http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (4): 729-734, 2006
ISSN 1028-8880

Somatic Embryogenesis and Plant Regeneration in *Brassica napus* L.

[1]A. Majd, [1]F. Chamandoosti, [1]S. Mehrabia and [2]M. Sheidai
[1]Department of Biology, Faculty of Science, Teacher Training University of Tehran
No. 49, Mofatteh Ave, Tehran Iran
[2]Department of Biology, Shahid Beheshti University, Tehran, Iran

Abstract: In this study a simple and repeatable method for somatic embryogenesis from hypocotyls of *Brassica napus* L. is described. Out of eighty different treatments included of different kinds and concentrations of Plant Growth Regulators (PGRs), different amount of sucrose and different explants were tested. Results of experiments showed that hypocotyls explants were more useful for somatic embryogenesis rather than other explants. The sucrose at 20 g L^{-1} concentration was more inducer for somatic embryogenesis than the sucrose at higher concentrations (for example 60 g L^{-1} concentration). On MS medium containing 20 g L^{-1} sucrose, 1 mg L^{-1} 2,4-D, 2 mg L^{-1} NAA, 2 mg L^{-1} BAP and 150 mM L^{-1} NaCl, explants produced somatic embryos. The somatic embryos germinated on medium that had the same formulation as above and produced shoots. Few shoots rooted but transfer of them on medium with 1 mg L^{-1} IBA caused to increasing root production. After adaptation to dry conditions regenerated of plantlets via somatic embryogenesis were successfully transferred to pots containing a mixture of soil and vermiculite.

Key words: *Brassica napus* L., somatic embryogenesis

INTRODUCTION

Somatic embryogenesis is a method in Biotechnology that in pathway of it plant cells, tissues and organs produce embryos *in vitro* conditions. A somatic embryo resembles a zygotic embryo with a view to general process of embryogenesis (globular, heart and torpedo stages, germination and production of new plantlet) but they developed via a different pathway (fecundation between sperm and oospher dose not occurred) (Von Arnold *et al*., 2002). Study about somatic embryogenesis is very important because having scientific and applied aspects. Somatic embryos are used as a model system in embryological studies. During embryogenesis large number of genes expressed with powerful regulations. This powerful regulations occur also in somatic embryogenesis. The greatest importance of somatic embryogenesis is application of it in large scale vegetative propagation (Von Arnold *et al*., 2002).

Brassica napus L. is a member of Brassicaceae family. It is one of the important oilseed in Iran, due to having edible and agricultural desirable specifications. Therefore cultivation of this important and economic crop has been increased in recent decade in Iran.

In this study we report a simple and economic method for somatic embryogenesis in *Brassica napus* L. because somatic embryogenesis is favoured over other methods of vegetative propagation because of the possibility to scale up the propagation by using bioreactors (Von Arnold *et al*., 2002). Besides induced variation is one of the method for crop improvement. One of the source of induced variation is somaclonal variation (Jaligot *et al*., 2000; Vendrame *et al*., 1999; Donovan *et al*., 1994). It is noticeable that there is few report about using of somaclonal variation for production of new lines in Brassicaceae member (Sacristan, 1982) and this subject must be studied more.

MATERIALS AND METHODS

This study conducted in developmental Biology laboratory in Faculty of Science, Teacher Training University, Tehran, Iran in November 2004-October 2005.

Preparation of explants: Seeds of *Brassica napus* L. were obtained from oilseed research and development Company, Iran. They were surfaced sterilized in commercial Sodium hypochlorite solution (5% available chlorine) for 8 min and washed thoroughly with sterile distilled water for 6-7 times under asceptic conditions and then cultured on half strength MS (Mourashige and Skoog, 1962) medium (basal salts) with 1% sucrose and 1% agar agar. The pH of medium was adjusted to 5.8 before adding agar agar and before

Corresponding Author: F. Chamandoosti, Department of Biology, Faculty of Science,
Teacher Training University of Tehran, No. 49, Mofatteh Ave, Tehran Iran

autoclaving at 121°C for 20 min. The cultures were grown under 16 h photoperiod at 200 $\mu mol^{-2}s^{-1}$. Hypocotyls (5-8), cotyledons (2-3) and roots (3-4) mm segments in length were excised from 7 days old aseptically grown green seedlings were and then were used for preparation of explants.

