobacterium* suspension medium containing 154.2 mg/L dithiothreitol to infect the half-seed cotyledonary explants (from mature seeds imbibed for 1 day), and co-cultured them for 5 days. The *Agrobacterium* infection efficiencies for soybean varieties Jack Purple and Tianlong 1 were higher than the other six varieties. Secondly, the rates of shoot elongation were compared among six different concentration combinations of gibberellic acid (GA3) and indole-3-acetic acid (IAA). The shoot elongation rate of 34 and 26% was achieved when using the combination of 1.0 mg/L GA3 and 0.1 mg/L IAA for Jack Purple and Tianlong 1, respectively. This rate was higher than the other five concentration combinations of GA3 and IAA, with an 18 and 11% increase over the original laboratory protocol (a combination of 0.5 mg/L GA3 and 0.1 mg/L IAA), respectively. The transformation efficiency was 7 and 10% for Jack Purple and Tianlong 1 at this optimized hormone concentration combination, respectively, which was 2 and 6% higher than the original protocol, respectively. Finally, GUS histochemical staining, PCR, herbicide (glufosinate) painting, and QuickStix Kit for Liberty Link (*bar*) were used to verify the positive transgenic plants, and absolute quantification PCR confirmed the exogenous gene existed as one to three copies in the soybean genome. This study provides an improved protocol for *Agrobacterium*-mediated transformation in soybean and a useful reference to improve the transformation efficiency in other plant species.