The use of paclobutrazol to stimulate multiple shoot formation in *Clivia miniata* (Lindley) Regel *in vitro*

5.1 Summary

Clivia has been propagated *in vitro* with varying degrees of success using embryos and fruit wall material as explants but the methods are relatively slow. However, commercial protocols for propagation exist, but have not been published. After observing the stimulatory effect which paclobutrazol (PAC) had on shoot formation *in vivo,* the same technique was attempted *in vitro*. Seeds were germinated on a MS medium supplemented with 20g / l sucrose, 3g / l agar, 0.6g / l Gelrite™, 1g / l myo inositol, 1.4mg / l 2,4-D, 2mg / l benzyl adenine and 3mg / l kinetin. After seven months, the seedlings were dipped in paclobutrazol (PAC) at concentrations of 125 - 1 000 ppm before being placed on fresh medium. Five months following application of PAC, there was no evidence of multiple shoot formation and mortality was approximately 70%.

5.2 Introduction

Moderate success has been achieved with the *in vitro* propagation of *Clivia*. Zygotic embryos from seeds could be used as the explant material for *C. miniata* (Wang, Li & Yang, 1995, Wang, 1998) and for *C. nobilis* (Min & Jinsheng, 1984). For purposes of propagating a mature plant, fruit wall material could be used (Finnie, 1998). However, seasonal availability of fruit walls at the right developmental stage together with slow plantlet regeneration hampered the development of a commercial protocol. The same conclusion was reached in another study (Chapman, 1999). Nevertheless, it appears that *Clivia* is successfully propagated *in vitro* on a commercial scale in Japan (Smithers, 2000) but the protocols used have not been published. The aim of this study was to examine whether paclobutrazol (PAC) could be used for generation of multiple shoots *in vitro*.

5.3 Materials and methods

Seeds were used as the explant material and were germinated *in vitro* following surface sterilisation. Disinfestation occurred in a 2.5% NaOCl solution (JIK™) for 10 minutes followed

by rinsing 3 times in sterile distilled water. A Murashige & Skoog (MS) medium supplemented with 20g / l sucrose, 3g / l agar, 0.6g / l Gelrite™, 1g / l myo inositol, 1.4mg / l 2,4-D, 2mg / l benzyl adenine and 3mg / l kinetin, adjusted to pH 5.6, was used. The higher relative cytokinin concentration was chosen in order to promote development of shoots and to suppress root growth. After seven months, plants were dipped in autoclaved PAC solutions and placed on fresh medium. PAC concentrations of 0,125, 250, 500 and 1000 ppm were used with 20 replicates of each treatment.

5.4 Results and discussion

After 5 months of culture there were no significant signs of new growth or any indication of formation of multiple shoots. Approximately 70 % of explants exhibited necrosis of the shoot in all treatments, including the control. The cause of the low survival rate was probably related to the medium composition, since much better growth was achieved when explants from the same batch were grown under the same conditions but with a different medium (Swanevelder, 2000). Furthermore, the formulation of PAC (Cultar™, 250g / l active ingredient) could not be filter sterilised and therefore necessitated autoclaving. The effect which this may have had on its efficacy is not known.

5.5 References

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