Preparation of media for Induction, maturation and germination of somatic embryos: MS medium plus 2% sucrose and 1% agar agar that supplemented with auxins (NAA, 2,4-D and IBA) cytokinins (BAP) and NaCl for induction somatic embryoid, maturation and development of them to plantlet were used (Table 1). MS medium free from PGRs and NaCl was also used for control. All of media were adjusted to pH 5.8 prior autoclaving at 120°C for 20 min. All cultures incubated at 24±2°C in a incubator under dark condition for induction and maturation of embryoid and 200 $\mu mol^{-2}s^{-1}$ for germination of somatic embryos and development of them to plantlets.

Transferring of plantlets to soil: Whole plantlets were washed and potted in sterile perlit. Plantlets were covered with a transparent plastic glass to maintain 90±0.05 relative humidity for 5 days and then this cover gradually perforated for adaptation of plantlets to dry conditions. Then plantlets were transferred to soil.

Statistical analysis: Experiments were set up in completely randomized design and repeated 3 times. Twenty four treatment has 4 replications. Observations on the number of somatic embryos per explants were recorded. Data were subjected to SD and ANOVA test.

RESULTS AND DISCUSSION

Callus and somatic embryo formation: After 3 weeks of culture hypocotyls explants produced small and creamy calli and after this stage (several days later) somatic embryos was formed. At first callus and somatic embryo induction did not affected by different concentration of media. Growth of calli was little and somatic embryos formed in them while diameter of them was 1-2 mm.

Effect of sucrose on callus and somatic embryo formation: Callus growth and somatic embryogenesis affected with different amount of sucrose. By increasing amount of sucrose a negative effect were seen in growth of callus and somatic embryogenesis. So that in 2% sucrose growth of callus and somatic embryogenesis was the highest and in 6% sucrose growth of callus and somatic embryogenesis was the least (data were not shown). Kirti and Chopra (1989) showed that the increase in amount of sucrose caused the decrease in somatic embryogenesis in *Brassica junceae* L.

Table 1: Effect of PGRs and NaCl on somatic embryogenesis in *Brassica napus* L.

Concentrations				
2,4-D ($mM\ L^{-1}$)	NAA ($mg\ L^{-1}$)	BAP ($mg\ L^{-1}$)	NaCl ($mg\ L^{-1}$)	Average number of somatic embryos per explant
1	1	2	0	$18.5\pm12.05^{f-h}$
1	1	2	50	$26.5\pm12.05^{f-h}$
1	1	2	75	$26.75\pm12.06^{f-h}$
1	1	2	100	$49\pm12.06^{a-d}$
1	1	2	125	$42.25\pm12.06^{b-f}$
1	1	2	150	69 ± 12.06^{a}
1	1	2	175	62.5 ± 12.06^{ab}
1	1	2	200	$53.5\pm12.05^{c-g}$
1	2	2	0	$44\pm12.06^{c-f}$
1	2	2	50	$42.5\pm12.06^{c-f}$
1	2	2	75	$49.5\pm12.06c^{a-e}$
1	2	2	100	$43\pm12.06^{b-g}$
1	2	2	125	$47.25\pm12.06^{a-d}$
1	2	2	150	$43.75\pm12.06^{b-e}$
1	2	2	175	$41\pm12.05^{b-f}$
1	2	2	200	$37.75\pm12.06^{c-g}$
2	2	2	0	$24.8\pm12.06^{f-h}$
2	2	2	50	$24.5\pm12.06^{f-h}$
2	2	2	75	$31.25\pm12.06^{e-g}$
2	2	2	100	$38\pm12.06^{c-f}$
2	2	2	125	$38.75\pm12.06^{d-g}$
2	2	2	150	$33.2\pm12.06^{d-g}$
2	2	2	175	$34\pm12.06^{c-g}$
2	2	2	200	$39\pm12.05^{c-e}$
0	0	0	0	0

Basal medium contained Murashige and Skoog (1962) salts supplemented with 20 gL^{-1} sucrose, 10 gL^{-1} Agar Agar. pH was 5.8 and cultures were kept in darkness at 25°C for induction of somatic embryos. Means of four replicate with the same letters are not significantly different at $p<0.01$

