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PRODUCTION OF PLANTLETS OF *ERUCA SATIVA IN VITRO*

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Cotyledonary nodes from two week old sterile seedlings of *Eruca sativa* were cultured on Murashige and Skoog's (MS) medium supplemented with different combinations of auxins and cytokinins. Multiple shoots were formed on BAP–IAA containing medium. The orientation of the explants influenced the type of response obtained. Shoots formed roots on medium containing low levels of cytokinins in combination with auxins. Flower buds, bearing normal corolla, were also induced in the plantlets.

Keywords : Micropropagation; *Eruca sativa; in vitro*

Introduction

Tissue culture technique is being used increasingly for crop improvement and clonal multiplication in the family *Brassicaceae* (Flick *et al.*, 1983). Although there are many reports of plant regeneration, *in vitro*, in this family, most of this work has been conducted on the genus *Brassica*, which includes the major group of oilseeds i.e. rapes and mustards (Bhattacharya and Sen, 1980; George and Rao, 1983; Klima Szewska and Keller, 1985; Maheshwaran and Williams, 1986 and Singh and Chandra, 1984a and b). *Eruca sativa* is another oilseed crop related to rape and mustard. Oil extracted from seeds is used in pickles, as a lubricant and also for illuminating purposes. The young plant is used as salad and vegetable. However, the cultivation of this crop is restricted to poor soil and therefore yield is very low. The present communication describes an ideal method to produce a large number of plants of *Eruca sativa* in a short period of time through a pathway in which there is no loss of morphogenetic potential.

Materials and Methods

Seeds of *Eruca sativa* were obtained from Agriculture Research Station, Durgapura (Jaipur). The seeds were surface sterilized with 0.1 percent $HgCl_2$ solution for 5 minutes. After repeated washings with sterilized distilled water, the seeds were cultured on hormone free half strengh MS nutrient medium (Murashige and Skoog, 1962) for germination. Coty-

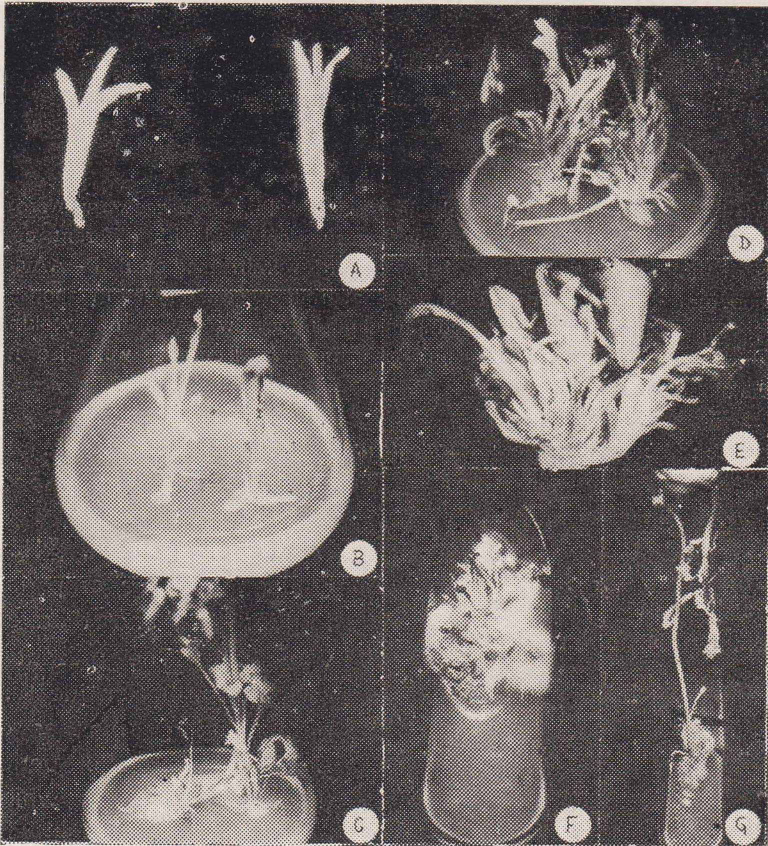

Fig. 1 Responses of cotyledonary node segment of *Eruca sativa*

A. Explant; B. Elongation of a single shoot at two weeks on MS + BAP (0.05 mgl^{-1}) + NAA (0.05 mgl^{-1}); C. Callusing and elongation of single shoot on MS + BAP (1.0 mgl^{-1}) + IAA (0 5 mgl^{-1}) when explant is placed horizontally; D,E. Multiple shoots from vertically placed explants on MS + BAP (1.0 mgl^{-1}) + IAA (0.5 mgl^{-1}); F. Rooting from isolated shoots on MS + NAA (1 0 mgl^{-1}(; G. Rooting, shoot elongation and flower bud induction on MS + kinetin (0.05 mgl^{-1}) + IBA (1.0 mgl^{-1}).

ledonary nodes of *Eruca sativa* excised from two week old asptically grown seedlings were cultured on MS medium, supplemented with various combinations of auxins and cytokinins. Cotyledons and apex were removed before culturing. pH of the medium was adjusted to 5.8 and it was solidified with 0.8 percent agar agar (BDH). All cultures were incubated under continuous fluorescent light at 26±2°C and 50 to 55 percent relative humidity. Observations were recorded four weeks after culture. For each experiment twenty replicates were used and all experiments were repeated twice.

