****

ORGANOGENESIS FROM IN VITRO CULTURE OF IMMATURE COTYLEDONS OF PHASEOLUS COCCINEUS

A. Allavena, L. Rossetti
Experimental Institute of Vegetable Crops,
Via Paullese, 28 - 20075 Montanaso Lombardo
(Milano) Italy.

Together with other grain legumes the genus Phaseolus appear recalcitrant to in vitro morphogenesis. Mature plants were produced by several authors through micropropagation of buds and meristems of common bean (P. vulgaris); also sexual embryos were grown in vitro and interspecific embryos rescued by tissue cultures. Unfortunately only one paper reports obtaining adventitious shoots from pieces of leaves of P. vulgaris (Crocomo et al., 1978); somatic embryos were obtained in the same species by Allavena and Rossetti (1983). In the last two cases no mature plants were recovered.

A project aimed to find a protocol for plant regeneration via organogenesis or somatic embryogenensis in P. vulgaris, which is the most cultivated species of the genus, was started at our laboratory in 1980. The effect of some components of the tissue culture medium and of the culture conditions on many common bean genotypes and on different sorts of explants were evaluated without significant results.

A wider range of genetic variability, including those related to the morphogenetic potential, is usually present in less domesticated, wild, weedy and perennial species and in the progenies of interspecific hybrids. Because it is genetically related to P. vulgaris, P. coccineus was the first material chosen for our in vitro experiments aimed to find genotypes able to regenerate; furthermore some of its cvs are potentially perennial and it has not undergone intense domestication by man.

Pods of the cv "Bianco di Spagna" containing immature seeds (less than 1 cm wide) were collected from field grown plants. After surface sterilization, with 1% NaOCl for 25 minutes, the seeds were aseptically removed; the cotyledons were excised from the embryo and cultured in 10 cm wide petri dishes containing 10 ml Murashige and Skoog (1962) basal medium (BM) plus one of the following growth factors: IAA (100 mg/l); NAA (100 mg/l); 2,4D (100 mg/l); NOA (100 mg/l); KINETIN (100 mg/l); BA (100 mg/l); GA_3 (100 mg/l); ABA (100 mg/l); TIBA (100 mg/l); SPERMIDINE (145 mg/l); SPERMINE (202 mg/l). After 48 hours of culture the cotyledons were recultured in 120 ml glass-jars containing 15 ml BM alone or with the addition of BA (1mg/l)or BA(1mg/l) and IAA (0.5 mg/l). The pH of the media was adjusted to 5.6 before autoclaving for 15' at 121°C; ABA and GA_3 were filter sterilized and added to the autoclaved medium. Cultures were kept in a growth chamber at 24±0.5°C; an illumination of 1500 lux was provided at the culture level by Gro-lux lamps on a 16 hours photo-period.

Cotyledons like structures, leaves, callus and shoots rose from the two cotyledons of the same seed cultured on the medium containing IAA and BA after the 24 hours treatment on medium containing ABA. No shoots at all were produced by the cotyledons treated with the other sequences of media.and the other cotyledons

Because the shoots were born in the distal part of the cotyledons, were preexisting buds were normally not present, they were of adventive origin.

Excised adventitious shoots were able to grow and to root on BM. These plantlets were micropropagate from single node cuttings on BM alone or with the addition of BA (1 mg/l). On BM alone 100% node cutting also rooted and complete plantlets were obtained. These were then transferred to pots of soil and are now growing to maturity.

The experiment will be repeated with immature embryos and other somatic tissue wich will be obtained from regenerated plants. If the new experiment succeeds we aim to transfer, by crossing, the regeneration potentiality into P. vulgaris.

References

- Allavena A., L. Rossetti (1983) Acta Horticulturae 131: 239-246
- Crocomo O.J., W.R. Sharp and J.E. Peters (1976) Z. Pflanzenphysiol. 78: 456-460
- Murashige T., F. Skoog (1962) Physiol. Plant 15: 473-497.

Appreciation is expressed to the Italian Council of Research (CNR-IPRA) for the financial assistance.