Effect of NaCl on callus and somatic embryos formation: As could be seen in (Table 1) similar treatments hormonal the highest frequency of somatic embryogenesis were seen in media with high concentration of NaCl. (the highest frequency of somatic embryogenesis was occurred in medium with 150 $mM\ L^{-1}$ NaCl. So we resulted that the presence of 75-200 mM^{-1} NaCl had a positive effect in somatic embryogenesis in *Brassica napus* L. There are different reports about effect of NaCl on somatic embryogenesis in different plants. For example Kirti *et al.* (1991) noticed that the Brassicas show interspecific variability for salt tolerance. It should therefore be possible to identify introgressesd tolerance by subjecting material from wild crosses to *in vitro* selection. This researcher efforted that with increasing of NaCl to induction medium somatic embryogenesis in *Brassica juncea* L. to obtain tolerant plants to salinity but failured and resulted that with limiting of NaCl from induction medium and adding of it to germination and growth media, plantlets with better root growth, shoot growth and fresh weight accumulation were obtained. But Pellegrineschi *et al.* (2004) emphasized that optimal callus induction and plant regeneration were obtained in bread and durum wheat by manipulating the NaCl concentration in the induction medium. On the other hand Zhang *et al.*

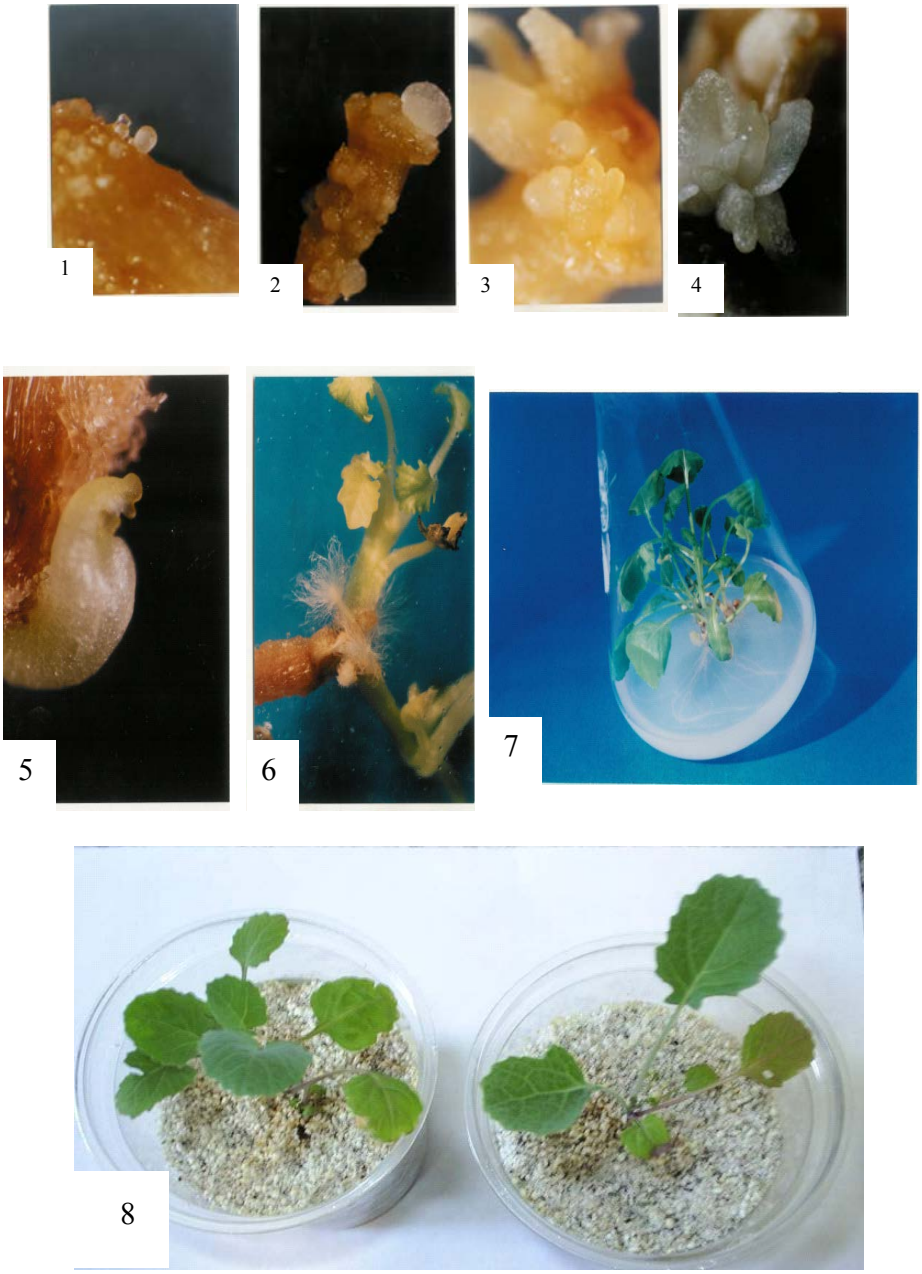

Fig. 1-8: Somatic embryogenesis and plant regeneration from hypocotyls of 7-days-old seedling in *Brassica napus* L. 1) globular embryo, 2) early heart embryo, 3) late heart embryo, 4) torpedo embryo, 5) mature or cotyledonary embryo, 6) germination of embryo on explants (Fig. 1-6 related to medium with 1 mg L^{-1} 2,4-D, 2 mg L^{-1} NAA, 2 mg L^{-1} BAP and 150 mM L^{-1} NaCl), 7) Plantlet regeneration resulting of embryos in medium with 1 mg L^{-1} IBA, 8) transfer of plantlets to soil

(2001) produced transgenic *Brassica napus* L. over expressing AtNHX1, a vacuolar Na+/ H+ antiport from *Arabidopsis thaliana*, were able to grow, flower and produced seeds in presence of 200 mM sodium chloride; Srivastava *et al*. (2004) indicated that transgenic *Brassica napus* L. plants that constitutively expressed *pea* PR10 gene are able to germinate and develop better in the presence of 75 mM NaCl when compared to control plants. It is interesting that we also produced plantlets resulting somatic embryogenesis in the same

concentration of NaCl therefore study about somaclonal variation in plantlets in this condition is very important and must be studied.

