PLANT REGENERATION *IN VITRO* FROM THE EMBRYONIC AXES OF COMMON AND TEPARY BEANS

Mohamed F. Mohamed, Paul E. Read and Dermot P. Coyne
Department of Horticulture, University of Nebraska, Lincoln, NE 68583-0724

Plant regeneration from embryonic axis explants has not been reported in *Phaseolus* species. This report describes a simple *in vitro* protocol for plant regeneration from embryonic axis tissues in four common bean (*Phaseolus vulgaris* L.) and two tepary bean (*P. acutifolius* A. Gray) genotypes. The effect of embryo size of explants and of media on shoot initiation and proliferation were investigated.

The genotypes used were four common beans, Great Northern (GN) 'UI59', 'PC50', 'GN Harris' (NE), 'Xan 159' (CIAT) and two tepary beans, T#81-3L179 and T#L242-45. All entries were planted twice at 10-day intervals. The collected pods were stirred in 70% ethanol alcohol for 1-2 mins. followed by 15-20 mins. in 15% (v/v) of 5.25%. Sodium hypochlorite solution with 2-3 drops of tween-20 per liter. Embryo sizes of 6-7 and 3-4mm were selected from the first and second planting dates, respectively. The embryonic plumules and radicles were cut off, and 1-2 mm of embryonic axes were cultured on Gamborg's B_5 basal medium with 0,5,10 or 20 μM of benzyladenine (BA). Each BA concentration was tested also in combination with 1 or 2 μM NAA. Cultures were maintained at 24-25° C under continuous light or were incubated for an initial dark period (2 weeks) before moving under light (25 μ Mol S^{-1} m^{-2} from cool-white fluorescent tubes). Three secondary media were used for the proliferation and elongation of the primary shoot initials. These secondary media were the basal medium without growth regulators or medium with reduced BA concentration (2 μM) alone or plus GA_3 (2 μM BA + 4 μM GA_3). Individual shoots proliferated on secondary media were transferred to medium without growth regulators for rooting. Mature plants growing in the greenhouse were observed for their growth and developmental characteristics.

Greatest percentages of explants regenerating multiple shoots (15-90%) for all genotypes were obtained on primary culture medium containing 5-10 μM BA. Visible multiple shoot initials were found on the meristematic regions on both sides and tips of embryonic axes on medium with 5-10 μM BA. Explants maintained under light, without receiving an initial dark period, showed early shoot formation and irregular shoot multiplication. Cultures on basal medium without growth regulators formed only roots ('UI 59', 'PC50' and 'Harris') or single shoots from the tip meristematic regions and roots (Xan 159 and the two tepary bean entries). Explants from smaller embryos were more regenerative than larger embryos on both 5-10 μM BA medium (50-90%) and 15-70%, respectively) and medium without BA (30-60% and 0-60%, respectively). The percentages of regenerative explants decreased on 20 μM BA medium for all genotypes, regardless of the embryo size used for explants. Media with NAA alone or plus 20 μM BA gave only callus. Shoots developed on media with NAA + 5 or 10 μM BA deteriorated and formed callus. Six to 8 shoots/explant from common bean and up to 20 shoots/explant from tepary bean were collected after 3 weeks on the secondary media. Better shoot proliferation and simulataneous elongation were observed on secondary medium with 2 μM BA. Shoots harvested from secondary medium with 2 μM BA formed roots easy after 7-10 days on medium without growth regulators. Seondary media with

BA/GA_3 enhanced shoot elongation but rooting was difficult for those shoots which were harvested after more than two weeks. All plants grown in the greenhouse were fertile and did not show apparent differences during the different stages of development.

In conclusion, the technique described in this report could be useful for the development of P. vulgaris transgenic plants. It may also be applied for increasing the efficiency of in vitro production of F_1 interspecific phaseolus hybrids.

Genetic Relationships among the Annual *Cicer* Species Using Allozyme Variation

R. Tayyar and J. G. Waines
Department of Botany and Plant Sciences
University of California
Riverside, CA 92521

The nine annual species of the genus *Cicer* include the cultivated chickpea (*Cicer arietinum*) and the wild relatives *C. reticulatum*, *C. echinospermum*, *C. bijugum*, *C. judaicum*, *C. pinnatifidum*, *C. yamashitae*, *C. cuneatum*, and *C. chorassanicum*. Chickpea or garbanzo constitutes an important source of proteins in developing countries of Asia, Africa, and Latin America. The wild species, however, are known to have useful characteristics which, if incorporated into the cultivated species, may lead to crop improvement. Some of these traits include resistance to diseases such as ascochyta blight (Singh *et al.*, 1981; Van der Maesen and Pundir, 1986) and botrytis grey mold (Singh *et al.*, 1982). In addition, higher vigor, large number of seeds per pod (Singh *et al.*, 1981) and cold tolerance (Singh *et al.*, 1990) are also found.

Most of the studies dealing with relationships among these species used cytological, seed protein, and crossability data (Ladizinsky and Adler, 1975; Ladizinsky and Adler, 1976). However, genetic relationships among them have not been fully assessed. In the projected study, we will be estimating genetic diversity within and among the nine annual *Cicer* species using allozyme variation. For this purpose, starch gel electrophoresis will be run to assay some enzyme systems in seed samples. The electrophoretic data will be used to calculate allele frequencies at the different loci and to determine average number of alleles per locus and average heterozygosity. The results will show the distribution pattern of genetic diversity. Then, the data will be used to estimate genetic distances using Nei's model and to construct a phenogram illustrating species relationships.