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Guar genetic transformation: an example of how to overcome recalcitrance in legumes

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Motivation and aim: Plant genetic transformation is a key biotechnological tool that allows us to apply modern technologies, such as genome editing, to improve the breeding efficiency in plants. Several reports on genetic transformation of legumes agree that recalcitrance and genotype-dependence are some of the main bottlenecks affecting the regeneration and transformation efficiency. Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is an economically important short-day legume. In Russia, the main obstacle to its cultivation is the long photoperiod during summer, which delays flowering in guar. The implementation of genome editing technologies in guar may provide a meaningful opportunity to precisely edit genes implicated in the flowering process to improve the adaptability of the crop to a long photoperiod. However, there is not a single publication describing experiments on gene editing in this plant. Therefore, there is an urgent need to develop a protocol for guar transformation to subsequently use this protocol for guar genome editing. In this study, we used different methods of plant transformation, including *in planta* and *in vitro* approaches to obtain guar transformed seedlings.

Materials and methods: Four transformation methods were tested to obtain guar transformants, these methods include floral dip, agro-injection transformation, *Agrobacterium*-mediated transformation using cotyledonary nodes and A*grobacterium*-mediated transformation using seed embryos. For the floral dip experiment, a suspension culture of *Agrobacterium tumefaciens* harboring the plasmid pBI121 with GUS as a reporter gene was prepared as overnight culture. The culture was centrifuged and the pellet was resuspended in the infiltration medium. Subsequently, guar spikelets containing several flowers were inoculated with the *Agrobacterium* suspension. In the case of the agro-injection, the transformation was carried out by injecting the guar pods with the same *Agrobacterium* suspension harboring the plasmid pBI121. In the *Agrobacterium*-mediated transformation using cotyledonary nodes, two different media compositions were tested for regeneration. Guar cotyledonary nodes were excised from germinated guar seedlings and transferred to the preculture medium. After preculture, the cotyledonary node explants were incubated in the *Agrobacterium* suspension harboring the plasmid pBI121 and transferred to solid co-cultivation medium for 24 h. Subsequently, the cotyledonary node explants were transferred to the shoot induction medium (SIM), then to shoot elongation (SEM) and finally to the rooting medium every 15 days, respectively. In the case of the *Agrobacterium*-mediated transformation using seed embryos, a preliminary experiment was performed to select the best regeneration medium. For this purpose, the seed embryos were isolated and transferred to the two regeneration media for 21 days. Subsequently, the regeneration efficiency was evaluated and the best medium was selected for the transformation experiment. In the transformation experiment, two suspension cultures of *Agrobacterium tumefaciens* harboring either plasmid pCambia 1302 (mGFP5 as reporter gene) or pCambia 1303 (gusA and mGFP5) were prepared. The seed embryo was isolated, incubated in *Agrobacterium* suspension and transferred to solid co-cultivation medium. After co-cultivation, the explants were transferred to the regeneration medium and incubated for 8 weeks to obtain fully regenerated plants. Transformation efficiency was assessed by verifying the presence and expression of the GFP/GUS reporter genes.

Results: As result of the floral dip and agro-injection experiments a total of 723 potentially transformed seeds were obtained. Therefore, 73 seeds were selected randomly, germinated and DNA was extracted to verify the presence of the GUS gene using PCR. However, after screening none of the samples were positive for the presence of the GUS gene. In the case of the transformation using cotyledonary nodes, shoots were obtained using the two media tested. However, after 21 days on SIM medium, many of the explants with shoots showed a pale green or white coloration in some regions, which may be an early sign of hyperhydricity or albinism. After transfer to SEM this condition worsened and the shoots continued to develop with a whitish color that eventually led to oxidation of the explant. Regarding the A*grobacterium*-mediated transformation using seed embryos, fully developed plants were obtained after 8 weeks in the regeneration medium. Therefore, the regenerated guar seedlings were screened for the expression of GFP using a microscope under blue light illumination or GUS gene expression using GUS staining. In both cases, the guar seedlings showed positive results for the expression of the GFP and GUS genes.

Conclusions: The transformation recalcitrance in legumes is one of the main problems during genome editing experiments, therefore several plant transformation methods should be tested to improve the efficiency of plant regeneration and transformation. In the case of Guar, recalcitrance to genetic transformation can be overcome by using seed embryos as explants to successfully obtain transformed plants.

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