

## **In vitro propagation and detection of somaclonal variation in *Phalaenopsis gigantea* as affected by chitosan and thidiazuron combinations**

### ABSTRACT

Protocorm-like bodies (PLBs) multiplication is one of the most preferable in vitro methods to increase the number of orchids that produce very few seeds or seeds that are not able to germinate. In the present study the effects of chitosan and thidiazuron (TDZ) combinations on multiplication, differentiation, and genetic stability of *Phalaenopsis gigantea* PLBs were investigated using different media. Initial PLBs were cultured in solid New Dogashima (ND) medium and Vacin and Went (VW) medium supplemented with different concentrations of chitosan (0, 5, 10, 15, 20, and 25 mg·L<sup>-1</sup>) and TDZ (0, 0.1, and 0.5 mg·L<sup>-1</sup>). The highest mean number of PLBs (353 PLBs) was observed in ND medium with 10 mg·L<sup>-1</sup> chitosan and 0.1 mg·L<sup>-1</sup> TDZ combination after 20 weeks of culture. Some PLBs differentiated into mature PLBs with a profusion of leaves on the apical region, and tiny plantlets started to develop after 10 weeks of culture. The highest mean number of shoots was observed in VW supplemented with 10 mg·L<sup>-1</sup> chitosan and 0.5 mg·L<sup>-1</sup> TDZ (16 shoots). Intersimple sequence repeat (ISSR) markers were used to determine the genetic fidelity among mother plant and PLBs obtained from each subculture stage of solid ND medium supplemented with 10 mg·L<sup>-1</sup> chitosan and 0.1 mg·L<sup>-1</sup> TDZ (the optimal treatment for PLB proliferation). Dissimilarity of 5% occurred between the mother plant and PLBs obtained after 16 weeks of culture. The range in the similarity coefficient varied from 0.80 to 1.0, and only 20% dissimilarity occurred between mother plant and PLBs after 20 weeks of culture.

**Keyword:** Chitosan; Intersimple sequence repeats; *Phalaenopsis gigantea*; Protocorm-like bodies; Thidiazuron; Vacin and went medium