Indian J.Sci.Res.10 (1): 63-67, 2015

ISSN: 2250-0138(Online)

INFLUENCES OF MEDIUM PARAMETERS ON SOMATIC EMBRYOGENESIS FROM LEAF EXPLANTS OF GLYCYRRHIZA GLABRA L

MANJUSHA SARKAR[1a] AND SATYESH CHANDRA ROY[b]

[ab]Ramakrishna Vivekananda Mission Institute of Advanced Studies, Kolkata, West Bengal, India

ABSTRACT

This study was conducted to optimize different types and concentrations of Carbon sources on somatic embryogenesis of leaf explants in *G. glabra*. Embryogenic efficiency and embryo development were promoted by high carbohydrate concentration. The best results were obtained with maltose (3%); the no. of somatic embryos per culture explants was higher compared to the control 3%sucrose. From these results an optimized induction medium is proposed.

Key Words – Callus, somatic embryo, nodal culture, suspension culture.

Licorice, the root of the leguminous *Glycyrrhiza* plant species (*Glycyrrhiza glabra* L.) has been used as food and traditional medicine over many years. Glycyrrhizin is a major biologically active flavanoid included specially in *Glycyrrhiza glabra* L. and has antioxidant property. The production of the wild *Glycyrrhiza glabra* L. was sharply decreased due to immoderate and ruinous utilization. Therefore, it is urgent to obtain sufficient products to supply the market by planting artificially. Somatic embryogenesis is a suitable process for the regeneration of plants from valuable genotypes obtained either *in vitro* manipulation or by breeding techniques. There are only few reports on the micropropagation of *Glycyrrhiza glabra* L. through shoot tip and nodal cultures.

MATERIALS AND METHODS

Material

Fully expanded young leaves were collected from the *in vitro* grown plantlet. The leaf as a whole or part of it was cultured. The leaf as a whole or part of it were inoculated on MS (Murashige and Skoog, 1962) solid medium supplemented with 1.5mgl^{-1}, 2, 4-D. This medium was used for callus induction and proliferation.

Establishment and Maintenance of Suspension Culture

One month old green calli were used for establishment of suspension cultures. About 0.5gm-1.0gm of callus was transferred into a 250ml Erlenmeyer flask containing 50ml of liquid callus induction media. The culture was maintained by several passages at 15 days interval and was incubated at 25±1^{o}C in diffused light on a horizontal shaker at 100rpm.

Induction of Embryogenesis

For induction of embryogenesis in liquid medium, 15 days old stock cultures were used as inoculums by sieving through a 100µm nylon sieve. These inoculums were then washed in MS basal medium without growth regulators. These inoculums of cell suspension (5ml) were added to 50ml of MS basal medium devoid of plant growth regulators in 250ml Erlenmeyer flask. For optimizing the process of embryogenesis and increasing the frequency of embryo formation, several culture components were manipulated .The work was repeated three times.

For the determination number of embryos in the media, cell suspensions from the dispersing culture flask poured into Petri dish marked with transparent checkered grid and then counted under the inverted microscope. Numbers were determined from the average counts of ten fields and data were presented as the mean value± the standard error.

Effect of 2, 4-D

Reducing the level of 2, 4-D from 2.0mgl^{-1} to 0.5mgl^{-1} and finally to 0.0mgl^{-1} in primary suspension culture inducing medium, its effect on embryogenesis was observed.

Effect of Carbon sources

In this set of experiment, different Carbohydrate sources like sucrose, glucose, fructose, maltose, manitol and sorbitol were used. These carbohydrates in three different concentrations were added individually in MS media with its usual 3% sucrose.

[1]Corresponding Author

Germination of Embryos

The most important step followed during in germination of embryo is its dehydration. Series of relative humidity were generated in desiccators using over saturated salt solution of Sodium Chloride (NaCl 78%), Ammonium Nitrate (NH_4NO_3 63%) and Potassium Carbonate (K_2CO_3 43%). The embryo in a petri dish, with a nutrient, are equilibrated at each humidity for 3 hours and then air-dried. The embryos were kept in between two sterile filter paper and air dried under Laminar flow for 2 hours.

