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Review

In vitro propagation of African violet: A review

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A B S T R A C T

African violet (*Saintpaulia ionantha* H. Wendl.) (Gesneriaceae) is a popular ornamental pot plant that is easy to culture *ex vitro* and *in vitro* relative to other herbaceous ornamentals. This quality makes it an ideal system for *in vitro* regeneration experiments. This review summarizes the studies that have been conducted on the *in vitro* culture and micropropagation of this plant. Although shoot regeneration from internodes, floral buds, anthers and protoplasts has been achieved, leaf blades and petioles have been a popular source tissue for regeneration. An effective and reproducible protocol for the direct induction of shoots, without the formation of any intermediary callus, involves the use of a cytokinin like BA or kinetin, usually in combination with an auxin like NAA, both within a concentration range of 0.1 to 1 mg/L, and on a Murashige and Skoog basal medium. Shoots can form in both light and darkness, but most effectively in the light, and rooting can be induced from shoots, even in the absence of an auxin, although a low concentration (0.1–0.5 mg/L) of NAA or IAA is recommended. African violets acclimatize easily, even in a simple soil-based substrate, and can flower within 4 months of transfer to *ex vitro* conditions. These protocols, including somatic embryogenesis and cryopreservation, may also benefit the *in vitro* culture and conservation of wild species of *Saintpaulia*.

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1. Introduction

The genus *Saintpaulia* belongs to the family Gesneriaceae, which consists mainly of tropical shrubs and herbaceous plants. Other popular

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ornamental plants from the same family include Cape primroses (*Streptocarpus*) and gloxinias (*Sinningia*). The genus name *Saintpaulia* was named after the Baron von Saint-Paul-Illaire, who introduced this plant from Tanginyika (Tanzania) to Europe in 1892 (Kimmins, 1980; Heywood, 1996; Miranto, 2005). The genus *Saintpaulia* was first described as new to science in 1893 by Hermann Wendland, after which the economic value of the genus was recognized (Miranto, 2005). This genus has attractive foliage and beautiful flowers, which are zygomorphic with two upper and three lower lobes, of various colors (Tatsuzawa and Hosokawa, 2016; Teixeira da Silva et al., 2016). *Saintpaulia* has a very short corolla tube and yellow protruding anthers, probably associated with buzz pollination (Harrison et al., 1999). All species of *Saintpaulia* are enantiostylous, *i.e.*, the style is strongly deflected to the left or right of the main floral axis, a feature often linked to buzz pollination (Miranto, 2005). The genus *Saintpaulia* is endemic to Kenya and Tanzania and includes 25 species (The Plant List, 2017). Eight species of *Saintpaulia*, including *S. ionantha* are listed in the IUCN Red List of Threatened Plants (IUCN SSC, 2014). They are listed as critically endangered (*S. watkinsii*, *S. ulugurensis*, *S. teitensis*), endangered (*S. goetzeana*, *S. inconspicua*, *S. shumensis*), and vulnerable (*S. pusilla*). *S. ionantha* is classified as near threatened as its natural populations are decreasing due to forest clearance for agricultural expansion. *S. ionantha* H. Wendl., commonly known as African violet (Moore, 1957), is a popular commercial and ornamental plant with many cultivars. The chemistry and medicinal uses of *Saintpaulia* have been poorly explored. The secondary metabolite, cyanidin, was identified in the leaves of *in vivo* and *in vitro* grown *S. ionantha* microshoots, and cyanidin content for *in vivo* and *in vitro* extracts was 98.16 and 85.04 mg/g dry tissue, respectively (Al-Sane et al., 2005). The presence of cyanidin in the leaves of *in vitro* grown *S. ionantha* highlights the potential of producing this valuable pigment *in vitro*.

The US potted plant market was valued at US$ 788 million in 2014 (US$ 810 million in 2015; USDA, 2016) of which African violets are accounted for US$ 4.07 million (US$ 4.16 million in 2015; USDA, 2016), with a 1% increase from 2013 (USDA, 2015). The characteristics that have made African violet such a popular houseplant are its visual appeal, shade tolerance, and ability to flower under artificial light (Grout, 1990). One of the reasons for the popularity of this plant is its ease of propagation. *Ex vitro*, roots form from leaves or pedicels when placed only in water or river sand, while few cultivars are seed-derived (Chen and Henny, 2009). Even though African violet cultivars have become an important asset to the floriculture industry, some wild *Saintpaulia* species face extinction (Eastwood et al., 1998). Thus, lessons learnt from the *in vitro* tissue culture of this ornamental may benefit the propagation of such wild species. The objective of this review is to shed light on the rich history of *in vitro* tissue culture of this popular potted plant as a way to offer perspectives for the sustained *in vitro* culture and conservation of African violet and of closely related *Saintpaulia* species.

