African Journal of Biotechnology Vol. 9(45), pp. 7583-7588, 8 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1031
ISSN 1684–5315 © 2010 Academic Journals

Review

The potential of developing an *in vitro* method for propagating Strelitziaceae

J. J. North, P. A. Ndakidemi* and C.P. Laubscher

Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa.

Accepted 30 September, 2010

***Strelitzia* spp. are highly valued as cut flowers and are of significant commercial value. Despite high demands, they have not been widely spread due to production constraints and are one of the few important cut flower plants for which no uniform cultivars are available. The conventional methods of propagation are very slow due to the plants low rate of multiplication. Large scale propagation and cloning is therefore needed to exploit its potential. Despite the plants commercial importance, a method for micropropagation has not yet been established. Tissue culture attempts of this plant have failed due to the oxidative browning of explants. Wounded tissues release polyphenolic compounds which are detrimental to further development of explants. Only partial success and a low rate of multiplication have been obtained. This review explores the possibilities of developing an *in vitro* method for the successful propagation of *Strelitzia* spp.**

Key words: *Strelitzia* spp., activated charcoal, antioxidants, auxins, cytokinins, dark incubation, immature embryos, media composition, wounding.

INTRODUCTION

The bird of paradise (S*trelitzia*) is an important ornamental monocotyledonous plant of South African origin (Chand, 2008). This tropical perennial is a beautiful plant of significant commercial value (Paiva et al., 2004). The exotic features of its colourful flowers, the long length of the stem and the high post-harvest durability result in it being highly valued as a cut flower (Wood, 1995). *Strelitzia reginae* has been one of the most sought after cut flowers destined for exportation from developing countries (Criley, 1988). However, its commercial exploittation and success is limited by its low rate of multiplication (Ziv and Halevy, 1983). Propagation is achieved either by seed or vegetatively by division. Both of these conventional propagation methods are very slow (Karnataka, 2008).

Propagation by seed is undesirable as *Strelitzia* presents a slow development, requiring 4 to 7 years to start producing flowers (Ziv and Halevy, 1983). Furthermore, from pollination to seed harvesting, a further 5 to 6 months is required (Criley, 1988). However, the greatest difficulty in the propagation of *Strelitzia* spp. via seed is the dormancy of seeds (Garcia, 2006). A germination inhibitor is thought to occur within the seeds (van de Venter and Small, 1975), which prolongs the time and results in a low percentage of seed germination (Garcia, 2006). The limited production of seeds obtained per plant must be taken into consideration. There is also a great degree of genetic variation in plants developed from seed (van de Pol and van Hell, 1988).

For vegetative propagation by division, plants of at least 10 years old must be used (Ziv and Halevy, 1983). This method of dividing naturally developed branches is limited by a low rate of multiplication, which was determined to be 0.5 to 1.5 divisions per branch per year (Vonk Noordegraaf and van der Krogt, 1976).

Thus, both methods limit the large scale production which is needed to exploit the plants potential and to enhance its improvement by cultivar development. Due to these constraints on production, it is one of the few important cut flower plants for which no uniform cultivars are commercially available (Ziv and Halevy, 1983).

*Corresponding author. Email: NdakidemiP@cput.ac.za.

Abbreviations: BAP, 6-Benzylaminopurine; **NAA,** naphthaleneacetic acid; **MS,** Murashige and Skoog.

Tissue culture could be more promising than other conventional methods of multiplying *Strelitzia* (Promtep, 1981). A reliable and advanced propagation and cloning method would greatly contribute to overcoming the limitations this species poses to the horticultural and landscaping industry worldwide (Ziv and Halevy, 1983). Despite the plants commercial importance, a reliable method for micropropagation has not yet been developed (Chand, 2008).