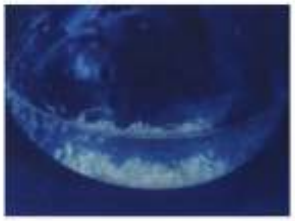

Fig. 1 Leaf callus in MS media containing 1.5 mgl^{-1} 2,4-D after 25 days.

Fig. 2 Cell suspension culture in liquid MS media without any growth regulator.

Fig. 3 Embryos in MS media containing 3% Maltose in presence of its usual 3% Sucrose after 30 days.

Fig.4 Germinating somatic embryos in petridish, MS media containing 1.0mgl^{-1} Gibberellic acid after 45 days

For germination, somatic embryos were transferred to hormone free MS medium and also to MS medium supplemented with ABA (0.5mgl^{-1} to 2.0mgl^{-1}) and GA_3 (0.5mgl^{-1} to 2.0mgl^{-1}) and Sorbitol (3%) separately. The time taken to germinate and the percentage of germination were noted to each case.

RESULTS AND DISCUSSION

Induction of Calli

For somatic embryogenesis calli were induced from leaves. Compact and greenish calli which were formed in callus inducing medium containing 1.5mgl^{-1} of 2, 4-D (Fig.1).

Induction of Embryogenesis in Suspension Culture

Cell suspensions were sieved through 100µm nylon mesh (Fig. 2) and were then transferred to MS medium without any growth regulator. The globular and heart shaped embryos appeared after 25-30 days of culture. Different chemical constituents were tried for the production of somatic embryo and no significant morphological changes were noted. However, quantitative changes were observed.

Effect of Carbohydrates

Carbohydrate type and concentration have been found to play important roles in the somatic embryogenesis. Although the majority of media used in plant tissue culture contain sucrose as the standard carbon and energy source. Different carbohydrate sources like sucrose, glucose, fructose, maltose, manitol and sorbitol were used .When these carbohydrates in three different concentrations were added individually in MS media with its usual 3% Sucrose, it was observed at 3% concentration the effect of maltose was highest (212.3±0.62) ((Fig.3) and lowest frequency of embryo (10.0±0.27) was found in Mannitol (Table-1).

Table-1: Effect of Carbohydrate on embryo formation (added with 3% Sucrose).

(Control= 160.2±0.57)

Carbohydrate Source	1%	3%	5%
Glucose	111.4±0.51	126.4±0.22	97.0±0.36
Fructose	58.5±0.76	64.0±0.42	40.1±0.27
Maltose	170.5±0.34	212.3±0.62	189.9±0.45
Mannitol	22.6±0.66	10.0±0.27	0
Sorbitol	87.3±0.76	104.6±0.53	90.5±0.37
Sucrose	134.0±0.66	154.4±0.70	33.5±0.61

Germination of Embryo

Somatic embryos, thus produced were transferred to MS semi solid media without any growth regulators and in other set MS media supplemented with GA_3 (0.5mgl^{-1} to 2mgl^{-1}), ABA (0.5 to 2.0mgl^{-1}) and sorbitol (3%) with usual 3% sucrose were used for their germination. The percentage of germination was 47% in GA_3 (1.5mgl^{-1}) (Fig.4) and lowest in sorbitol (Table-2).

Table-2: Influence of different plant growth regulators on germination of somatic embryo

Medium	Time taken To Germination	Percentage of germination
Hormone free MS	14days	23.3%
MS+ABA (0.5mgl)	13 days	43.3%
MS+ABA (1.5mgl)	10days	47.0%
MS+ABA (3.0mgl)	10days	13.0%

DISCUSSION

Somatic embryogenesis is the most promising technique for plant multiplication because of it higher proliferation potential. The ability to produce normal embryos and whole plants from undifferentiated somatic cells in culture through the process of somatic embryogenesis is a unique feature in plant system. The initiation and development of embryos from somatic tissue in plant culture rather than the maturation of excised zygotic embryos were first recognized by Steward (1958) and Reinert (1958, 1959) in culture of *Daucus carrota*.

