The establishment of high frequency somatic embryogenesis and plant regeneration system of *Eruca sativa* Mill

ZHANG Tao[1,2]

[1]*College of Life Sciences, ChongQing Normal University, ChongQing, 400047, China*
[2]*Gansu Academy of Agricultural Science, Lanzhou, 730070, China Email: zhto2@st.lzu.edu.cn*

Abstract

A protocol was developed for high frequency somatic embryogenesis and plant regeneration of *E. sativa.* Cotyledon explants grown on MS medium supplemented with 1.0mg/L 2,4-D formed embryogenic callus after 4 weeks culture. Secondary somatic embryos were also produced from that on MS medium containing 0.2mg/L2, 4-D. Somatic embryos differentiated into mature embryos on N_6 medium. Mature somatic embryos developed into plantlets by culturing the mature somatic embryos on 1/2MS medium containing 0.1mg/L IBA.

Key words: *Eruca sativa*; Somatic embryogenesis; Plant regeneration

Introduction

Eruca sativa Mill. is a minor crop of the Brassicaceae family. The leaves are used as parts of mixed salads and for herbal remedies (Yaniv etal., 1998). The seed is a source of 4-methylthiobutyl glucosinolates (Iori et al., 1999), which is believed to be beneficial to human health (Mithen et al., 2000). The seed oil rich in erucic acid, a long chain (C22) fatty acid, is exclusively used as industrial lubricant (Kumar and Tsunoda, 1980). This plant is also resistant to drought, mustard aphid and the stem canker pathogen (Tewari et al., 1995). Because of these characteristics, *E. sativa* has been considered as a promising genetic resource for improvement of other crucifers, and also used as a model plant for study of long chain (C22) fatty acid metabolism, glucosinolates biosynthesis and stress resistance. To use *E. sativa* for study leading to crucifer improvement, it is essential to establish an efficient tissue culture system for high frequency plant regeneration.

Somatic embryogenesis has been considered as an effective pathway of plant regeneration and foreign gene introduction because of its highly regenerative potential and lower risk of chimeric mutations. The work reported in this study describes a process of high frequency somatic embryogenesis and plant regeneration from cotyledon explants of *E. sativa.*

Materials And Methods

Plant materials and culture conditions. Seeds of *E. sativa* cv. Qing Cheng, Ling Tao, Jing Ning and Tian Shui were surface-disinfected in 70% ethanol for 30s and 0.1% (w/v) $HgCl_2$ for 10min, then washed 3-4 times with sterile distilled water, and germinated on MS medium (Murashige and Skoog, 1962) supplemented with 2% (w/v) sucrose, and solidified with 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. All the cultures were incubated at 25±2°C with 16-h photoperiod under fluorescent light with the light intensities of 20 μmol $m^{-2}s^{-1}$.

Induction of embryogenic callus. The cotyledons explants from five-day-old seedlings were cut into 4–6mm^2 segments and cultured on MS medium supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-butyric acid (IBA), indoleacetic acid (IAA), α-naphthaleneacetic acid (NAA) and benzyladenine (BA). Each treatment consisted of 30 explants and repeated three times. MS medium without hormones was used as control. After 4 wk of culture, the frequencies of somatic embryogenesis were calculated.

Maintenance of embryogenic callus. After 4 wk, embryogenic callus initiated on the induction medium was subcultured on MS medium containing 0.2mg/L 2,4-D for proliferation. Callus was subcultured at 3-wk intervals.

Maturation of somatic embryos. Embryogenic callus grown on the maintenance medium was transferred to N6 medium for maturation. Each treatment consisted of five replications and was repeated twice. After 4 wk, the mature somatic embryos were desiccated by placing in non-sealed Petri dishes in the laminar air flow for 48 h. After desiccation, embryos were transferred to hormone-free MS medium for germination.

Plant regeneration. The germinated embryos were transferred to 1/2MS medium containing 0.1mg/L IBA for plant regeneration. After 4 wk, the regenerated plantlets were removed from the medium, washed with tap water to remove the agar sticking to roots, and transplanted to autoclaved vermiculite in pots. After 2 wk, the plantlets were transplanted to garden soil in large pots and acclimated for 2 wk before transferring to outdoors for further plant development.

Results And Discussion

After cultured on MS medium with 2,4-D for 2 wk, the cotyledon explants from *E. sativa* expanded 2-3 fold in size and some embryogenic callus formed at the cut surfaces (Fig. 1A). The embryogenic callus developed quickly and covered the whole explants after 4–6 wk, the callus was light, yellowish and compact, forming somatic embryos at the globular stage (Fig. 1B). As shown in Table 1, the frequencies of somatic embryogenesis from cotyledon explants ranged from 34.4% to 85.3%,

respectively depending on the concentration of 2,4-D and genotypes of *E. sativa*. The best results were observed on 1.0mg/L 2,4-D, at which 85.3% of cotyledon explants from Qing Cheng gave rise to embryogenic callus. Embryogenic callus formation gradually reduced or inhibited when the 2,4-D concentrations was lower or higher than 1.0mg/L. On hormone-free MS medium, cotyledons and hypocotyl explants show no response after 4–6 wk of culture.

Table 1 Effects of 2,4-D on somatic embryogenesis from cotyledon explants of *E.sativa* .