The lack of successful tissue culture techniques for the propagation of *S. regina* was due to oxidative browning of the explants as reported earlier by Ziv and Halevy (1983). Wounded tissue releases polyphenolic compounds, which diffuse into the culture medium (Strosse et al., 2009). These undesirable exudates were found to be detrimental to the development of the explants, as they promoted the onset of necrosis (Ziv and Halevy, 1983). Terminal and axillary meristems of *S. reginae* were used by Ziv and Halevy (1983) but success depended on the use of antioxidants to prevent browning. This is a destructive method as plants are destroyed when their terminal and axillary buds are excised. Thus, it is an unsuitable method when starting material is limited or in the culture of rare plants. Paiva et al. (2004) reported the failure of *in vitro* development using axillary buds and leaf segments, irrespective of the applied treatments. Phenolic oxidation was identified as a crucial problem. Thus, it was not possible to reproduce the protocol developed by Ziv and Halevy (1983) for the *in vitro* propagation of *S. reginae* from axillary buds. However, the germination of immature embryos inoculated *in vitro* resulted in well-formed and complete plants (Paiva et al., 2004). There are no reports on success or attempts made in the stimulation of axillary bud proliferation from embryo-derived plantlets.

In several investigations, only partial success and a low rate of multiplication were obtained, indicating major problems with growing and multiplying this plant *in vitro*. Furthermore, the successful regeneration from zygotic embryo explants has not been reported, suggesting that the protocols developed so far have not been efficient with respect to the growth and large scale multiplication of this plant.

The objective of this review is to discuss possible techniques for the rapid clonal propagation of *Strelitzia* spp., using embryos as the starting material. Zygotic embryo culture could induce faster growth and a higher germination rate (Chang and Yang, 1996; Bürün and Çoban Poyrazoğlu, 2002). The use of embryos as explants will allow the variation in seeds (Foolad and Jones, 1992; Larkin et al., 1984) to play an important role in the breeding cycle and development of cultivars, reducing seed dormancy. Furthermore, the limited wounding of the explants may remedy the production of phenolic exudates and the excessive need for antioxidants during the crucial initial stages of plant development. In addition, it is not a destructive method. There is no need to destroy plants in order to gain starting material for the culture.

Thus, indicating the possibility of developing an efficient method of clonal propagation and mass production of this kind of plant in the future.

POSSIBLE EFFECTS OF VARIOUS MEDIA COMPOSITIONS ON THE *IN VITRO* GERMINATION OF IMMATURE EMBRYOS OF *STRELITZIA* SPP.

The germination of excised embryos is influenced by the maturity of embryos at excision, the composition of the culture medium (Johri and Rao, 1984; Diro and van Staden, 2004) and genetic variation (Pierik, 1979).

Embryos excised from developing seed at or near maturity are completely autotrophic (Gamborg and Phillips, 2002), whereas, immature embryos require a far more critical medium composition compared with mature embryos (Pierik, 1979). The younger the embryo, the more complex is its nutritional requirements (Bajaj, 1977). The chances of success in this type of culture depend largely on the developmental stage of the excised embryo (Razdan, 1993). Paiva et al. (2004) reported that the best time for seed collection and embryo excision for *S. reginae* was 20 weeks after pollination. However, the most important aspect in culturing embryos is to develop and clearly define a culture medium that can sustain growth and development (Chawla, 2002). In less than optimum medium, the immature embryos may fail to survive, turn into undifferentiated callus, or germinate prematurely (Gamborg and Phillips, 2002).

A variation of macro- and micro-nutrients

The MS medium of Murashige and Skoog (1962) is a salt composition that supplies the needed macro- and micronutrients. To achieve growth differentiation, concentrations of inorganic nutrients must be optomized such that the medium meets the requirements of the cells or tissues used (Chawla, 2002). This is applicable to the nutritional requirements of an embryo and its developmental stage. The *in vivo* development of an embryo consists of two phases:

(1) The heterotrophic phase: An early phase wherein the embryo is nutritionally dependent on the endosperm and maternal tissues.
(2) The autotrophic phase: A later phase in which the embryo is metabolically capable of synthesising substances required for its growth, thus is fairly independent for nutrition.

