Somatic embryogenesis and plant regeneration from bulb, leaf and root explants of African blue lily (Agapanthus praecox ssp. minimus)

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Abstract

The African blue lily (Agapanthus praecox ssp. minimus) is a valuable plant, reported to contain medicinal compounds such as saponins, sapogenins and phytoecdysteroids, besides gaining popularity as an ornamental and landscape species. This paper reports on high efficiency and rapid in vitro propagation of Agapanthus praecox ssp. minimus via somatic embryogenesis from tissues derived from sterile root, leaf and bulb explants obtained from one-month-old aseptic seedlings of this species. Detailed observations on developmental stages of somatic embryos (from globular to coleoptilar) of this monocotyledonous species were also reported in the present investigation. Friable callus which gave rise to high plant regeneration rate (95%) was lucratively produced within 1-2 months after the explants were cultured on Murashige and Skoog (MS) medium supplemented with picloram, 2,4-D, TDZ and combinations of NAA and BAP (0.5 mg/L to 2.0 mg/L). Subsequently, the proliferated calli were transferred onto the same media compositions at 2 weeks interval to encourage extensive production of somatic embryos, followed by transferring to plant growth regulator-free MS medium for complete plant regeneration. Analysis of results showed that explant types highly influenced the degree of response to hormone treatments, whereby root explants were the most responsive. Picloram (a systemic herbicide) was the most effective in inducing somatic embryogenesis from leaf explants, while 2,4-D had excellent influence on root and bulb explants. Further development of somatic embryos to complete plantlets was achieved on MS basal medium. Regenerated plantlets were then hardened and acclimatized on black (peat) soil with 86.67 ± 6.31% survival rate. Scanning electron microscopic studies on leaf tissues showed no morphological variations between the in vivo and in vitro grown plants, hence mass propagation through tissue culture for true-to-type production of this species is feasible.

Keywords: Agapanthus praecox ssp. minimus; somatic embryos; in vitro regeneration; scanning electron microscopy; acclimatization.

Abbreviations: BAP - 6-benzyl aminopurine; 2,4-D - 2,4- dichlorophenoxyacetic acid; NAA - α-naphthalene acetic acid; PIC – picloram; TDZ – thidiazuron.

Introduction

Originating from South Africa, the Agapanthus species, especially the violet/blue blooming flowers of Agapanthus praecox ssp. minimus have been increasingly popular around the world for ornamental and landscaping purposes. A. praecox, or the ‘African lily’ or the ‘lily of the Nile’ (Suzuki et al., 2001; Mor et al., 1984) have long been used by the native South African for medicinal purposes, especially to treat prolonged labour (Varga and Veale, 1997), and has been dubbed as the plant of fertility and pregnancy. The plants within the genus Agapanthus were also found to possess antifungal properties (Pretorius et al., 2002; Singh et al., 2008; Tegegne et al., 2008) as well as phytoecdysteroids, although lesser in A. praecox compared to other species within the genus (Savchenko et al., 1997). Furthermore, A. praecox flowers were also found to contain anthocyanin, with potentials to be utilized as natural colourant (Bloor and Falshaw, 2000; Yaacob et al., 2011). Somatic embryogenesis offers many advantages as compared to conventional breeding, especially in terms of mass propagation of selected genotypes through the use of bioreactors. Furthermore, somatic embryos can be utilized for production of synthetic seeds, whereby the somatic embryos were encapsulated and germinated as natural seeds (Norgaard et al., 1993). Somatic embryogenesis also allows higher yield of plants to be produced in shorter time and may be cryopreserved for future uses (Norgaard et al., 1993). In addition, somatic embryogenesis also serves as a very good model to study embryo development in plants, especially in terms of the biochemical and morphological changes that may have occurred due to the changes in gene expression patterns resulting from somatic embryogenesis process (Santos et al., 2005). Somatic embryogenesis is also a very beneficial tool for rapid production of clonal elite cultivars, where an embryo or a plant can be derived from a single somatic cell (Vicent and Martinez, 1998). Somatic embryogenesis had been reported in Picea abies (Hakman and Von Arnold, 1985; Chalupa, 1985) as well as in various conifers and cypads (Jain et al., 1995). On the other hand, the use of bioreactors for mass propagation through somatic embryogenesis had been successful, as reported for Coffea canephora (Robusta) and Coffea arabica (Ducos et al., 2007). Li et al. (1998) had also achieved somatic embryogenesis in Theobroma cacao grown on DKW (Driver