Pak. J. Bot. 33 (special issue) 2001

CALLOGENESIS, EMBRYOGENESIS AND ORGANOGENESIS IN CHRISTMAS CACTUS (*SCHLUMBERGERA BRIDESI*)

AAMIR ALI, SHAGUFTA NAZ, FAYYAZ AHMAD SIDDIQUI AND JAVED IQBAL

Department of Botany, University of the Punjab
Quaid-e-Azam Campus, Lahore, Pakistan

Abstract

For callus induction explant from young joints of mature plant of Christmas cactus (Schlumbergera bridesii) were cultured on MS medium supplemented with different hormones. However MS medium containing 3 mg/l of 2,4-D exhibited maximum percent callus induction. The shoot regeneration was achieved by sub culturing the calli on MS medium containing 2 mg/l of BAP and 1 mg/l of Kinitin. Shoot formation was observed in compact calli. The regenerants were rooted on MS medium containing 1.0 mg/l NAA and then acclimatized in green house.

Introduction

Christmas cactus (Schlumbergera bridesii) is a group of epiphytic cactus native to the South American jungles. It is a succulent plant and can store a reasonable quantity of water. It is not a true cactus and is not quite as drought tolerant as the name infers. More abundant blooms are produced on plant that has been exposed to high light intensity. Cacti and other plants which possess CAM usually present limited reproductive capacities and very slow growth rates (Malda G *et al*; 1999). This plant is commonly propagated by taking short Y- shaped cuttings of the stem tips. But this practice of cutting requires skilld labour and considerable time, therefore limits the speed of plant propagation. Moreover this conventional way of shoot cutting is a slow process of propagation and they are subjected to many diseases and environmental hazards that cause gradual degeneration of cultivars. However, it is being carried out because it was not possible to obtain satisfactory number of plants by any other method. It is therefore necessary to tap alternate methods of propagation.

In recent years cell and tissue culture techniques have been successfully used for mass scale production of Cacti. This technique enables us to have mass scale production of healthy and premium quality planting material which is a prerequisite of commercialization (Qureshi *et al.*, 1993). (Malda *et al.*, 1999) studied the comparison of *in vitro* and *ex vitro* growth rates in Cacti and stated that *in vitro* environment notably accelerates the growth of Cacti. The object of this study was to develop methods for the mass scale production of healthy and premium quality planting material of Christmas cactus.

Production of different species of Cacti by using plant tissue culture techniques have also been reported by different scientists (Boyle, 1997; Eliasrocha *et al.*, 1998; Bhau, 1999).

Materials and Methods

Surface sterilization: Explants from young joints of mature plant of Christmas Cactus were cut into pieces and washed thoroughly with tap water.

Mohamedyasseen *et al.* (1995) also used young joints of mature plant as an explant for micropropagation of Tuna. Surface sterilization was carried out by immersing explants in 15 % sodium hypochlorite solution for 15 minutes. The explants were then rinsed three times with autoclaved distilled water. Murashiage and Skoog (1962) basal medium was used containing 3 % sucrose and 1.5 % phytogel. The pH of the medium was adjusted to 5.7 and was autoclaved at 121 °C and 15 lb pressure for 15 minutes

Callus induction: Different concentrations and combination of 2,4 – D and Kin were added to MS medium for initiation of callus. The leaf discs were transferred to the medium and the cultures were incubated with 10 hours photo period at the temperature of 27 ± 1°C.

Plant regeneration & rooting: Different conations of auxins alone and in combination with cytokinins were used for regeneration. Regenerated plants were transferred to media containing different concentrations of NAA for rooting. Rooted plantlets were transferred in autoclaved sand in green house for hardening.

Results and discussions

Callogenesis: For callus induction phylloclade explant of different sizes was used. Callus formation was found to be good in 10-15 mm long phylloclade explant. The effect of different concentrations and combinations of auxin (2, 4–D) and cytokinin (Kinitin) was studied for callus induction. Significantly greater amount of callus was formed when 2, 4 – D at the concentration of 3 mg/l was added to MS basal medium. 2,4–D at the concentration of 1mg/l or 2 mg/l did not show good results and induced least amount of callus. Similarly results were not good when the concentration of 2,4-D higher than 3mg/l were used. In fact, the writer has not come across any literature regarding the callogenesis in Christmas cactus. However Mangolin (1999) obtained maximum amount of callus in Cereus peruvianus by using a combination of 4 mg/l 2,4-D and 8 mg/l kinetin. Bhau (1999) also used the combination of 2,4-D and kinetin for callus induction in Coryphantha elephantidens (cactaceae). But in case of Christmas cactus, the combination of 2,4–D and Kinetin did not express significant effect on callus formation.