Thus, while relatively mature embryos can grow on a simple inorganic salt medium supplemented with a carbohydrate source, the nutritional requirements of relatively immature embryos is complex (Bajaj, 1977). The critical stage at which the embryo passes from the heterotrophic phase into the autotrophic phase varies with the species (Razdan, 1993).

The developmental phase and nutritional requirements

of *Strelitzia* embryos 20 weeks after pollination is uncertain. Therefore, it is necessary to evaluate a variation in inorganic nutrients (macro and micronutrients) and vitamins to obtain optimum embryo growth and development.

The use of activated charcoal as a phenolic adsorbent

The use of tissue culture for the propagation of *S. reginae* has invariably failed due to oxidative browning of the explants (Ziv and Halevy, 1983). During the initial stages of culture development, the production of polyphenols is excessive (Pan and van Staden, 1998). The polyphenol exudate that diffuses into the medium was found to be detrimental to the further development of the explants, which become necrotic and die (Ziv and Halevy, 1983).

The identified problem of phenolic oxidation has also been reported in *Musa* and *Ensete* spp. (Zeweldu and Ludders, 1998; Diro and van Staden, 2004; Birmeta and Welander, 2004), which are related to *Strelitzia* (Strosse et al., 2009). Activated charcoal, used in tissue culture media to adsorb inhibitory substances, has been used with success in Musaceae and Strelitziaceae (Diro and van Staden, 2004; Ziv and Halevy, 1983). In *Ensete*, a genus of Musaceae, the use of activated charcoal enabled zygotic embryos to regenerate healthy seedlings. The inclusion of activated charcoal into the media reduced oxidative browning, promoted germination of the embryos and improved the growth of seedlings (Diro and van Staden, 2004). In *S. reginae*, Ziv and Halevy (1983) found activated charcoal in combination with antioxidant treatments, effective in controlling oxidative browning of terminal and axillary buds. However, there are no reports on the use of activated charcoal on the zygotic embryos of *S. reginae* or other *Strelitzia* spp.

Strelitzia competence in responding to *in vitro* culture is dependent on reducing oxidative browning of the explants (Ziv and Halevy, 1983). Thus, an investigation into the addition of activated charcoal to culture media is of vital importance in the development of a suitable technique for the germination of excised embryos, as has been done in *Ensete*.

POSSIBLE EFFECTS OF AUXIN AND CYTOKININ CONCENTRATIONS ON INDUCING MULTIPLE SHOOTS FROM AXILLARY BUDS

This involves the use of plant growth regulators in an attempt to stimulate the development of axillary buds, which are usually present in the axil of each leaf (Chawla, 2002). Axillary bud proliferation exploits the normal ontogenetic route for branch development by lateral meristems (Gamborg and Phillips, 2002). However, many axillary meristems remain dormant *in vivo* (in nature) if the type of branching for a particular species displays apical dominance (Razdan, 1993). Apical dominance suppresses the growth and development of axillary buds in the presence of a terminal bud (Chawla, 2002).

In *Strelitzia*, there is an absolute absence of branching from axillary buds *in vivo*. This may be as a result of a strong apical dominance effect (van de Pol and van Hell, 1988). Therefore, a method of eliminating apical dominance *in vitro* to promote branching, is required to increase the multiplication rate of *Strelitzia*.

Since the mechanism of apical dominance has been demonstrated to be under the control of various growth regulators, the proportions of these substances in the media can be manipulated to break dormancy and enhance shoot formation (Razdan, 1993). The growth regulators, auxins and cytokinins, are of particular significance in *in vitro* culture (Pierik, 1979). Cytokinins are concerned with the modification of apical dominance (Razdan, 1993). A high cytokinin concentration promotes axillary shoot formation by decreasing apical dominance (Pierik, 1979; Dodds and Roberts, 1995; Chawla, 2002). Although cytokinin induces the growth of buds, auxin is required in the culture media. Most cultures require a combination of these two compounds for growth regulation (Dodds and Roberts, 1995). Usually the manipulation and variation of auxin and cytokinin levels can successfully alter growth behaviour in plant cultures (Dixon and Gonzales, 1994). For axillary shoot formation, a low auxin concentration, together with a high cytokinin concentration is required (Pierik, 1979; Razdan, 1993).

