of Science and Technology, Lucknow, for financial support.

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1. Chattopadhyay, S., Chattopadhyay, U., Shukla, S. P. and Ghosal, S., *Pharm. Res.*, 1984, **61**, 237.
2. Chaudhuri, R. K. and Ghosal, S., *Phytochemistry*, 1971, **10**, 2425.
3. Ghosal, S., Biswas, K. and Chattopadhyay, B. K., *Phytochemistry*, 1978, **17**, 689.
4. Ghosal, S., Srivastava, A. K., Srivastava, R. S., Chattopadhyay, S. and Maitra, M., *Planta Med.*, 1981, **42**, 279.
5. Farns Worth, N. R., Bingel, A. S., Cordell, G. A., Crane, F. A. and Fong, H. H. S., *J. Pharm. Sci.*, 1975, **64**, 535.
6. Kihlman, P. P., *Adv. Cell. Mol. Biol.*, 1971, **1**, 59.

CALLUS INITIATION AND REGENERATION POTENTIAL IN DIFFERENT GENOTYPES OF *ERUCA SATIVA*

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ERUCA SATIVA Mill. 'taramira' ($n=11$, genome formula–EE) is highly resistant to aphids (*Lipaphis pseudobrassicae*) and thrives well under both rainfed and drought conditions[1]. Besides, its oil is rich in erucic acid[2], an industrially useful compound. Direct utilization of *E. sativa* in a breeding programme has been limited owing to the presence of strong incompatibility barriers with other *Brassica* species. Attempts are now being made to use *in vitro* techniques for the genetic amelioration of this crop species[3,4]. The present study was undertaken to identify genotypes responsive to *in vitro* culture and to develop regeneration protocols from cotyledonary explants of *E. sativa*.

Seeds of *E. sativa* cvs. T-27, TMH-46 and TMH-48 were surface-sterilized for 20 sec with 90% ethanol and for 7 min with $HgCl_2$ solution, washed thoroughly in sterilized distilled water, and germinated on agar (0.8%)/sucrose (2.0%) medium under a light and dark cycle of 16/8 h at 25 ± 1°C. After 7–8 days, the cotyledonary leaves were excised and cultured on MS[5] medium supplemented with cytokinins and/or auxins (table 1). About 100 cotyledons were cultured per medium/genotype tested. The number of explants regenerating shoots and the number of shoots per regenerating explant were recorded after 30 days of culture. All the cultures were kept under continuous fluorescent light of 5000 lux intensity at 25 ± 1°C.

Cotyledonary explants enlarged significantly within a week of culture and exhibited three kinds of response: callus, root formation and shoot formation (table 1). Callusing was observed at the cut ends of the explants in all the cultivars and on all the media, although with different efficiencies. Callus proliferation could be increased by causing multiple injuries

Table 1 *Effect of different media on callus induction and root and shoot regeneration in cotyledonary explants of different cultivars of Eruca sativa*

Medium	Cultivar	Cotyledons (%) forming calli	roots	shoots
MS + 0.05 mg/l NAA	T-27	64.6	89.6	2.1 (0 1)*
	TMH-46	57.8	85.6	1.1 (0 1)
	TMH-48	60.0	90.8	1.5 (0 1)
MS + 0.5 mg/l NAA + 2 mg/l BAP	T-27	87.1	77.0	20.7 (0 10)
	TMH-46	90.0	84.5	15.6 (0 10)
	TMH-48	93.3	85.0	8.3 (0 5)
MS + 0.2 mg/l NAA + 2.0 mg/l kinetin	T-27	95.8	91.5	15.3 (0 10)
	TMH-46	93.1	85.4	10.4 (0 5)
	TMH-48	91.4	81.4	12.9 (0 6)
MS + 2 mg/l NAA + 10 mg/l kinetin	T-27	3.9	11.8	1.0 (0 1)
	TMH-46	10.7	14.6	1.0 (0 1)
	TMH-48	33.4	36.2	1.0 (0 1)

*Number of shoots per regenerating explant.

to the cotyledonary leaves. Callus initiation and its further growth was maximum on MS medium supplemented with either kinetin (2 mg/l) or benzylaminopurine (BAP, 2 mg/l) and a low concentration of naphthaleneacetic acid (NAA). Kinetin and NAA at higher concentration caused little or no callus formation. NAA alone could support moderate callus growth only.

