INDUCTION OF SOMATIC EMBRYOS IN PEA, PISUM SATIVUM L.

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Successful application of tissue culture technology to the improvement of crop plants depends upon the ability to regenerate plants in vitro. Plant regeneration, however, has been quite difficult with seed legumes as peas and beans (Glycine, Phaseolus, Vicia). In pea, an attempt was made to overcome the regeneration problem by screening several genotypes for their in vitro behavior in order to select embryogenic cell lines.

Ten genotypes of pea were tested, including the variety 'Dippes Gelbe Viktoria' (DGV), five X-ray and neutron-induced mutants of the variety DGV (A89C, 251A, 37A, 39, and 176A), three recombinants from crosses with the fasciated mutant 489C and DGV (R 1115, R 15±1, R 20±1 —the last two are named according to the number ol their sterile nodes), and a line of Pisum arvense.

For callus formation leaf explants (6 mm in diameter) of 13-day-old pea seedlings were transferred to a modified MS-medium (MS-salts, 2 mg/1 thiamine HC1 and 250 mg/1 inositol) supplemented with 3% sucrose and 0.8% agar. The callus medium with 0.06 mg/1 picloram (4-amino-3,5,6--trichloropicolinic acid) in combination with 0.1 mg/1 BA (benzyl-adenine) was found to be optimal. The pH of the media was adjusted to 5.8. Callus formation was performed at 27C in the dark. For suspension cultures, the same modified MS-medium was used.

The genotypes showed different genetic behavior, not only for callus growth (Fig. 1) but also for callus morphology. The recombinants R 1115, R 15±1, and R 20±1 as well as the accession of P. arvense developed a compact and nodular embryogenic callus whereas the other genotypes produced only a compact callus which however exhibited nodular structures upon transfer to liquid MS-medium. The callus cultures of genotype 37A segregated into a slow growing, yellowish, soft, and compact callus and into a fast growing, yellowish-white, compact callus.

In suspension cultures, some of the nodular callus structures developed into embryos (Fig. 2, 3). When torpedo-shaped embryos were transferred to hormone-free solid MS-medium, some of them showed root formation, but no shoot morphogenesis has yet occurred. In a limited number of cases, an additional root was formed in the apparent shoot apical region after 2-5 days. Growing the embryos in liquid MS- media with added soluble starch, casein hydrolysate, activated charcoal, or culturing with varying combinations of BA alone or in combination with IBA (indole-3-butyric acid) or PAA (pheny1-acetIc acid) did not result in shoot morphogenesis.

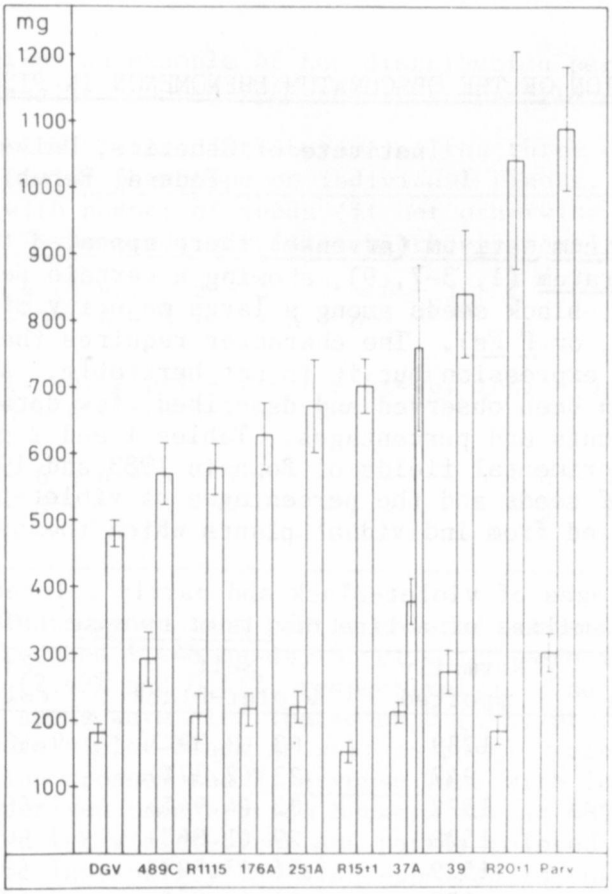

Fig. 1. Mean fresh weight of leaf-derived callus after a culture period of 5 and 10 weeks. Bars give ± S.E. of 9-12 replicates.

Fig. 2. Embryogenic callus with embryoids and embryos in suspension (vertical bar : 1 cm).

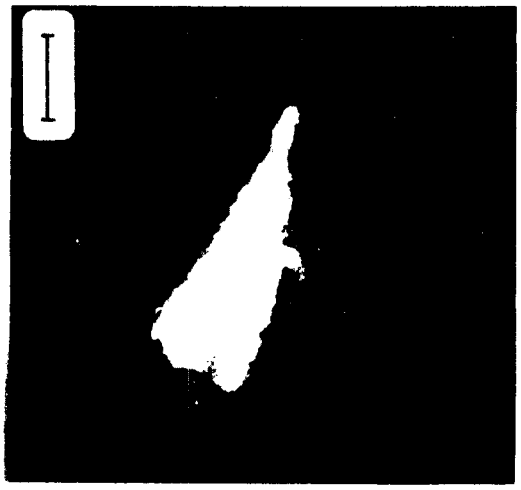

Fig. 3. Single embryo (vertical bar : 1 mm).