The cytokinin treatment can be varied, that is, the selection of the type of cytokinin and concentration (Pierik, 1979). In *Musa,* a relative of *Strelitzia*, 6-benzylaminopurine (BAP) is said to be the preferred cytokinin (Banerjee and de Langhe, 1985). It has dramatically influenced axillary shoot formation in various *Musa* spp. (Wong, 1986; Arinaitwe et al., 2000; Srangsam and Kanchanapoom, 2007). Earlier reports revealed that there is a strong synergistic effect of BAP-1-naphthaleneacetic acid (NAA) interactions (Novak et al., 1989; Okole and Schulz, 1996; Cote et al., 2000; Khalil et al., 2002; Srangsam and Kanchanapoom, 2007).

The concentration and combination of auxin and cytokinins in the nutrient medium is a key factor which determines successful plant regeneration (Razdan, 1993). To improve the success of axillary bud proliferation for *Strelitzia* spp., the optimal balance between these two groups of growth regulators needs to be determined. Thus, indicating the importance of a study into BAP-NAA interactions.

POSSIBLE EFFECTS OF WOUNDING ON SUPPRESSING APICAL DOMINANCE IN ORDER TO INDUCE MULTIPLE BUD DEVELOPMENT

Apical dominance inhibits the development of axillary meristems (Razdan, 1993). In nature, axillary meristems are generally the source of bud formation when leaders

are damaged (Burrows, 1989). This indicates the positive effects of meristem wounding and even apical bud removal on stimulating the growth of axillary buds.

In *Strelitzia*, the absence of branching from axillary buds, may be due to a strong apical dominance effect. The introduction of *in vivo* branching can increase the multiplication rate of *Strelitzia.* This can be achieved by the removal of the apical dome. This method of eliminating apical dominance is practised in vegetative propagation by the division of branches known as fans (van de Pol and van Hell, 1988).

Similarly to *in vivo* methods, an *in vitro* method to reduce apical dominance and promote axillary bud development is needed. In *Musa* and *Ensete*, success has been achieved through wounding of the meristem region (Jarret et al., 1985; Gupta, 1986; Birmeta and Welander, 2004). There are no reports on the effects of *in vitro* wounding in Strelitziaceae. However, it is hopeful that axillary meristem development as observed in the *in vivo* methods as well as in the *in vitro* cultures of its relative Musaceae, can be applied for improving axillary bud development in Strelitzia. An investigation into the wounding effects on *Strelitzia* spp. may be significant in the clonal propagation of this plant.

POSSIBLE EFFECTS AN ADSORBENT AND ANTIOXIDANT MAY HAVE ON REDUCING OXIDATIVE BROWNING OF WOUNDED TISSUES

As previously mentioned, the excessive production of polyphenols is a problem frequently encountered during the initial stages of culture develoment (Ziv and Halevy, 1983; Pan and van Staden, 1998; Birmeta and Welander, 2004; Diro and van Staden, 2004). Tissue injury, during explant excision, stimulates the production of phenols (Dodds and Roberts, 1995) as a form of a defence mechanism (Pan and van Staden, 1998). The incorporation of activated charcoal to initiated cultures is most effective in controlling polyphenol oxidation (Ziv and Halevy, 1983; Pan and van Staden, 1998; Chawla, 2002; Birmeta and Welander, 2004; Kiong et al., 2007; Karnataka, 2008). The adsorption of toxic phenols prevents the browning and death of the tissues (Horner et al., 1977; Fridborg et al., 1978; Weatherhead et al., 1979; George and Sherrington, 1984; Madhusudhanan and Rahiman, 2000; Chawla, 2002).

