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MORPHOGENIC POTENTIAL OF FLAX (*LINUM USITATISSIMUM*) EMBRYOS *IN VITRO*

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Flax (*Linum usitatissimum* L.), one of the major oil seed crop, is extensively grown in the Northern hemisphere,and is well-known for its use in industry & medicine. The information on tissue culture of *L. usitatissimum* is limited and the explants used have been the hypocotyl (Crooks, 1933; Link and Eggers, 1946; Gamberg and Shyluk, 1976), cotyledons (Rybozynski, 1975), stem segments (Murray *et al.*, 1977; Chlyah *et al.*, 1980), and radicle, pedicel and calyx (Singh and Govil, 1982). The present investigation deals with the morphogenic potential of zygotic embryos *in vitro*. Direct plantlet formation, callus mediated multiple shoot induction, and whole-plant regeneration were achieved from the embryos cultured on MS (Murashige and Skoog, 1962) medium supplemented with various growth regulators and adjuvants.

The seeds of *L. usitatissimum* were soaked in distilled water for 12 hours, and surface sterilized with 0.1% mercuric chloride solution for 5 minutes followed by repeated washings with sterilized distilled water. The excised embryos from the seeds were inoculated on MS fortified with BAP (6-Benzyl amino purine), KN (Kinetin) NAA (Naphthalene acetic acid), CM (Coconut milk), YE (Yeast extract), and CH (Casein hydrolysate) in various combinations and concentrations. The cultures were maintained at 25±2°C with 12 hours diffuse light at about 1000 lux. For each treatment 36 cultures were raised, and all the experiments were repeated twice.

The embryos on medium without growth regulators produed normal plantlets (Fig. 1 A), whereas in the medium containing BAP 1 mg/l+YE 5% only callus developed all over the surface (not shown in figure). Callusing and subsequent differentiation of multiple plantlets was observed in the medium fortified with KN 1 mg/l +CM 20% (Fig. 1B); the callus was cream coloured & nodular. After three weeks it produced green protuberances on the surface and these differentiated into shoot buds after another four weeks. The shoot buds developed into plantlets upon subculture on MS + NAA 0.5 mg/l and attained a size of 5-10 cm after six weeks (Fig. 1C). On MS+KN 1 mg/l +YE 5% the excised embryos produced callus and then

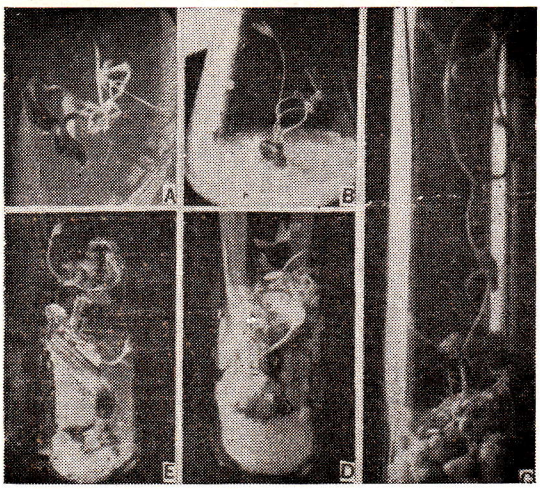

Fig 1

A—Plantlet foramation from excised embryos MS medium; B—Callogenesis and multiple plantlet induction from excised embryo on MS+KN 1mg/l+CM 20%; C—Rhizogenesis of shoots on MS + NAA 0.5mg/l; D—Callus mediated multiple plantlet regeneration from embryo on Ms+KN 1mg/l+YE 5%; E—Flat hypocotyl showing adventitious shoot buds and shoots

multiple plantlets without subculture (Fig. 1D). Multiple plant regeneration was maximum (ca 85%) on KN 1 mg/l+YE 5%, ollowed by multiple shoot induction (ca 60%) on KN 1mg/l+CM 20%. Profuse rhizogenesis was also observed from the embryos as well as callus derived from them in the medium fortified with NAA 1mg/l+CH 10%. The roots, however, were negatively or positively geotropic (with root pockets, a feature characteristic of aquatic plants). In some combinations of growth regulators, the hypocotyl of the regenerated plant was stout and flat and produced adventitious shoot buds and shoots on its surface (Fig. 1E).

Our results illustrate the potential of zygotic embryos of flax to produce multiple plantlets by manipulation of KN, YE and CM levels in the medium. Induction of plantlets is due to the synergism of YE and KN; BAP and IAA were ineffective and produced only callus.

This technique can be utilised for the production of variable plantlets and, hence, in the utilisation of hybridisation programme, and even for the production of salt-resistant plants (Mc Hughen and Swartz, 1984).

This protocol may enable the plant breeders in raising interspecific and intergeneric crosses for generating variability, salt-resistance, high-yield, and rich-oil-content.

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