For *in vitro* culture of plant tissues, Sucrose is generally regarded as the standard carbohydrate component of culture media (Evans et al., 1981; Dunwell, 1985; Strickland et al., 1987; Scott and Lyne, 1994). Furthermore, when sucrose is supplied to non-photosynthetic plant tissues, it is frequently hydrolyzed by cell wall invertases to glucose and fructose before entering the cell in which it is metabolized (Lucas and Modre, 1988). Maltose has shown to be better carbohydrate source for *in vitro* culture of certain plant tissue culture.

In the present study, different carbohydrates (i.e. Sucrose, Glucose, Fructose, Maltose, Mannitol and Sorbitol) were used. When different carbohydrates at the concentration of 1% were added individually to MS media containing 3% Sucrose, the effect of Maltose was highest (170.5±0.34) followed by Sucrose,Glucose , Sorbitol, Fructose and lastly by Mannitol (22.6±0.66). When 3% concentration of different carbohydrates were added together with 3% Sucrose in the media the highest frequency was observed in Maltose(212.3±0.62) and lowest in mannitol (10.0±0.27) and other carbohydrates showing an intermediate value (Table-8). When these carbohydrates were added at 5%concentrations, the highest frequency were observed in Maltose (189.9±0.45) which is rather less with respect to 3% concentrations. Increased sucrose concentrations promoted somatic embryogenesis in *Carica pappya* (Litz and Conover, 1983); Norway spruce (Von Arnold, 1987); Sunflower (McCann *et al.,* 1988).

The stimulatory effect of Maltose in the initiating and development of somatic embryogenesis was also reported in *Solanum tuberosum* L. (Batty and Dunwell, 1989); *Triticum aestivum* (Last and Bretell, 1990).

High osmotic concentrations produced by additional Sucrose such as Inositol or Sorbitol promoted normal development of somatic embryos in carrot and *Sium suave* somatic embryos (Ammirato and Steward, 1971; Ammirato, 1983) in a way that they prevent precocious germination. Sorbitol and Mannitol in medium with Sucrose increased embryogenic efficiency in primary and long term cultures.

In the present study, MS medium with GA_3 (1.5mgl^{-1}), ABA (0.50mgl^{-1}) and 3% Sorbitol (with usual 3% Sucrose) proved to be the most suitable combinations for the germination of embryos. It was evident from different studies that ABA was a critical factor in promoting maturation of embryo and normal development of plants (Von Arnold and Hakman, 1988; Roberts *et al.*, 1991).It was suggested that favorable effect of ABA was due to an increase in storage reserves, such as storage proteins, triglycerides and lipids. The role of ABA on the germination of somatic embryos has

been reported in a series of publications-interior spruce (Webster *et al.,*1990,Roberts, 1991); hybrid larch (Lelu *et al.,*1994, Fujii *et al.,*); *Simarouba glauca* (Rout and Das, 1994). The somatic embryos so developed due to exogenous application of ABA closely resembled Zygotic embryos both in structure and behavior (Ammirato, 1988).

The osmoticum of the cultural medium was another factor involved in the development of somatic embryos Roberts (1991) reported that the osmoticum had effects similar to those of ABA on the development of hybrid larch somatic embryos. Germination frequency could also be increased by using a high osmoticum (Sorbitol) in *Brassica napus* (Finkelestien and Crouch, 1986); and Celery (Nadel *et al.,* 1990) somatic embryos.

REFERENCES

Ammirato, P.V. and Steward,F.C. 1971. Some effects of the environment on the development of embryos from cultured free cells. Bot. Gaz. 132: 149-158.