In the (multiplication stage) of culture, wounding techniques will be employed in efforts to induce axillary bud proliferation. Polyphenol exudation is exag-gerated in response to wounding (Birmeta and Welander, 2004). Thus, indicating the need for an adsorbent or the addition of an antioxidant in the culture media.

The beneficial use of activated charcoal as a culture component for the adsorption of toxic substances is established (Teixeira et al., 1994; Veramendi and Navarro, 1996; Pan and van Staden, 1998; Gallo-Meagher and Green, 2002). However, its addition to shoot proliferation media may have adverse effects on growth and development as activated charcoal is able to adsorb high concentrations of growth regulators (Fridborg et al., 1978; Ebert and Taylor, 1990; Nissen and Sutter, 1990; Ebert et al. 1993; Pan and van Staden, 1998; Thomas, 2008), thus reducing their effectiveness in tissue culture. The ratio and concentration of auxins and cytokinins in the medium is a key factor in determining successful plant regeneration (Razdan, 1993). The non-selective adsorption of these compounds by activated charcoal may result in inhibitory effects of growth *in vitro* (Pan and van Staden, 1998; Thomas, 2008).

Due to the instrumental role of auxin-cytokinin supplements on regulating plant growth, it is questionable whether to rely on activated charcoal. Although the complete effects of activated charcoal in the medium are unknown, its addition to the medium, may retard plant growth. This will require the need to evaluate an alternative treatment into experiments involving *Strelitzia* spp. The use of activated charcoal can make a difference in the success or failure of this culture attempt.

Ascorbic acid is an antioxidant used to control oxidation of phenols (Bharadwaj and Ramawat, 1993; Chawla, 2002; Abeyaratne and Lathiff, 2002). Its addition to the medium has reduced blackening of the medium to an acceptable level (Almaz et al., 2001). However, it has been reported not as effective as activated charcoal (Birmeta and Welander, 2004). A comparative study of these two phenolic reducing agents would gain further insight into the adsorption of the growth regulators, BAP and NAA, and identify the most successful way of reducing oxidative browning of wounded tissues. In determining the optimal agent, there is a trade-off between efficient control of browning and the instrumental role of auxin-cytokinin supplementation in the medium.

EFFECTS OF A DARK INCUBATION PERIOD ON REDUCING OXIDATIVE BROWNING OF WOUNDED TISSUES

The limitations of polyphenols on *in vitro* growth and development are evident as discussed previously. Tissues containing relatively high concentrations of phenolic compounds are difficult to culture (Scalbert et al., 1990; Dodds and Roberts, 1995; Khatri et al., 1997; Zweldu and Ludders, 1998; Abeyaratne and Lathiff, 2002; Titov et al., 2006). Success is often dependent upon the ability to reduce the phenolic oxidation reaction to wounding and cutting.

As discussed, adsorbents and antioxidants can be used as a method in reducing browning. Another useful technique is the incubation of cultures in darkness for the initial culture period (Bajaj, 1977; Durand-Cresswell and Nitsch, 1977; Birmeta and Welander, 2004; Titov et al., 2006; Kiong et al., 2007). Maintaining cultures in

darkness suppresses the metabolic sequence as it is known that phenolic oxidation products are formed under illumination (Chawla, 2002).

In *Musa*, *Ensete* and *S. reginae*, the incubation of cultures in darkness is reported to be effective in reducing browning (Ziv and Halevy, 1983; Birmeta and Welander, 2004; Strosse et al., 2009). However, success in these cases was achieved when the dark incubation period was used together with an antioxidant treatment. Obtaining optimal techniques in reducing oxidative browning of *Strelitzia* spp., requires a study into the dark incubation period in conjunction with the use of an adsorbent or antioxidant and the variation of medium composition. The type of phenol reducing agent, its concentration and the specific species are of importance in reducing oxidative browning of wounded tissues. The optimal collaboration of a dark incubation period together with a phenol reducing agent would increase the potential of *Strelitzia* spp. *in vitro* (Ziv and Halevy, 1983; Birmeta and Welander, 2004; Strosse et al., 2009).