Regeneration & Differentiation: The callus developed on the medium containing 3 mg/l of 2,4- D were shifted for regeneration. MS media containing different concentrations and combinations of growth regulators was used. Significant differences between the treatments in respect of plant regeneration were observed. Maximum regeneration of callus was obtained in MS media containing 1 mg/l BAP + 0.5 mg/l kinetin. Elias Rocha (1998) used the combination of BAP and kinetin for shoot induction in Mammillaria candida (cactaceae). BAP when used alone did not respond well even at the concentration of 4mg/l. However, Bahu BS (1999) reported that combination of 2,4-D and kinetin has proved more effective than other growth substances for regeneration in Cacti. But in Christmas cacti omission of 2,4-D results in good regeneration. According to Ammirato (1985) and Vasil (1988) 2,4-D at relatively higher concentration promotes callus induction and inhibits organ differentiation. Flick *et al* (1983) and Talwar & Raashid (1989) also reported that depletion or omission of 2,4-D induces the growth and morphogenesis.

Root Induction: After regeneration when plantlets attain considerable size were transferred to the media supplemented with different concentration and combination of auxins. Bhau obtained roots in simple MS media in Coryphantha elephantidens. Eliasrocha and Santos (1998) used ½ MS media supplemented with 3.24mM CaCl2. It was observed that Significantly greater number of plants were rooted when transferred to MS medium containing 1 mg/l of NAA. Increase in the concentration of NAA did not show any improvement in rooting. Similar results were obtained when concentration of NAA lower than 1mg/l was used. Rooting was also observed in simple MS basal medium but by the addition of NAA rapid and good results were obtained.

Effect of Auxins and cytokinins on callus induction

Sr No	Media	Concentration (mg/l)	No of explant cultured	%age of callus induction
		1	5	30
		2	5	75
1	MS + 2,4 –D	3	5	100
		4	5	90
		5	5	85
		2,4 –D 2.0 + Kin 0.5	5	20
		2,4 -D 2.0 + Kin 1.0	5	20
2	MS + 2,4 D + Kin	2,4 -D 3.0 + Kin 1.0	5	85
		2,4 -D 3.0 + Kin 2.0	5	75
		2,4 -D 3.0 + Kin 4.0	5	70

Effect of different hormones on regeneration of calli.

Sr No	Media	Concentration (mg/l)	No of cultures used	% age of regeneration
1	MS + BAP	1	5	0
		2	5	20
		3	5	35
		4	5	35
2	MS + BAP + Kin	BAP 0.5 + Kin 0.5	5	45
		BAP 1.0 + Kin 0.5	5	85
		BAP 2.0 + Kin 1.0	5	70
		BAP 3.0 + Kin 2.0	5	75
		BAP 3.0 + Kin 3.0	5	65
3	MS + 2,4 –D + Kin	2,4 -D 1.0 + Kin 0.5	5	0
		2,4 -D 1.0 + Kin 1.0	5	45
		2,4 -D 2.0 + Kin 1.5	5	25
		2,4 -D 3.0 + Kin 2.0	5	25
		2,4 -D 4.0 + Kin 4.0	5	15

Effect of different hormones on rooting.

Sr No	Media (mg/l)	No of cultures used	% age of rooting
1	MS basal	5	70
2	MS + NAA 0.5	5	85
3	MS +NAA 1.0	5	95
4	MS + NAA 1.5	5	90
5	MS + NAA 2.0	5	90

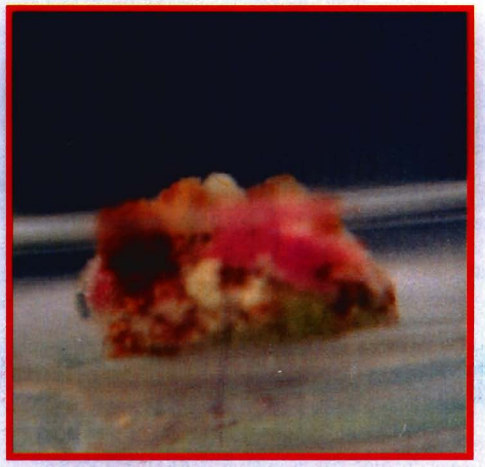

Figure 1. Callus formation flower pigment.

