Callus induction on standard type *Cymbidium* cultivars

INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM Publishing House, Hungary

Jánvári L.[1], Bisztray Gy.[1], Füzesi T.[2] and Velich I.[1]

[1]Szent István University, Faculty of Horticultural Science, Department of Genetics and Horticultural Plant Breeding, H-1118 Budapest, Ménesi út 44. Hungary
[2]Plantlab; H-9722 Perenye, Béke u 140

Key words: *Cymbidium0hybrida*, micropropagation, callus induction, benzyladenine, naphtylacetic acid, thidiazuron

Summary: Tissue cultured *Cymbidium* PLBs (protocorm-like body) were used as starting material to induce embryogenic callus which could serve as objects of genetic transformation. We obtained callus using two methods. The first method was culturing the PLB segments for one month in liquid MS medium in the presence of 0.5 mg/l benzyladenine and 0.05 mg/l naphtylacetic acid followed by cultivation on the same composition solid medium with 0.5 g/l activated charcoal for an additional month. Callus formation was observed on 30% of the explants. The second way was to propagate the PLB segments on solid MS medium supplemented with 1 mg/l thidiazuron. In these cultures we also observed callus formation on 20% of the explants.

Abbreviations: benzyladenine – BA, naphtylacetic acid – NAA, thidiazuron – TDZ, protocorm-like body – PLB

Introduction

Cymbidiums are propagated via tissue culture routinely since Dr. George Morel has tried to culture *Cymbidium* shoot tip meristems *(Morel* 1960) and realised its capability for mass propagation. Following this technique additional ones also have been devised using shoot tips or axillary buds to form PLBs in tissue culture. These PLBs are divided afterwards in each propagation cycle and the segments develop new PLBs on the surface. This could result millions of clones in one year in case of multiplication by 4 in every monthly cycle, but for a single PLB this means only 4 immediate progeny. It should be noted that only the outer cell layers of the PLB are able to regenerate plantlets, the inner ones do not divide and has abnormal endoploidization. *(Morel* 1974., *Nagl* 1972., *Nagl & Rucker* 1972.).

We have to introduce the transgenes into the regenerating cells to produce transformed plants. On each PLB section only a limited number of cells proliferate to form the new plant against a background of numerous inactive cells. For an efficient transformation system this level of regenerability is too low.

Our aim was to induce regeneration in large number of transformable cells on embryogenic way.

Material and methods

Plant material: Aseptic cultures of three standard type *Cymbidium* varieties were obtained from the kindness of *Tamás Füzesi*. Cultures were in continuous growth in PLB stage.

Basal medium: For all medium the salts of MS medium *(Murashige & Skoog* 1962) was used supplemented with the following basic components: 100 mg/l inozitol, 0.5 mg/l niacin, 0.1 mg/l thiamine, 0.5 mg/l pyridoxine, 2 mg/l glycin, 0.5 g/l pepton, 20 g/l sucrose. The pH of the medium was adjusted to 5.5 with 1N KOH or 1N HCl prior to autoclaving at 121°C for 20 min. Solid medium was prepared with 7 g/l agar. All cultures were maintained at 25±2°C with a light/dark cycle of 16/8 h. Culturing periods were 4 weeks.

First method:
We used two medium.

1. 50 ml liquid basal medium in 250 ml erlenmeyer flasks containing 0.5 mg/l BA and 0.05 mg/l NAA. Cultures were maintained under continuous agitation (100 rpm) on horizontal shaker.
2. solid medium in baby food jars with 0.5 mg/l BA and 0.05 mg/l NAA and 0.5 g/l activated charcoal.

Second method: 1 mg/l thidiazuron was incorporated in the basal solid medium.

Results and discussion

Germinating *Cymbidium* seed forms protocorm in a stage of development, which later differentiates to form a plant. The same structures, protocorm-like bodies are formed from buds or meristems in vitro. Initial cultures were in this stage. Plant material was maintained on agar solidified medium with 0.5 mg/l BA, 0.05 mg/l NAA and 0.5 g/l activated charcoal (propagation medium). Propagation was continuous and intensive. Subcultures of PLBs resulted numerous new PLBs from all varieties. Frequent sectioning and subculturing minimised the plant formation that would require longer undisturbed period.

During standard propagation we haven't observed callus formation, or initiation of high number of PLBs on the explants surface. In order to induce callus we tested the procedures of *Steward & Mapes (Steward & Mapes* 1971.) and *Chang & Chang (Chang & Chang* 1998). Using these methods we failed to induce callus. This may be the consequence of the differences in the used genotypes. Although Steward and Mapes have not reported the variety they used, Chang and Chang induced callus on *Cymbidium ensifolium var. misericors*. The species *Cymbidium ensifolium* is a member of the subgenus *Jensoa (Du Puy & Cribb* 1988). Members of this subgenus have an unusual developmental sequence producing rhizome from seed instead of protocorm and the shoot buds differentiates from its epidermal cells *(Morel* 1974).

In order to develop an effective composition numerous hormon compositions were tested (not presented). Two treatments gave positive results. The switch from hormone containing liquid culture to solid medium with the same hormones and activated charcoal. Callus was also induced using thidiazuron, a highly active synthetic cytokinin in solid medium.

The first method

PLBs were grown on the propagation medium. The sectioned PLBs were placed in the liquid culture and grown under continuous agitation. Numerous PLBs developed on the sections forming clumps. No callus formation was observed on them. PLBs were halved and placed on the charcoal containing solid medium. 70% of them continued to develop PLBs, 30% grew slowly forming callus on the upper surface of the explants in 4 weeks *(Fig. 1)*. The callus was granulous consisting of homogenous cells. These cultures were left undisturbed and numerous PLBs developed from them *(Fig 2)*. The PLBs were separated and plants differentiated from them on the propagation medium.

Figure 1 Callus induced on the explants

Figure 2 Callus deriver PLBs

Second method

PLBs were grown on the propagation medium. The sectioned PLBs were placed on agar solidified medium containing 1 mg/l thidiazuron. The explants grew rapidly and numerous shoots protruded from the swollen tissues. *(Fig. 3)*

Figure 3 TDZ induced shoot production

On 20% of the explants granulous callus developed *(Fig. 4)* and followed the same developmental sequence as the one induced by the first method.

Figure 4 TDZ induced callus

These two methods provide plant material for transformation experiments. Further investigations are needed in order to compare the mutagenic effect of the different methods and their use as sources of *Cymbidium* cell suspension cultures for large scale propagation of embryogenic cells.

Acknowledgements

We thank *Mária Lujza Reményi & László Udvardy* for their help in preparing the photographs and *Annamária Mészáros* for her help and advices.

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