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SOMATIC EMBRYOGENESIS FROM CALLUS CULTURE AND PROTOPLAST ISOLATION AND CULTURE IN *DATURA INNOXIA* MILL.

V.K SHARMA and S.L. KOTHARI
Department of Botany. University of Rajasthan, Jaipur-302004, India.

Anthers of *Datura innoxia* Mill were cultured on MS medium and large number of embryoids differentiated and they could germinate to form plantlets, shoots or callus from the entire surface and showed further embryogenesis. Prolific embryogenesis occurred when this callus was sub-cultured on a medidm with kinetic—2,4-D. For protoplasts cultre, leaf material was taken from the *in vitro* differentiated plants.

Keywords : Embryoids; Callus; Embryogenesis; Protoplast culture; Plant regeneration.

The production of large number of haploid plants from cultured anthers or isolated pollen grains has been demonstrated in many angiosperms (Maheshwari, *et al.*, 1982). The phenomenon was first discovered in *Datura innoxia* (Guha and Maheshwari, 1964), which now is consider-as an ideal material for somatic cell genetics using deploid or haploid protoplasts or cell suspensions (Schieder, 1980). Present study deals with the somatic embryogenesis from callus cultures and protoplast isolation and culture in *Datura innoxia* Mill.

Datura innoxia Mill. plants growing in the University campus were used as a source of explant materials. Flower buds, 4–5 cm in length, were collected and anthers, 5–8 mm in size, dissected aseptically after surface sterilizing the buds in 0.1% $HgCl_2$ solution for 5 min and washing them thrice with sterile distilled water. Excised anthers were cultured on MS Medium (Murashige and Skoog, 1962) supplemented with 0.8% agar, 3% sucrose, 0.005 to 0.05 mg/l Kinetin (K) (Kukreja, 1979) and adjusted to pH 5.8. All cultures were incubated in a growth room at a temperature of 26±2°C and light intensity (16th light per day) of 1200 lux.

Callus formed from the entire surface of the embryoids was subcultured on a medium with K (0 05

mg/l) + 2,4-dichlorophenoxy acetic acid (2,4-D) (0.5 mg/l).

Fig. 1-3 : 1. Shoot and plantlet formation from the cultured anthers on MS medium with K (0.005 mg/l); 2. Plantlet formation from the germinated embryoids on MS+IAA (0.2 mg/l); 3. Leaf mesophyll protoplasts.

Leaf material from the *in vitro* differentiated plants was taken for protoplasts isolation. About 1 gm of the larger leaves were cut and placed in sterilized glass petridish (100 mm in diameter), and some droplets of a 0.3 M mannitol solution was added to prevent desiccation. Leaves were cut into very small pieces (3-5 mm^2 in diameter) and 10 ml of the 0.3 M mannital solution was added After shaking and removing the mannitol solution, leaf material was transferred into a flask containing 50 ml of the enzyme solution for digesting at 24°C in dark in a BOD incubated the cell walls. The enzyme solution consisted :—

1% cellulase Onozuka R-10, 0.2% Macerozyme R-10 (both kinki Yakult Co., Nishinomiya, Japan, 0.6M Mannitol, pH 5 5, adjusted (Schieder, 1981).

After 4-5 hr incubation large number of protoplasts were isolated (Fig. 3). Enzyme solution, containing the protoplasts and undigested leaf material was filtered through a steel sieve (Pore size 100 μm). Protoplasts containing enzyme solution were diluted with culture media and then centrifuged for 3 min to sediment the protoplasts. The sedimented protoplasts were resuspended with culture media and centrifuged 2-3 times for removing the enzyme solution and other debris. Such protoplasts were then transferred to MS liquid medium

(20 ml medium in 50 ml flasks) (Culture media) containing 0.6 M mannitol, 1.5 mg/l α-Naphthaleneacetic acid (NAA) and 0.4 mg/l 6-benzylaminopurine (BAP). The cultures were incubated under illumination of about 1500 lux at 25-27°C.

Anthers showed swelling after 3 days which continued upto 2 weeks. Large number of embryoids differentiated in about 20 days from the cultured anthers. On the same medium the embryoids germinated to form plantlets and shoots (Fig. 1) and callus if kept for 5-6 weeks. Callus on the same medium showed further embryogenesis and growth upto 90 days.

Growth of the callus continued and more embryoids differentiated while the older embryoids germinated While keeping the kinetin concentration to 0.05 mg/l if 2,4-D level was raised to 3.0 mg/l the growth of callus and further development of embryoids was inhibited. For best germination of embryoids and root formation from *in vitro* differentiated shoots, MS solid medium without any growth regulator or with IAA (0.2 mg/l) was most suitable (Fig. 2). However, on liquid medium of similar composition the plants became transluscent and root system developed also was very poor.

The protoplast started dividing after 5-6 days of incubation and in 3-8 weeks small cell colonies wer formed. After a further 3-4 weeks the colonies were visible macroscopically.

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