CONCLUSION

The *Strelitzia* is an ornamental plant of significant horti-cultural commercial value (Paiva et al., 2004). Despite high demands, it is not widely spread due to the constraints on its production and is one of the very few important cut flower plants for which no uniform cultivars are available. Its horticultural success is limited by the slow conven-tional propagation methods currently used (Ziv and Halevy, 1983). It is commonly propagated vegetatively by division or by seeds. Both these methods of propagation are slow (Karnataka, 2008). From this background an alternative propagation and cloning method is required for the large scale production of *Strelitzia* to exploit its potential as an ornamental plant. The development of a reliable *in vitro* method for propagating *Strelitzia* spp., through the culture of excised embryos and multiple shoot formation, would overcome the constraints this plant poses to the horticultural industry worldwide, thus, greatly contributing to the commercial production of *Strelitzia* spp.

REFERENCES

Abeyaratne WM, Lathiff MA (2002). *In-vitro* propagation of *Rathambala* (*Musa* AAA) and the occurrence of phenotypic variations in the pseudostem. Ann. Sri Lanka Department of Agric. 4: 191-197.

Almaz N, Krens F, Schaart J, Visser B (2001). *In vitro* conservation of enset under slow-growth conditions. Plant Cell Tissue Org. Cult. 66: 107-111.

Arinaitwe G, Rubaihayo PR, Magambo MJS (2000). Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. Sci. Hortic. 86: 13-21.

Bajaj YS (1977). Applied and fundamental aspects of plant cell, tissue and organ culture. Springer, Verlag Berlin Heidelberg.

Banerjee N, De Langhe E (1985). A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (Banana and Plantain), Plant Cell Rep. 4: 351-354.

Bharadwaj L, Ramawat KG (1993). Effect of antioxidants and adsorbents on tissue browning associated metabolism in *Cocculus pendulus* callus cultures. Indian J. Exp. Biol. 31: 715-718.

Bürün B, Çoban Poyrazoğlu E (2002). Embryo culture in Barley (*Hordeum vulgare* L.). Turk. J. Biol. 26: 175-180.

Birmeta G, Welander M (2004). Efficient micropropagation of *Ensete ventricosum* applying meristem wounding: a three-step protocol. Plant Cell Rep. 23: 277-283.

Burrows GE (1989). Developmental anatomy of axillary meristems of *Araucaria cunninghamii* released from apical dominance following shoot apex decapitation *in vitro* and *in vivo*. Bot. Gaz. 150(4): 369-377.

Chand M (2008). Multiple shoot formation from dissected embryos of *Strelitzia reginae* (Bird of Paradise). http://www.urop.uci.edu/ symposium/past_symposia/08/program_a-g.pdf

Chang SH, Yand JC (1996). Enhancement of plant formation from embryo cultures of *Taxus mairei* using suitable culture medium and PVP. Bot. Bull. Acad. Sin. 37: 35-40.

Chawla HS (2002). Introduction to Plant Biotechnology 2nd rev. ed. Science Publishers Inc., Enfield, New Hampshire.

Cote FX, Folliot M, Domergue R, Dubois C (2000). Field performance of embryogenic suspension-derived banana plants (*Musa* AAA, cv. Grande naine). Euphytica, 11: 245-251.

Criley RA (1988). Propagation of tropical cut flowers: *Strelitzia*, *Alpinia* and *Heliconia*. Acta Hort. 226: 509-518.

Diro M, van Staden J (2004). Germination of zygotic embryos and *in vitro* growth of seedlings of wild types of *Ensete ventricosum*. S. Afr. J. Bot. 70(4): 635-639.

Dixon RA, Gonzales RA (1994). Plant Cell Culture*:* A Practical Approach, 2nd rev. ed. Oxford University Press, Oxford.

