

**ESTABLISHMENT OF OPTIMUM NUTRIENT MEDIA FOR *IN VITRO* PROPAGATION OF  
*CYMBIDIUM* SW. (ORCHIDACEAE) USING PROTOCOL-LIKE BODY SEGMENTS**

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**REFERENCES**

- Arnon D. J., Hoagland D. R. (1940). Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. *Soil Science*, 50: 463-485.
- Begum A. A., Tamaki M., Tahara M., Kako S. (1994). Somatic embryogenesis in *Cymbidium* through *in vitro* culture of inner tissue of protocorm-like bodies. *Journal of the Japanese Society for Horticultural Science*, 63: 419-427.
- Chang C., Chang W. C. (1998). Plant regeneration from callus culture of *Cymbidium ensifolium* var. *misericors*. *Plant Cell Reports*, 17: 251-255.
- Choi H.-W., Lemaux P. G., Cho M.-J. (2000). High frequency of cytogenetic aberration in transgenic oat (*Avena sativa* L.) plants. *Plant Science*, 156: 85-94.
- Chu C. C., Want C. C., Sun C. S., Hsu C., Yin K. C., Chu C. Y., Bi F. Y. (1975). Establishment of an efficient medium for anther culture of rice, through comparative experiments on the nitrogen sources. *Scientia Sinica*, 18: 659-668.
- Fujii K., Kawano M., Kako S. (1999). Effects of benzyladenine and  $\alpha$ -naphthaleneacetic acid on the formation of protocorm-like bodies (PLBs) from explants of outer tissue of *Cymbidium* PLBs cultured *in vitro*. *Journal of the Japanese Society for Horticultural Science*, 68: 35-40.
- Fukai S., Hasegawa A., Goi M. (2002). Polysomaty in *Cymbidium*. *HortScience*, 37: 1088-1091.
- Gamborg O. L., Miller R. A., Ojima K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50: 151-158.
- Hinnen M. G. J., Pierik R. L. M., Bronsema F. B. F. (1989). The influence of macronutrients and some other factors on growth of *Phalaenopsis* hybrid seedlings *in vitro*. *Scientia Horticulturae*, 41: 105-116.
- Huan L. V. T., Tanaka M. (2004). Callus induction from protocorm-like body segments and plant regeneration in *Cymbidium* (Orchidaceae). *Journal of Horticultural Science and Biotechnology*, 79: 406-410.
- Kano K. (1965). Studies on the media for orchid seed germination. *Memoirs of the Faculty of Agriculture, Kagawa University*, 20: 1-68.
- Kishi F., Takagi K. (1997). Analysis of media components used for orchid tissue culture. *Lindleyana*, 12: 158-161.
- Knudson L. (1946). A new nutrient solution for the germination of orchid seeds. *American Orchid Society Bulletin*, 14: 214-217.
- Kubota S., Yoneda K., Suzuki Y. (2000). Effects of ammonium to nitrate ratio in culture medium on growth and nutrient absorption of *Phalaenopsis* seedlings *in vitro*. *Environment Control in Biology*, 38: 281-284.
- Linsmaier E. M., Skoog F. (1965). Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum*, 18: 100-127.
- Lloyd G., McCown B. H. (1981). Commercially-feasible micropropagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *International Plant Propagator's Society Proceedings*, 30: 421-427.
- Mariat F. (1948). Influence des facteurs de croissance sur le développement et la différenciation des embryons d'orchidées. *Revue Genetique et Botanie*, 55: 229-243.
- Mishiba K., Mii K. (2000). Polysomaty analysis in diploid and tetraploid *Portulaca grandiflora*. *Plant Science*, 156: 213-219.

- Morel G. M. (1960). Producing virus-free cymbidiums. *American Orchid Society Bulletin*, 29: 495-497.
- Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15: 473-497.
- Nitsch C., Nitsch J. P. (1967). The induction of flowering *in vitro* in stem segments of *Plumbago indica* L. II. The production of reproductive buds. *Planta*, 72: 371-384.
- Oyamada T. (1989). Effects of mineral nutrient composition on the proliferation of *Cymbidium* PLBs cultures *in vitro*. *Scientific Reports of the Faculty of Agriculture, Meijo University*, 25: 27-34.
- Quorin M., Lepoivre P. (1977). Improved media for *in vitro* culture of *Prunus* sp. *Acta Horticulturae*, 78: 437-442.
- Samantaray S., Rout G. R., Das P. (1995). An *in vitro* study on organogenesis in *Trema orientalis* (Blume) Linn. *Plant Science*, 105: 87-94.
- Schenk R. U., Hildebrandt A. C. (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Canadian Journal of Botany*, 50: 199-204.
- Sharma A., Tandon P. (1992). *In vitro* culture of *Dendrobium wardianum* Warner: morphogenetic effects of some nitrogenous adjuvants. *Indian Journal of Plant Physiology*, 35: 80-85.
- Shimasaki K., Uemoto S. (1990). Comparative organogenesis between terrestrial and epiphytic *Cymbidium* species. *Journal of the Faculty of Agriculture, Kyushu University*, 32: 31-39.
- Shimasaki K., Ishikawa K., Fukumoto Y. (2003). Effect of ultrasonicated water treated with quartz porphyry "bakuhan-seki" on organogenesis in protocorm-like body (PLB) of *Cymbidium* and *Phalaenopsis* species cultured *in vitro*. *Environmental Control in Biology*, 41: 295-299.
- Stancato G. C., Faria R. T. (1996). *In vitro* growth and mineral nutrition of the lithophytic orchid *Laelia cinnabarina* Batem. (Orchidaceae) I. Effects of macro and microelements. *Lindleyana*, 11: 41-43.
- Tsukamoto Y., Kano K., Katsuura T. (1963). Instant media for orchid seed germination. *American Orchid Society Bulletin*, 32: 354-355.
- Vacin E., Went F. W. (1949). Some pH changes in nutrient solution. *Botanical Gazette*, 110: 605-613.
- White P. R. (1933). Plant tissue cultures. Results of preliminary experiments on the culturing of isolated stem-tips of *Stellaria media*. *Protoplasma*, 19: 97-116.
- Wimber D. E. (1963). Clonal multiplication of cymbidiums through tissue culture of the shoot meristem. *American Orchid Society Bulletin*, 32: 105-107.