

POLYPLOIDY INDUCTION AND PROTOPLAST ISOLATION FROM PROTOCORM LIKE BODIES IN ORCHIDS

GROSSO V.*, NARDI L.** , FARINA A.* , ARACRI B.*** , PASHKOULOV D.*** , GIORGI D.* ,
LUCRETTI S.*

*) ENEA Centro Ricerche Casaccia, Unità Tecnica AGRI, Laboratorio GEN, Via Anguillarese 301,
00123 Roma (Italy)

**) ENEA Centro Ricerche Casaccia, Unità Tecnica RAD, Laboratorio FARM, Via Anguillarese 301,
00123 Roma (Italy)

***) AMBIOTECH-FLORAMIATA, Via Vera19, 00142 Roma (Italy)

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Orchidaceae is one of the most highly developed monocotyledonous families, their pot plant and cut flower production have high economical value in international flower markets. So the genetic improvement in orchids is very important to obtain new varieties and hybrid combinations commercially attractive. One way to introduce new variability is to double the ploidy level of the wild and commercial varieties. Polyploidy is a very useful tool in ornamental plant breeding. It may allow to overcome barriers to hybridization, to restore hybrid fertility by the creation of allopolyploids, to enhance pest resistance and disease tolerance in allopolyploids or to create larger plants with an enhanced vigor. In orchids has been reported that polyploid plants have larger flowers, show extended blooming time and develop flowers several times a year. Polyploids may be generated through the use of spindle formation inhibitors.

In this work we use colchicine and amiprofos-methyl [APM; O-methyl-O-(4-methyl-6-nitrophenyl)-N-isopropyl-phosphorothioamidate] as spindle inhibitors on culture of *Dendrobium* protocorm like bodies (PLBs). PLBs cultures were developed into liquid and solid medium and their ploidy was assessed in combination with the flow cytometric approach that allow us to do a fast and early screening of the treatment effects. We were able to identify which treatments were more effective and less harmful to our explants thus avoiding unnecessary mutagenic treatments and subculturing of unaffected materials .

We have also developed a protocol for the protoplasts isolation from PLBs with the final aim to isolate polyploid PLB protoplasts by means of flow sorting, This procedure allows us to recover a viable subpopulation of protoplasts enriched for higher ploidy level for further attempts on regeneration and whole plant recovery, thus reducing the chance for chimera formation.

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