2,4-D (mg/L)	Frequency of Somatic embryogenesis (%)			
	Qing Cheng	Ling Tao	Jing Ning	Tian Shui
0.2	44.5±2.2[a]	38.2±1.6	36.8±2.0	34.4±1.6
0.5	68.7±2.8	59.6±2.5	58.5±2.5	56.2±2.4
1.0	85.3±3.1	72.0±3.6	70.4±3.1	68.5±3.3
2.0	72.4±2.6	56.1±3.1	49.0±2.3	47.0±2.7

[a]Means of three replications ± S.E, each replicate contains 30 explants.

It has been reported that NAA and BA are beneficial for somatic embryogenesis from zygotic embryos of *E. sativa* (Ahloowalia, 1987). However, both growth regulators were not effective in this study. This may be due to the use of different genotypes and explants. IAA and IBA alone was no effective on somatic embryogenesis, but they induced adventitious root formation at high frequencies (65-100%). BA neither induced somatic embryogenesis nor adventitious root formation.

On MS medium containing 0.2mg/L 2,4-D, secondary embryos could be produced profusely within 10d of culture, but the development of somatic embryos was arrested at the globular stages (Fig. 1C), indicating that 2,4-D was effective for somatic embryogenesis but inhibitory for further embryo development. On N6 medium, somatic embryos at various stages could be observed in a single somatic embryos cluster because of asynchronous development (Fig. 1D). The positive effects of desiccation on embryo germination have been observed in many plant species, including rapeseed (Kott and Beversdorf, 1990) and grape (Gray, 1989). In this study, we observed that desiccation was useful for promoting somatic embryos germination and conversion, compared to embryos that cultured on maturation medium but not subjected to desiccation showed low rate of germination and conversion (data not presented). Desiccation improved embryo germination after rehydration, as a result, more than 70% embryos survived and underwent further shoot development.

Most of the mature somatic embryos converted into normal plantlets after transferred to 1/2MS medium with 0.1mg/L IBA. After transplanted to pots with autoclaved vermiculite and then with garden soil in greenhouse for one month, 75% of the regenerated plantlets survived and grew well. The plants were indistinguishable with the plantlets from seeds in morphology, and they flowered and set fruits after transferred to outdoors.

In conclusion, we have developed a system for plant regeneration from cotyledons explants of *E. sativa* via somatic embryogenesis. This protocol is simple and will be useful for studying the mechanism of long chain (C22) fatty acid metabolism and glucosinolates biosynthesis. It can also be used for plant breeding and genetic studies.

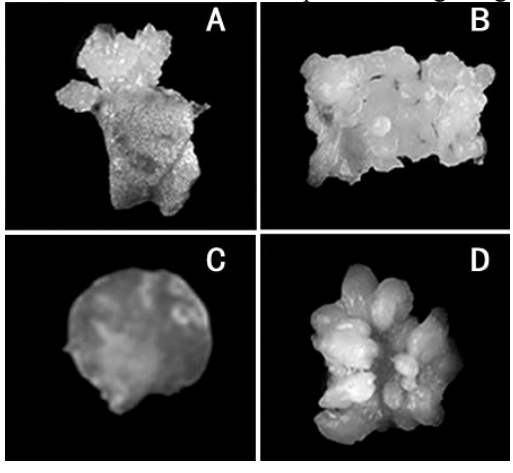

Fig.1 Somatic embryogenesis of *E. sativa*

A, Cotyledon explant cultured on MS medium with 1.0mg/L 2, 4-D for 2 wk; B, Somatic embryos cultured on MS medium with 1.0mg/L 2, 4-D for 4 wk; C, Globular stage somatic embryos on MS medium with 0.2mg/L 2, 4-D; D, Numerous somatic embryos at various stages on N6 medium.

Acknowledgements

This work was supported by Natural Science Foundation Project of CQ CSTC(2006BB1262) and ChongQing Normal University (06XLB014).

References

Ahloowalia, B.S. Somatic embryogenesis and plant regeneration in *Eruca sativa*. Crop Sci. 27: 813-814; 1987.

Gray, D.J. Effect of dehydration and exogenous growth regulators on dormancy, quiescence and germination of grape somatic embryos. In Vitro Cell. Dev. Biol. 25: 1173–1178; 1989.

Iori, R.; Bernadi, R.; Gueyrard, D.; Rollin, P.; Palmieri, S. Formation of glucoraphanin by chemoselective oxidation of natural glucoerucin: a chemoenzymatic route to sulforaphane. Biorg. Med. Chem. Lett. 9: 1047–1048; 1999.

Kott, L.S.; Beversdorf, W.D. Enhanced plant regulation from microspore-derived embryos of *Brassica napus* by chilling partial desiccation and age selection. Plant Cell Tiss. Organ Cult. 23:187–192; 1990.

Kumar, P.R.; Tsunoda, S. Variation in oil content and fatty acid composition among seeds from Cruciferae. In: Tsunoda S et al. (ed.) Brassica crops and wild allies. Tokyo: Japan Scientific Societies Press; 1980: 235-252.

Mithen, R.F.; Dekker, M.; Verkerk, R.; Rabot, S.; Johnson, I.T. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. J. Sci. Food Agric. 80: 967–984; 2000.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497; 1962.

Tewari, J.P.; Bansal, V.K.; Stringam, G.R.; Thiagarajah, M.R. Reaction of some wild and cultivated *Eruca* accessions against *Leptosphaeria maculans*. Can. J. Plant Pathol. 17:362-363; 1995.

Yaniv, Z.; Schaerman, D.; Amar, Z. Tradition, uses and biodiversity of rocket (*Eruca sativa*, Brassicaceae) in Israel. Econ. Bot. 52: 394–400; 1998.