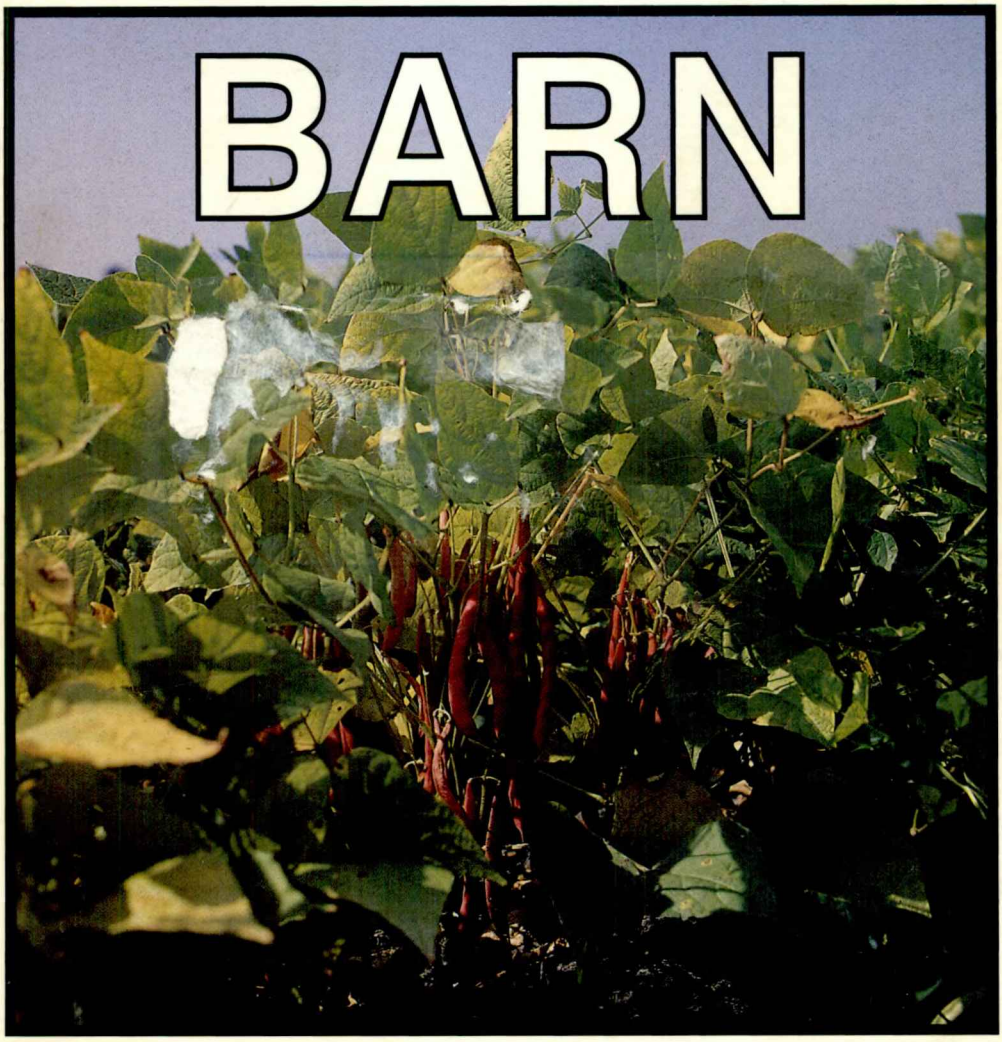
Proceedings

Phaseolus Beans Advanced Biotechnology Research Network



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Attempts to transform *Phaseolus vulgaris* with bean golden mosaic virus DNA sequences using a helium driven device

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ABSTRACT

There is considerable interest in the transformation of common beans for introducing useful genes for crop improvement such as disease and insect resistance as well as protein quality. The traditional methods utilized for plant transformation, including direct DNA transfer, the use of *Agrobacterium*, electroporation of DNA into protoplasts have not revealed to be useful for common beans. This is due to the lack of regeneration of bean tissues. Recent reports (Russell, *et al.*, 1993, Plant Cell Rep 12: 165-169; Smith, Jofre-Garfias and Sanford, 1992, Phytopathology 82:1167 Abstr.) indicate that the biolistic technology has been successfully used for transformation of common beans. In the present work we are using a helium driven microprojectile bombardment system, as developed by Sanford (1992). The vector consists of the antisense of the ORF's AL1-AL2-AL3, and the antisense of the ORF BL1, of bean golden mosaic virus, Brazil isolate (Gilbertson *et al.*, 1993, Phytopathology 82: in press), in front of the independent promotor CAMV 35S, besides the marker gene GUS, also driven by the 35S promotor. Apical domes were excised from seeds soaked in water for 10-12 hours, positioned with the dome upward in an agar medium. Bombardment was done with DNA coated particles using the vector above associated with another plasmid vector containing the Kan gene as a marker. The experiments were conducted with the bean variety Olathe (Pinto type). Initial results indicate very high levels of transient expression of the GUS gene in bean apical dome cells (meristem), embryonary axis, and cotyledonary tissues. Frequency of transient expression varied with the technique used to prepare the particles, keeping the amount of DNA at 1.8 ìg per shot. The calcium-spermidine method, which requires a much lower particle concentration, is now routinely used in the laboratory. The calcium-phosphate method has resulted in high levels of particle aggregation, with the consequence of causing some damage to the target tissue. From 90 to 250 gold particles per shot yielded up to 600 GUS expressing points (Aragao *et al.*, 1993 Pl. Cell Rep 12:483-490). We are currently regenerating the first set of plants showing chimeric tissue for GUS. So far, from near 3000 embryos bombarded, 3 explants showed to be transformed based on the GUS activity in a ring of the embryo axis, after shoot elongation.

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