Dodds JH, Roberts LW (1995). Experiments in Plant Tissue Culture, 3rd rev ed. Cambridge University Press, Cambridge.

Durand-Cresswell R, Nitsch C (1977). Factors influencing the regeneration of *Eucalyptus grandis* by organ culture. Acta Hortic. 78: 149-155.

Ebert A, Taylor HF (1990). Assessment of the changes of 2,4 dichlorophenoxyacetic acid concentrations in plant tissue culture media in presence of activated charcoal. Plant Cell Tissue Org. Cult. 20: 165-172.

Ebert A, Taylor F, Blake J (1993). Changes of 6-benzylaminopurine and 2,4-dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. Plant Cell Tissue Cult. 33: 157-163.

Foolad MR, Jones RA (1992). Models to estimate maternally controlled genetic variation in quantitative seed characters. Theor. Appl. Genet. 83(3): 360-366.

Fridborg G, Pedersen M, Landstorm LE, Eriksson T (1978). The effect of activated charcoal on tissue cultures adsorption of metabolites inhibiting morphogenesis. Physiol. Plant. 43: 104-106.

Gallo-Meagher M, Green J (2002). Somatic embryogenesis and plant regeneration from immature embryos of saw palmetto, an important landscape and medicinal plant. Plant Cell Tissue Org. Cult. 68: 253-256.

Gamborg OL, Phillips GC (2002). Plant Cell, Tissue and Organ Culture (eds): Fundamental Methods. Springer, Verlag Berlin Heidelberg.

Garcia AS, Branquinho EGDA, Menuchi ACTP, Erlacher KC, Domingues MCS (2006). Efeito de reguladores vegetais na germinção e desenvolvimento da semente *Strelitzia reginae*. Thesis São Paulo ano III, 5: 161-176.

George EF, Sherrington PD (1984). Plant Propagation by Tissue Culture. Exegetics Ltd., Eversley.

Gupta PP (1986). Eradication of mosaic disease and rapid clonal multiplication of bananas and plantains through meristem tip culture. Plant Cell Tissue Org. Cult. 6: 633-639.

Horner M, McComb JA, McComb AJ, Street HE (1977). Ethylene production and plantlet formation by *Nicotiana* anthers cultured in the presence and absence of charcoal. J. Exp. Bot. 28: 1366-1372.

Jarret RL, Rodriguez W, Fernandez R (1985). Evaluation, tissue culture propagation, and dissemination of Saba and Pelipita in Costa Rica. Sci. Hortic. 25: 137-147.

Johri BM, Rao PS (1984). Experimental embryology. In: Johri BM (ed). Embryology of Angiosperms. Springer-Verlag, Berlin.