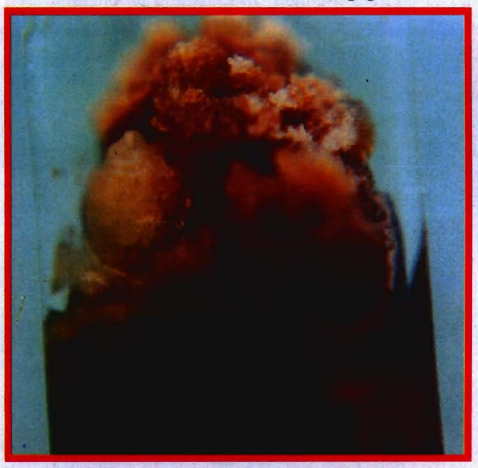

Figure 2. Callus formation from leaf tissues.

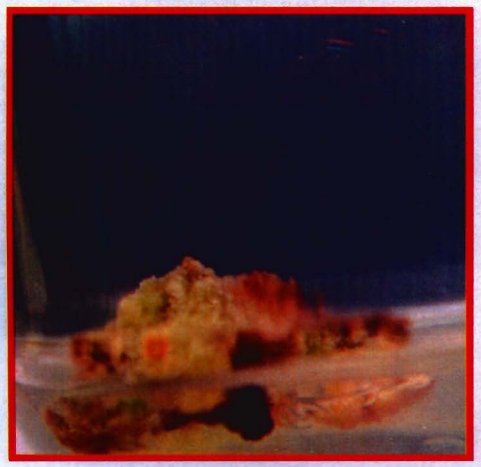

Figure 3. Development of embryogenic callus.

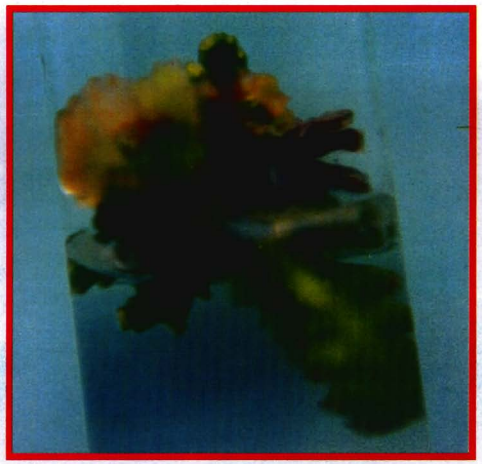

Figure 4. Shoot formation from embryogenic callus.

Figure 5. Harden Plant.

Figure 6. Plant developed through tissue culture.

References

Ammirato,P.V. 1985. Pattern of development in culture. In: Tissue culture in Forestry and Agriculture. New york. pp.9-29,Plenum.

Bhau, BS. 1999. Regeneration of Coryphantha elephantidens (Lem.) Lem. (Cactaceae) from root explants. *Scientia Horticulturae*, 81(3): 337- 344.

Boyle, T.H. 1997. Influence of Benzyladenine and gibberellic acid on organogenesisi Crimson giant easter cactus. *Plant Growth Regulation*, 22(2) : 131-136.

Eliasrocha, M.A., M.D. Santosdiaz and A. Arredondogomez. 1998. Propagation of Mammillaria candida (Cactaceae) by tissue culture techniques. *Haseltonia Iss.*, 6: 96-101.

Flick, C.E., D.A. Evans and W.R. Sharp. 1983. *Organogenesis. In: Handbook of plant cell culture*. Volume 1. McMillan Publications Co., New york, pp13-18.

Malda, G., H. Suzan and R. Backhaus. 1999. In vitro culture as a potential method for the conservation of endangered plants possessing Crassulacean acid metabolism. *Scientia Horticulturae*. 81(1): 71-87.

Mangolin, C.A., L.M.M. Ottoboni and M.D.P.S. Machado. 1999. 2 – dimensional electrophoresis of Cereus Peruvianus (Cactaceae) Callus- Tissue Proteins. *Electrophoresis*. 20(3): 626-629.

Mohamedyasseen, Y., S.A. Barringer, W.E. Splittstoesser and R.J. Schnell. 1995. Rapid propagation of Tuna and plant establishment in soil. *Plant cell tissue and organ culture*. 42(1) : 117- 119.

Murashige, I. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant*. 15: 473-487.

Qureshi, A., Hidayat ullah, M. Bhatti and S. Ahmad. 1993. An improved method for in vitro multiplication of Potato. *Advances in plant tissue culture Pakistan*. Pp 55-60.

Talwar, M. and A. Rashid. 1989. Somatic embryo formation from unemerged inflorescence and immature embryos of a germinaceous crop Echinochla. *Annal of Botany*. 64: 195-199.

Vasil, I. K. 1988. Progress in the regeneration and genetic manuplation of cereal crops. *Bio/technology*. 6; 397-402.