Ammirato, P. V. 1983. Embryogenesis. In: Evans *et. al*. (eds.) Handbook of Plant Cell culture. Vol. 1 MacMillan, New York. 82-123.

Batty, N. and Dunwell,J.M.1989.Effect of carbohydrate source on the response of potato anthers in culture. *Plant Cell Tissue Organ Culture. 18: 221-226.*

Dunwell,J.M.and Thurling, N.1985. Role of sucrose in microspore embryo production in *B. napus*, sp. Olcifera. J. EXP. Bot. 36: 1478- 1491.

Evans ,D.A., Sharp, W.R. and Flick,C.E.1981. Growth and behavior of cell cultures: Embryogenesis and Organogenesis. In: Thorpe, T.A.(ed.).Plant Tissue Culture: Methods and application in agriculture. Academic Press. New York. pp. 45- 113.

Fujii, J., Slade, D., Olsen, R., Ruzin, S,.E. and Redenbough, K.1990. Alfa alfa somatic embryo maturation and conversion to plants. Plant Sci. 72: 92-100.

Last,D.I and Bretell, R.S.I.1990. Embryo yield in wheat anther culture is influenced by the choice of sugar in the culture medium. Plant Cell Rep. 9: 14-16.

Lelu.M.A. Bastein,C., Klimaszewska, K. and Charest, P.J.1994. An improved method for somatic plant production in hybrid larch. Part 2: Control of germination and plantlet development. Plant Cell Tissue Organ Culture. 36: 117-127.

Litz, R.E. and Conover,R.A. 1983. High frequency somatic embryogenesis from *Carica* suspension cultures.Ann. Bot.51: 683-686.

Lucas, W.J.and and Modre,M.A.1988. Sugar uptake in higher plants. In: Preiss, J. (ed.). The biochemistry of plants, Academic press, New York. Vol. 14. Pp 35-84.

McCann, A.W., Cooley, G. and Van Dreser, J.1988. A system for routine plantlet regeneration of sunflower (*Helianthus annus* L.) from immature embryos derived callus. Plant Cell Tissue Organ Culture.14: 108-110.

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

Reinert, J.1958. Morphogenesis und ihre Kontrolle an Gewebekulturn aaus Karotten. Naturwissenschaften. 45: 344-345.

Reinert, J.1959. Uber die kontrolle der Morphogenese und die indukttion von Advienticeembryonen an Gewebekulturen aus Karotten. Planta. 58: 318-333.

Roberts, D.R., Flinn, B.S., Webb, D.T. Webster, F.B. and Sutton, B.C.S.1991. Abscisic acid and Indole-3-butryic acid regulation of maturation and accumulation of storage protein in somatic embryos interior spruce.

Rout, G.R. and Das, P. 1994. Somatic embryogenesis in *Simarouba glauca*. Plant Cell Tissue Organ Culture. 37: 79-81.

Scott, P. and Lyne,R.L.1994. The effect of different carbohydrate sources upon the initiation of embrygenesis. Plant Cell Tissue Organ Culture 43:163-170.

Steward, F.C. 1958. Growth and Development of cultivated cells. III. Interpretations of growth from free cell to carrot plant. Am. J. Bot. 45: 709-713.

Strickland, F.C. Nichol, J.W. McCall, C.M. and Stuart, D.A.1987. Effect of Carbohydrates Source on alfa alfa somatic embryogenesis. Plant Sci.48: 113-117.

Von Arnold, S.1987.Improved efficiency of somatic embryogenesis in mature embryos in *Picea abies*. J. Plant Physiol. 128: 233-244.

Von Arnold,S.,and Hakman,I. 1988 Regulation of Somatic embryo development in *Pieca abies* by abscisic acid (ABA). J. Plant Physiol. 132: 164-169.

Webster, F.B., Roberts, O.R., McInnis, S.M. and Sutton, B.C.S.1990. Propagation of interior spruce by somatic embryogenesis. Can.J. For. Res. 20: 1759-1765.