Results and Discussion

Culture of cotyledonary nodes—cotyledonary nodes from which Cotyledons and apex were removed (Fig. 1A) showed better shoot proliferation response on concentrations of IAA in combination with kinetin/BAP as compared to kinetin/BAP and NAA combinations. Kinetin (0.05, 0.5 and 1.0 mg/l) induced restricted elongation of a single shoot and a dense mat of thin white roots. Callus was induced from the cut end on MS medium supplemented with 1.0 mg/l each of kinetin and NAA. Similar results were obtained on kinetin (0.05, 0.5 and 1.0 mg/l) and IAA (0.05, 0.5 and 1.0 mg/l) supplemented media. Shoot and root induction was accompanied by moderate callus initiation. BAP (0.05 and 0.5 mg/l) in combination with auxins (IAA and NAA 0.05, 0.5 and 1.0 mg/l) evoked the elongation of a single shoot and the induction of few short roots (Fig. 1B). Shoot clusters were formed when explants were placed on media containing BAP (1 0 mg/l) and IAA (0.05, 0.5 and 1.0 mg/l). The hypocotylar end inside the medium gave rise to a knob-like compact callus mass (Fig. 1E). Optimum proliferation occurred on BAP (1.0 mg/l) and IAA (0 5 mg/l) supplemented media. Higher concentrations of NAA (0.5 and 1.0 mg/l), in combination with 1.0 mg/l of BAP induced callus and thick roots along with the elongation of a single shoot.

The effect of orientation of the explant was also studied. Cotyledonary nodes were placed horizontally as well as vertically on MS medium containing BAP (1.0mg/l) and IAA (0.5 mg/l). In horizontally placed explants, callusing was observed at the cut end and 2-3 shoots were formed (Fig. 1C) while vertically placed explants gave rise to shoot clusters and callus from the base of the explant which was embedded inside the medium (Fig. 1D, E) These shoots could be maintained by subculturing on the same medium.

Rooting of Shoots—Shoot clusters obtained by the proliferation of the cotyledonary node were separated

into single shoots from the base by making a cut with a sharp scalpel. Single shoots were transferred to MS medium supplemented with different levels of IAA, IBA or NAA (1.0, 3.0, 5.0 mg/l). Combinations of auxins (1.0 mg/l) and low cytokinin concentrations (kinetin or BAP 0.05 mg/l) were also tried. On hormone-free basal MS medium and half strength MS medium rhizogenesis was not observed, leaves turned yellow and the shoots eventually became necrotic.

IAA (1.0, 3.0 and 5.0 mg/l) did not induce good rooting in the shoots. However, IBA (3.0 and 5.0 mg/l) evoked a better response. Roots formed were long, white, hairy and thin. NAA (1.0, 3.0 and 5 0 mg/l induced the formation of very short roots (Fig. 1F). IBA (1.0 mg/l) was unable to induce rooting in any of the shoots even in six weeks time. Combinations of kinetin/BAP (0.05 mg/l) and IAA/NAA (1.0 mg/l) induced very few short roots. However, kinetin/BAP and IBA combinations evoked a good rhizogenic response in 80 percent of the shoots (Fig. 1G). Flower buds bearing normal corolla, were also seen in the plantlets (Fig. 1 G).

The present study demonstrates that cotyledonary node culture could be used for the *in vitro* propagation of *Eruca sativa*. Meristematic tissues or organs have proved to be ideal material for clonal propagation and conservation of germplasm (Hu and Wang, 1983). During the present studies also totipotentiality of cotyledonary nodes of *Eruca sativa* was observed by repeated and constant proliferation into shoot clusters thus being ideal for clonal propagation. Similarly axillary buds have been used for shoot multiplication in *Brassica campestris* (Kuo and Tsay 1977) and *Brassica oleracea* var *capitata* (Mascarenhas *et al.*, 1978). In *Eruca* optimal results were obtained on BAP (1 0 mg/l) in combination with IAA (0.5 mg/l) when nodes were placed vertically. Horizontally placed explants gave rise to 2–3 shoots which elongated but did not proliferate further. However, a knob like callus mass was observed at the lower cut end. The orientation of the explant in culture is the most important factor influencing organogenesis and callus formation since gradients are known to exist along the length of plants (Huges, 1981). We conclude that the placement of the explant and the quantity of hormone, are the chief factors governing organogensis *in vitro*.

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