2. Organogenesis *in vitro*

Organogenesis *in vitro* of any plants species is affected by explant type, source, and disinfection procedure, culture medium (types and concentrations of salts, plant growth regulators (PGRs), carbon source, gelling agent), temperature, photoperiod, light source and type, among other factors, including in African violet (Teixeira da Silva et al., 2016). Organogenesis *in vitro* was first studied by Kukułczanka and Suszyńska (1972) who induced adventitious shoot regeneration from leaf explants of *S. ionantha* var. *alba*, although only morphological observations were made. In 1973, Kukułczanka and Kwiecień (1973) noted that the addition of morphactin IT 3456 inhibited the regeneration from leaves and caused developmental abnormalities in shoot buds. Vazquez et al. (1977) reported that intact ovaries and leaf discs cultured on PGR-free MS medium could produce adventitious shoots within 60 days whereas sepals and petals did not shows any sign of morphogenesis. The addition of 0.2 or 0.4 mg/L BA could induce adventitious shoots from sepals and petals (Vazquez et al., 1977). Since these early studies, over 30 studies on the *in vitro* regeneration of African violet and related *Saintpaulia* species have been conducted, with details described in Supplementary Table 1. Broadly speaking, the *in vitro* culture of African violet is most effective through one of two paths (Figs. 1, 2) where shoots are regenerated directly from the explants (Fig. 2A, C) or indirectly *via* callus formation (Fig. 2B).

The easy regeneration ability of African violet was extensively explored *in vitro* (see details for different explants in Supplementary Table 1) with regeneration protocols having been reported for leaves or leaves with petioles (Fig. 1), petioles, internodes, floral buds, anthers, sub-epidermal tissue, and protoplasts derived from petiole-derived callus, leaves, or young shoots from leaf cultures. Lercari et al. (1986) were able to induce shoot buds from *in vitro* leaves on Linsmaier and Skoog (1965) medium in the absence of PGRs simply by manipulating the spectral light quality.

Thin cell layers (TCLs) (Teixeira da Silva and Dobránszki, 2013, 2014, 2015), longitudinal TCLs being the most effective, have seen limited use in African violet tissue culture: petiole TCLs were employed by Murch et al. (2003) for somatic embryogenesis, and Kosari-Nasab et al. (2014) used petiole and pedicel TCLs to induce callus and shoots.

In a fairly unique experiment involving rare earth elements (REEs), Xie (1999) found that MS with cerium nitrate ($Ce(NO_3)_3$) facilitated shoot and root induction, inducing more and longer roots as well as roots with more root hairs in the presence of this REE. Lower concentrations (10, 20 mg/L) of $Ce(NO_3)_3$ improved shoot differentiation, leaf and root induction and leaf growth but higher concentrations (>20 mg/L) suppressed shoot differentiation and root growth. Wang et al. (2005) discovered that REEs had a definite stimulating effect on tissue culture and rapid propagation of *S. ionantha* with different REEs showing different effects. MS with 1.0 mg/L BA and 0.1 mg/L NAA supplemented with 100 mg/L Pr or 200 mg/L Nd chlorate, respectively, were most suitable concentrations for callus proliferation, inducing 10.4% and 59% more than the control, respectively. Half MS with 0.01 mg/L NAA supplemented with 200 mg/L Pr or 800 mg/L Nd chlorate were most suitable concentration for root induction in 100% of explants compared with 39.74% on 1/2 MS with 0.01 mg/L NAA without REEs after 10 days of culture. However, the range of effective concentrations on shoot growth was limited: shoot growth occurred only when the concentration was too high (1000 or 1200 mg/L) while concentrations under 800 mg/L induced shoots to grow or had some stimulative effect on shoot formation.

The South Korean literature also provides a niche of information that supplements studies from other countries (see Supplementary Table 1 for details). Pak et al. (1983) examined three detergents with or without Tween-20 for surface sterilization and found that Clorox 10% with 1% Tween-20 caused least microbial contamination (7% contamination with fungi or bacteria *vs.* 24% contamination without Tween-20). So (1983) reported that 1.0 mg/L BA and 0.25 mg/L NAA in MS medium was the best PGR combination for the induction and growth of adventitious shoots as well as callus formation: 23.8–24.3 shoots/explant, 3.9–4.1 g fresh weight, and abundant callus formation. Pak and Kwack (1987c) noted that MS medium containing 100 mg/L *myo*-inositol and 880 mg/L Ca (double the concentration in standard MS medium) produced highest shoot formation and callus growth: 25.3 *vs.* 17.9 shoots/explant, 4.42 *vs.* 3.90 g/explant, excellent *vs.* good callus development. Lee (1986) noted that pretreatment with 200 mg/L BA for 18 min followed by 0.2 mg/L NAA in MS medium was the best combination for shoot formation and callus growth while 0.2 mg/L NAA with 0.2 mg/L BA and 0.2 mg/L NAA were the most effective PGR combinations for shoot and root growth, respectively. Application of TDZ at a low concentration (0.001–0.002 mg/L) formed shoots more effectively than BA in both petioles and leaves (Lee, 1992). In terms of somaclonal variation of micropropagated plantlets, Pak and Kwack (1987b) observed less somaclonal variation (2% and 3%, respectively in

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