Winthin 2–3 weeks, the callus started differentiating into nodular structures and formed shoot buds, which subsequently gave rise to multiple shoots. Frequency of cotyledons responding to plant regeneration depended upon the plant genotype and the hormonal constitution of the medium (table 1). MS medium supplemented with BAP and NAA was most effective and gave highest frequency of shoot regeneration. The frequency of shoot regeneration declined when BAP was substituted with kinetin. Media containing kinetin and NAA together at high concentration or NAA alone gave poor regeneration frequencies.

When 10–15-day-old regenerated shoots were separated and subcultured individually onto fresh medium, multiple shoots (1–25) could be seen regenerating from the callus formed at the base of the transferred shoot. In one case as many as 98 multiple shoots could be obtained from a single cotyledon explant in two subcultures.

There were significant differences in the shoot regeneration response of the three cultivars of *E. sativa*. The best regeneration was observed in cv. T-27, which gave an average of 9.8% explant cultures with shoots. TMH-46 and TMH-48 gave 7% and 5.9% explant cultures with shoots respectively.

Most of the regenerated shoots developed roots in the parent medium. The remaining shoots could be easily rooted when cultured on MS medium supplemented with NAA, after giving a cut at the base.

The study showed that genotype of the plant and hormonal constitution of the medium determine the shoot regeneration response of explant tissue in *E. sativa*. A proper balance of cytokinin and auxin is necessary to achieve high regeneration frequencies. The procedure and the mode of plant regeneration are similar to that reported for other *Brassica* species[6,7]. By using such techniques it may be possible to select genetically modified *E. sativa* through *in vitro* culture and to develop agronomically improved strains of this crop.

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1. Prakash, S., In: *Brassica Crops and Wild Allies*, (eds) S. Tsunoda, K. Hinata and C. Gomez-Campo, Japan Scientific Societies Press, Tokyo, 1980, p. 152.
2. Kumar, P. R. and Tsunoda, S., In: *Brassica Crops and Wild Allies*, (eds.) S. Tsunoda, K. Hinata and C. Gomez-Campo, Japan Scientific Societies Press, Tokyo, 1980, p. 235.
3. Ahloowalia, B. S., *Crop Sci.*, 1987, **27**, 813.
4. Sikdar, S. R., Chatterjee, G., Das, S. and Sen, S. K., *Plant Cell Rep.*, 1987, **6**, 486.
5. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.
6. Narasimhulu, S. B. and Chopra, V. L., *Plant Cell Rep.*, 1988, **7**, 104
7. Jain, R. K., Chowdhury, J. B., Sharma, D. R. and Friedt, W., *Plant Cell Tissue Organ Culture*, 1988, **14**, 197.

THIN-LAYER CHROMATOGRAPHY ON CELLULOSE

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THIN-LAYER chromatography (TLC)[1,2] is a rapid, simple and versatile method for separation of mixtures of phytochemicals because of its speed and sensitivity as well as the availability of a variety of adsorbents like silica gel, alumina, celite, calcium hydroxide, magnesium phosphate, polyamide, Sephadex, polyvinylpyrrolidone and cellulose, or a mixture of two or more of them.

Ready-to-use TLC plates are not yet common in Indian laboratories, especially plates coated with silica gel, cellulose and polyamide. The preparation of TLC plates coated with silica gel[3] is comparatively easy but their preservation is difficult; on the other hand the preparation of cellulose plates[4] suited for a specific purpose is difficult though the prepared plates are comparatively easily preserved and handled. Under these circumstances and in view of the fact that cellulose TLC shows much higher resolution and speed than PC, it was decided to develop and test cellulose plates for qualitative and preparative analysis. The present study relates to the preparation of cellulose TLC plates and examination