Karnataka J (2008). Effect of Antioxidant on *In vitro* Establishment of *Strelitzia reginae* Through Shoot Tip Explants. J. Agric. Sci. 21(2): 324-325.
Khalil SM, Cheah KT, Perez EA, Gaskill DA, Hu JS (2002). Regeneration of banana (*Musa* spp. AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. Plant Cell Rep. 20: 1128-1134.
Khatri A, Khan IA, Siddiqui SH, Ahmed M, Siddiqui KA (1997). *In vitro* culture of indigenous and exotic banana clone for maximizing multiplication. Pak. J. Bot. 29: 143-150.
Kiong ALP, Nee TC, Hussein S (2007). Callus induction from Leaf Explants of *Ficus deltoidea* Jack. Int. J. Agric. Res. 2(5): 468-475.
Larkin PJ, Ryan SA, Brettell RIS, Scowcroft WR (1984). Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67(5): 443-455.
Madhusudhanan K, Rahiman BA (2000). The effect of activated charcoal supplemented media to browning of *in vitro* cultures of *Piper* species. Biol. Plant, 43(2): 297-299.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.
Nissen SJ, Sutter EG (1990). Stability of IAA and IBA in nutrient medium to several tissue culture procedures. Hort. Sci. 25: 800-802.
Novak FJ, Afza R, Van Duren M, Perea-Dallos M, Cronger BV, Xiaolang T (1989). Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.) Nat. Biotechnol. 7: 154-159.
Okole BN, Schulz FA (1996). Micro-cross sections of banana and plantains (*Musa* spp.): morphogenesis and regeneration of callus and shoot buds. Plant Sci. 116: 185-195.
Paiva PDO, Paiva R, Pasqual M, Paiva LV (2004). In vitro establishment of Strelitzia (*Strelitzia reginae* Banks.). Ciênce Agrotecnol. 28(5): 1031-1037.
Pan MJ, Van Staden J (1998). The use of charcoal in *in vitro* culture. A review. Plant Growth Regul. 26: 155-163.
Pierik RLM (1987). *In Vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht.
Promtep K (1981). Embryo and tissue culture of *Strelitzia reginae* ait. Agris record: XB8110894.
Razdan MK (1993). Introduction to Plant Tissue Culture. Intercept Limited, Andover, Hampshire.
Scalbert A, Monties B, Favre JM (1990). Polyphenols of *Quercus robur* adult tree and *in vitro* grown calli and shoots. Phytochemistry, 27: 3483-3488.
Srangsam A, Kanchanapoom K (2007). Establishment of *in vitro* Culture of *Musa* AA Group 'Kluai Sa' and *Musa* AA Group 'Kluai Leb Mue Nang' and the Analysis of Ploidy Stability. Sci. Asia. 33: 437-442.
Strosse H, Van den Houwe I, Panis B (2009). Banana Improvement. FAO Corporate Document Repository. mhtml:file://E: Zingiberaceae\Banana Improvement.mht
Teixeira JB, Söndahl MR, Kirby EG (1994). Somatic embryogenesis from immature inflorescences of oil palm. Plant Cell Rep. 13: 247-250.
Thomas TD (2008). The role of activated charcoal in plant tissue culture. Biotechnol. Adv. 26: 618-631.
Titov S, Bhowmik SK, Mandal A, Alam SM, Uddin SN (2006). Control of Phenolic Secretion and Effect of Growth Regulators for Organ Formation from *Musa* spp. cv. Kanthali Floral Bud Explants. Am. J. Biochem. Biotechnol. 2(3): 97-104.
van de Pol PA, van Hell TF (1988). Vegetative Propagation of *Strelitzia reginae*. Acta Hortic. 226: 581-586.
Van de Venter HA, Small JGC (1975). Evidence for the presence of a germination inhibitor in seeds of *Strelitzia* Ait. S. Afr. J. Bot. 41: 221-223.
Veramendi J, Navarro L (1996). Influence of physical conditions of nutrient medium and sucrose on somatic embryogenesis of date palm. Plant Cell Tissue Org. Cult. 45: 159-164.
Vonk Noordegraaf C, Van Der Krogt TM (1976). Produktie en bloei van *Strelitzia*. Vakblad. Bloemisterij. 31(49): 18-19.
Weatherhead MA, Burdon J, Henshaw GG (1979). Effects of activated charcoal as an additive to plant tissue culture media. Z. Pflanzenphysiol. 94: 399-405.
Wong WC (1986). *In vitro* propagation of banana (*Musa* spp.): initiation, proliferation and development of shoot-tip cultures on defined media. Plant Cell Tissue Org. Cult. 6: 159-166.
Wood T (1995). Ornamental Zingiberaceae. Revista Brasileira de Horticult. Ornam. 1: 12-13.
Zeweldu T, Ludders P (1998). Preliminary investigation in *Ensete* (*Ensete* spp.). J. Appl. Bot. 72(1-2): 25-27.
Ziv M, Halevy AH (1983). Control of Oxidative Browning and *in Vitro* Propagation of *Strelitzia reginae*. Hort. Sci. 18(4): 434-436.