Enhanced Differentiation of Somatic Embryoids in Callus Cultures of Common Bean

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The induction of somatic embryogenesis in cell and tissue cultures of common bean (Phaseolus vulgaris L.) were reported by Allavena and Rossetti (1983), Martins and Sondahl (1984), and Saunders et al. (1987). Explants from bean seedlings were used previously to induce embryogenic callus. On other hand, enhanced competence for shoot differentiation in primary cultures occurred in explants excised from seedlings preconditioned on benzyladenine (BA) containing medium [Malik and Saxena (1991) in common bean and Wright et al., (1986) in soybean (Glycine max L. Merr.)]. Forchlorfenuron (CPPU) was more effective than BA for preconditioning donor seedlings and in subsequent cultures of nodal explants of common and faba beans (Mohamed et al., 1992). The use of the latter pretreatments to enhance the competence of callus from explants of common bean seedlings to differentiate and develop somatic embryos needed to be determined. Our objective, therefore, was to investigate the effect of preconditioning explant source seedlings on medium containing different concentrations of CPPU and the use of CPPU in the medium on induction, maturation and conversion of somatic embryos.

Materials and Methods. Dry seeds of two bean lines, Great Northern (GN) ‘Harris’ and Xan-159, were surface disinfested then germinated on L-6 solidified medium (Kumar et al., 1988a) with 3% sucrose. The medium was used either with or without 2 /µM forchlorfenuron (CPPU). The pH of all media was adjusted to 5.7 before autoclaving. Cotyledonary leaf explants were prepared from 2 week-old seedlings. The explants were cultured for callus induction on medium containing 4% sucrose and 0.2 g.liter⁻¹ casein hydrolysate, and supplemented with combinations of 2,4-D (4, 8 or 16 /µM) and kinetin (0, 1, 2 or 4 /µM).

All explant cultures were incubated at 25°C in darkness. Callus samples for use in a subsequent study were selected at random representing the induction media with 4 /µM 2,4-D or plus 1 or 2 /µM kinetin since these supplements produced the most prolific callus. The callus samples were either inoculated in liquid or solidified medium; both were supplemented with 2, 4 or 6 /µM 2,4-D alone or plus kinetin or zeatin (filter-sterilized) or CPPU at 0.25 or 0.50 of the concentration of 2,4-D. All cultures were kept under cool-white light (12 μE.m⁻².s⁻¹, 16h/day). The cultures in liquid medium were maintained on a gyratory shaker (120 rpm). Four to five weeks later, samples from the liquid and solidified cultures were observed for somatic embryogenesis. The somatic embryos and the putative embryogenic callus were then transferred to solidified medium containing 3% sucrose and enriched with CPPU or zeatin or kinetin (0, 1, 5 or 10 /µM). Different concentrations (1, 3, or 6 /µM) of naphthalenacetic acid (NAA) or filter-sterilized gibberellic acid (GA₃) (1, 2, or 4 /µM) were used in combinations with the various levels of each of CPPU, zeatin and kinetin. The cultures were placed under cool-white light (25 μE.m⁻².s⁻¹, 16h/day). After 4 weeks, the somatic embryos were subcultured either on the same corresponding medium or on medium lacking plant growth regulators. Based on the results from the above mentioned study, the following protocol was used in subsequent investigations unless otherwise stated: the solidified medium contained 4 /µM 2,4-D plus 1 /µM kinetin for callus induction, the liquid medium was supplemented with 2/µM 2,4-D plus 1 /µM zeatin for initiation of somatic embryos, and the solidified medium was used with 5 /µM CPPU alone or plus 1 /µM GA₃ for shoot differentiation. To determine the differential embryogenesis of various seedling explants, hypocotyl, petiole and shoot tip segments, in addition to leaf explants were used. Subsequently, increased concentrations of CPPU (4 and 6 /µM) in the seedling preconditioning medium were investigated. Induced somatic embryos in this latter experiment were placed on solidified medium with 1, 5 or 10 /µM parachlorophenoxy isobutyric acid (PCIB) alone or plus 5 /µM CPPU or on medium without PCIB and CPPU but containing 2 or 4 g charcoal/liter.

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Results and Discussion. Up to 200 heart and cotyledonary somatic embryos (derived from callus cultures of leaf explants of seedlings grown on CPPU containing medium) were observed after 4 to 5 weeks in liquid, but not solidified medium only with 2,4-D plus zeatin. The number of the heart shaped somatic embryos decreased and no cotyledonary stages developed in cultures with 2,4-D but lacking zeatin. Only globular structures or few heart shaped somatic embryos were observed in callus derived from leaf explants of seedlings grown on medium lacking CPPU regardless of zeatin supplements in the liquid cultures with 2,4-D. When CPPU or kinetin was added to 2,4-D liquid medium at the same concentrations used for zeatin, then only globular structures were initiated regardless of the preconditioning treatments for the donor seedlings. Martins and Sondahl (1984) indicated that high levels of 2,4-D inhibited the differentiation of embryogenic cells; however, increased concentrations of kinetin combined with reduced concentrations of 2,4-D induced embryogenesis in liquid medium for callus cultures. Therefore, higher levels of kinetin than we used with 2,4-D may be useful; CPPU may be decreased in the liquid medium of 2,4-D since it has strong cytokinin-like effects (Mohamed et al., 1992). Somatic embryos of the early cotyledonary stage in the liquid cultures of Xan-159 developed distinct cotyledons, shoot meristem, and elongated radicle 2 weeks after transferring to solidified medium enriched with CPPU (5 or 10 μM) either alone or plus 1 μM NAA or GA3. Leaf-like structures but no roots formed 2 weeks later. Kumar et al., (1988b) using a selected cell line derived from suspension cultures of tepary bean (P. acutifolius A. Gray), developed somatic embryos through an intermediate phase of culture on BA containing medium indicating a role for some effective cytokinins in their development and conversion. However, we did not observe further normal shoot development, whether the embryos were kept on the same medium (containing CPPU alone or plus GA3) or subcultured on medium free of these supplements. The somatic embryos formed only thick primary and secondary roots when transferred from the liquid to solidified medium containing 5 or 10 μM from cytokinins less effective than CPPU (zeatin or kinetin) alone or plus 1, 3 or 6 μM NAA. These embryos on solidified medium other than as discussed above either turned brown and died or formed long thin primary roots. Subsequent experiments indicated that more somatic embryos were differentiated in petiole-derived than in leaf and hypocotyl-derived callus but similar to callus initiated from shoot tips. Use of more than 2 μM CPPU in seedling preconditioning medium did not enhance further somatic embryoid differentiation. This is due to the effectiveness of CPPU at low concentrations in tissue culture of common bean (Mohamed et al., 1992). Neither the increased concentrations of CPPU (4 and 6 μM) during the preconditioning nor culturing the somatic embryos on medium containing PCIB plus CPPU or use of charcoal improved the differentiation of normal shoots and plantlets.

Literature Cited