Approaches to Develop an *Agrobacterium*-mediated Transformation System via Direct Shoot Organogenesis in Common Bean (*Phaseolus vulgaris* L.)

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Gene transfer technology requires an efficient regeneration of whole plants from *in vitro* tissue culture. Direct regeneration from explants via direct shoot organogenesis, avoiding the callus phase as much as possible, is certainly recommendable for this purpose. The best-characterized and most commonly used transformation system for plants is the *Agrobacterium tumefaciens* (*At*)-mediated DNA transfer system. However, *At*-mediated transformation of *P. vulgaris* has not been successful. Common bean has been transformed using the biolistic DNA delivery system (Aragão et al., 1996; Russell et al, 1993). Specific and crucial parameters affecting successful *At*-mediated transformation of common bean need to be determined. We investigated here the effects of several important factors on the regeneration and infection efficiency of *P. vulgaris*, and established an efficient and reproducible procedure for the regeneration and infection using optimal combinations of those factors with a GUS (β-glucuronidase) reporter gene system.

Materials and Methods

The binary vector pTJ101 containing 35S *bar* and 35S GUS intron cassettes with TEV (tobacco etch virus) translational enhancer element was used in this study. The binary vector was mobilized into *At* strain EHA101 or other strains by either the triparental mating method or by the freeze-thaw method. Putative plant transformation and successful regeneration procedures were based on a direct shoot organogenesis protocol previously described by Mohamed et al. (1991) and Zhang et al. (1997) with some modifications. To prevent the escape of chimeric or non-transformed buds and shoots, 2mg/l glufosinate was added to shoot induction medium (SIM) as a selection agent. In an effort to increase the infection rate of *At* on the regenerated buds and shoots including differentiating tissues around the nodal region, 100μM acetosyringone (AS) was added to either the YEP medium or the YEP medium and the germination medium (GM). Also two antioxidants L-cysteine and L-ascorbic acid were added to the GM and to the cocultivation medium (CM).

Results and Discussion

Several important factors affecting the regeneration and infection rates of common bean were evaluated for the optimization of a stable transformation procedure. Among 12 cultivars derived from Andean and Middle American origins, the multiple-disease resistant cultivar GN Weihing (Coyne et al., 2000) showed high regeneration ability with our optimized culture methods and media (B5 salts and vitamines) containing BAP (5μM in GM and 11.25μM in CM and SIM) and the highest susceptibility to the *At* strain EHA101 with 3- or 5-day cocultivation under 16-hour light. The genotype dependent susceptibility to *At* strains has been reported previously in common bean (Brasileiro et al., 1996; Lewis and Bliss, 1994; Zhang et al., 1997). GN Weihing was significantly more susceptible to the *At* strain EHA101 than the other three strains A2760,

EHA105, and C58C1. The optimal level for selection was 2mg/l glufosinate. The *At* inoculum in the exponential phase (0.8-1.0 at OD_{650nm}) significantly increased the infection rate and GUS expression of GN Weihing without any loss of regeneration rate. The length of cocultivation was limited to a maximum of 5 days since longer cocultivation resulted in overgrowth of the *At* strain EHA101 on the cotyledonary node explants and subsequent losses during shoot induction stage due to contamination by *At*. Dark treatment of the explants during cocultivation reduced the infection rate and GUS expression at 3, 5 and 7 days and also caused more overgrowth of *At* on the explants than light treatment. The lack of effect of the dark treatment on the infection could be due to the orientation of explants (abaxial-side down) on semi-solid CM overlaid with a filter paper after inoculation thus making a shade over the wounded region of explants under both light or the dark. The combination treatment of 400mg/l cysteine and 200mg/l ascorbic acid supplemented in GM and CM significantly increased GUS expression on the regenerated explants although there was no significant increase in infection rate between control and antioxidant treatments. Sixteen percent of the total explants retained GUS expression on the differentiating tissues in this treatment, whereas no explant showed GUS expression on the differentiating tissues in the control. The antioxidant compounds supplemented in GM and CM minimized necrosis on the explants during wounding and inoculation, especially on the differentiating cells at the nodal region. The supplement of 100μM AS in YEP medium to increase the virulence of *At* slightly increased infection rate and GUS expression thus enhancing the antioxidant treatments.

Further studies are necessary to optimize factors in additional steps such as shoot elongation of putatively transformed tissues using an appropriate selection level.

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