Effect of kind and hormonal balance on callus and somatic embryos formation: In present study kind and hormonal balance had a critical effect on callus and somatic embryos formation. MS medium with 1 mg L^{-1} 2,4-D, 2 mg L^{-1} NAA and 2 mg L^{-1} BAP was a good and effective medium for somatic embryogenesis in *Brassica napus* L. (Table 1). In this medium after callusing somatic embryos produced and were seen in different developmental stages (Fig. 1-5). Kirti and Chopra (1989) resulted that on MS medium supplemented with 2% sucrose and 0.25 mg L^{-1} 2,4-D, 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BAP-R the highest frequency of somatic embryogenesis produced in *Brassica juncea* L. This medium for purpose of hormonal balance resemble above medium. Somatic embryos germinated on medium that had the same formulation as above medium and few of them rooted in this medium. On medium with 1 mg L^{-1} IBA after 2-3 days all shoots regenerated resulting from germination of somatic embryos rooted and whole plantlets were obtained (Fig. 6 and 7). Dan *et al.* (1998) noticed to the positive effect of 12.5-29.2 μm L^{-1} IBA in rooting of Rudraswamy and Reichert (1998) resulted that the Rice micro shoots obtaining in MS media with different concentrations of BA root in MS medium with IBA within 10 days. Plantlets were adapted to dry conditions and potted (Fig. 8).

The main objective of this research is presentation a simple, short and economic method for somatic embryogenesis in *Brassica napus* L. As were mentioned earlier we resulted somatic embryos from hypocotyls explant in this plant. Also Kirti and Chopra (1989) Kirti *et al.* (1991) and Koh and Loh (2000) produced somatic embryos from vegetative explants in *Brassica genus*.

It is clear that somatic embryogenesis from vegetative explants such as hypocotyls is easier and faster from micro spore explants that there are many reports about it in *Brassica napus* L. In these cases mother plants need to different pretreatments and categorize pollen at specific developmental stages (Venkatachalam *et al.*, 1999). Therefore since the useful micro spore for somatic embryogenesis are not always readily available, in this research attempts have been made to somatic embryogenesis from other explants including hypocotyls of young seedlings [Micro spore culture and plant regeneration of *Brassica napus* L. this important economic crop has been investigated extensively because of its high frequency of androgenesis from isolated late uninuclear-micro spores and early binuclear pollen. As high as 70% micro spores may undergo androgenesis in this species and the culture protocols have been progressively optimized over many years (Tian *et al.*, 2004)].

