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“Bridging the gap between increasing knowledge and decreasing resources”

Regeneration and Agrobacterium-Mediated Transformation of Cowpea

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Abstract

Cowpea (*Vigna unguiculata* L. Walp.), a widely grown multipurpose legume, is attacked by many insect pests during its life cycle and under storage. Insect resistance breeding has been limited by the lack of resistance traits in the cowpea gene pool. Alternatively, transgenic approaches can be used to close this gap via introducing genes from other pools. The recalcitrance nature of the cowpea to *in vitro* regeneration and transformation and varietal dependence of the existing protocols make, however, the transgenic approach quite challenging. Thus, in this study, we have optimised different regeneration and transformation conditions such as (a) the media for inoculation, co-cultivation and regeneration, (b) type of explants and (c) bacterial concentration. Transformation vectors containing GUS and GFP genes were used for transient transformation studies.

Cowpea was regenerated from cotyledonary node (CN) explants on MSB5 medium containing varying level of BA alone or in combination with Kinetin or NAA. The optimal medium for multiple shoot production from CN explants was MSB5 medium supplemented with 3 μ M BA and 0.5 μ M Kinetin. CN explants obtained from BA supplemented pre-conditioning medium showed better shoot production than medium supplemented with TDZ. Rooting of *in vitro* shoots was obtained on media with or without IBA. The *in vitro* rooted plantlets were successfully acclimatized and transferred to greenhouse. Based on transient transformation, decapitated embryo explants from dry seeds showed better transformation efficiency as compared to CN explants from 3–4 days old germinated seedling (33–56 % and 2–6 %, respectively) in relation to the media used for inoculation and co-cultivation. Adjustment of the overnight bacterial concentration to higher optical density value (up to 2.0) in the inoculation medium seemed to improve transient transformation both in terms of efficiency and the strength of the GUS gene expression. The optimised protocol could facilitate the stable transformation of cowpea for economical traits of interest such as insect resistance.

Keywords: Resistance breeding, transformation