On the other hand the explant such as microspors, ovules and zygotic embryos have potential of direct embryogenesis while somatic embryogenesis is a method that in pathway of it somatic cells produced embryonic structure.

Briefly we resulted that there is possibility of somatic embryogenesis in *Brassica napus* L., this important economic plant and utilities of it (induced variation, somaclonal variation, large scale propagation) and it is very simple, fast and economic.

REFERENCES

Dan, Y.H., M.M. Mcdougald, M. Tyler and N.A. Reichert, 1998. Organogenenic regeneration of soybean from hypocotyls and progeny analysis. *In vitro* Cell Dev. Biol., 34:3, part II.

Donovan, A.M. R. Morgan, C.V. Piagnani, M.S. Ridout, James and C.M.E. Garrett, 1994. Assessment of somaclonal variation in apple.I. resistance to the fire blight pathogen, *Erwinia amylovora*. J. Hortic. Sci., 69: 105-113.

Jaligot, E., A. Rival, T. Beule, S. Dussert and J.L. Veridiel, 2000. Somaclonal variation in oil palm (*Elaeis guineensis* Jacq.): The DNA methylation hypothesis. Plant Cell Reports, 19: 684-690.

Kirti, P.B., S. Hadi, P.A. Kumar and V.L. Chopra, 1991. Production of sodium-chloride-tolerant *Brassica juncea* plants by *in vitro* selsction at the somatic embryo level. Theor. Applied Genet., 83: 233-237.

Kirti, P.B. and V.L. Chopra, 1989. A simple method of induction somatic embryogenesis in *Brassica juncea* (L.) Czern and Coss. Plant Breed., 102: 73-78.

Koh, W.L. and C.S. Loh, 2000. Direct somatic embryogenesis and plant regeneration and *in vitro* rapid-cycling *Brassica napus*. Plant Cell Reports, 19: 1177-1183.

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

Pellegrineschi, A., R.M. Brito, S. McLean and D. Hoisington, 2004. Effect of 2,4-Dichlorophen-oxyacetic acid and NaCl on the establishment of callus and plant regeneration in durum and bread wheat. Plant Cell. Tissue Organ Cult., 77: 245-250.

Rudraswamy, V. and N.A. Reichert, 1998. Microshoot proliferation of *Oryza sativa* L. for successful clonal propagation. *In vitro* Cell Dev. Biol., 34: 3, part II.

Sacristan, M.D., 1982. Resistance response to *Phoma lingam* of plants regenerated from selected cells and embryogenic cultures of haploid *Brassica napus*. Theor. Applied Gene., 62: 193-200.

Srivastava, S., B. Fristensky and Nat, N.V. Kav, 2004. Constitutive expression of a PR10 protein enhances the germination of *Brassica napus* under saline condition. Plant Cell Physiol., 45: 1320-1324.

Tian, H.C., Y. Yi and X.S. Meng, 2004. High frequency conversion of microspore-derived embryos of *Brassica napus* cv. Topas by supplemented calcium and vitamins. Plant Cell Tissue and Organ. Cult., 79: 159-165.

Venkatachalam, P., N. Geetha, A. Khandelwal, M.S. Shaila and G.L. Sita, 1999. Induction of direct somatic embtyogenesis and plant regeneration from mature cotyledon explants of *Arachis hypogaea* L. http://www.ias.ac.in/currsci/jul25/articles18.htm.

Vendrame, W.A., G. Kochert and Wetzstein, 1999. AFLP analysis of variation in pecan somatic embryo. Plant Cell Reports, 18: 853-857.

Von Arnold, S., I. Sabala, P. Bozhkov, J. Dyachok and L. Filonova, 2002. Developmental pathways of somatic embryogenesis. Plant Cell. Tissue Organ Cult., 69: 233-249.

Zhang, X.H., N. Joanna, H. John, P. Williams and E. Blumwald, 2001. Engineering salt-tolerant *Brassica* plants: Characterization of yield and seed oil quality in transgenic plants with increased vacular sodium accumulation. Eblumwald@ucdavis.edu.