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Review

Micropropagation protocols for the mass propagation of over-exploited medicinal plants in South Africa

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South Africa has a very rich plant biodiversity, many of which are medicinally useful. The rich resource is decreasing at an alarming rate as a result of over-exploitation. Plant *in vitro* regeneration is a biotechnological tool that offers a potential solution to this problem as it provides a means of putting the plants onto the market at lower prices. We present in this paper a review of micro propagation protocols developed for selected medicinal plants of South Africa and highlight the need for the utilization of this technology for the mass propagation of over-exploited medicinal plants in South Africa.

Key words: Tissue culture, protocols, micro propagation, medicinal plants, herbal medicine.

INTRODUCTION

Plants have been an important source of medicine for thousands of years. The World Health Organization estimates that up to 80 percent of people still rely on herbal remedies for their health care (Farnsworth et al., 1985). Phytomedicine has been part of Africa indigenous peoples' cultural heritage and medicinal plants have become an important element in health care delivery system in both the urban and rural African communities. Several factors are responsible for this, namely, the high cost of drugs, drug resistance which often lead to treatment failure, prolong and expensive treatment of some chronic diseases which the general populace cannot afford.

The demand for medicinal plants is therefore very high leading to their over-exploitation from the wild. In fact, the collection of medicinal plants has become a form of rural self-employment in Africa which is generating a lot of income for the rural poor.

South Africa has the richest plant biodiversity in the world, many of which are medicinally useful. The rich resource is decreasing at an alarming rate as a result of over- exploitation. It is estimated that over half a million people are directly involved in medicinal plant trade in the country. A case study of the trade in medicinal plants in the Eastern Cape Province revealed a minimum of 166 plant species providing 525 tonnes of plant material valued at R27 million annually (Dold and Cocks, 2002). Of the species documented, 93% are being harvested unsustainably and 34 species have been prioritized for conservation management. Despite legislations for

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Abbreviations: MS: Murashige and Skoog's medium; BA: Benzyl-6-adenine; BAP: 6-Benzylaminopurine; IBA: Indole-3-butyric acid; NAA: α- Naphthaleneacetic acid; IAA: Indole-3 acetic acid; 2,4-D: 2,4-Dichlorophenoxyacetic acid; ABA: Abscisic acid.

restriction (Netshiluvhi, 1996; Mander, 1998), the extraction pressure on the natural populations of these medicinal plants in the wild is still very high indicating that the enforcement is incapable of curbing over-exploitation (Cunningham, 1988). With the current rate of harvesting, the plant supplies will, in time dwindle and many of the species will eventually become extinct. The rural dwellers that are the most dependent on these plants will be the most affected.

Plant *in vitro* regeneration is a biotechnological tool that offers a potential solution to the problem of medicinal plants decimation in South Africa. We present in this paper a review of micro propagation protocols developed for selected medicinal plants in the country. We also highlight the need for the utilization of this technology for the mass propagation of over-exploited medicinal plants.

AMARYLLIDACEAE

Crinum variabile (Jacq.) Herb is restricted to the Namaqualand, Bokkeveld mountains and western Karoo regions of South Africa. Although its potential as a horticultural and medicinal plant is well recognized, the seeds of this plant are recalcitrant and produce vegetative offsets too slowly and infrequently. As an alternative to the conventional methods, *C. variabile* was successfully propagated *in vitro* using twin-scale explants (Fennell et al., 2001). Shoots developed in the axes of twin-scales when placed on Murashige and Skoog's (MS) medium. Plant growth regulators were not required for the induction of shoots but Benzyl-6-adenine (BA), in the absence of α-Naphthaleneacetic acid (NAA), promoted their outgrowth. The inclusion of 5 g l^{-1} activated charcoal improved development by increasing the bulblet size and the frequency with which shoots formed bulblets. These bulblets were used to initiate further cultures.

ARALIACEAE

The genus *Cussonia* consists of 22 species most of which are indigenous and well distributed in South Africa (Strey, 1973). *C. paniculata* is widely used in traditional medicine against pain, inflammation, infections and malaria (Hutchings et al., 1996) Micro propagation protocol for this plant was developed by Tetyana and van Staden (2001). Seeds were germinated *in vitro* on MS medium. The survival of the seeds in culture was 70%. Explants were obtained from the seedlings for micro propagation. Axillary node explants produced multishoots when cultured on MS medium supplemented with 2.5 mg l^{-1} BA. Roots were obtained by individually transferring shoots to the medium containing 0.75 mg l^{-1} Indole-3-butyric acid (IBA) or 1 mg l^{-1} NAA. Rooted plantlets were acclimatized for planting *ex vitro*. This protocol had 63% plantlets survival after acclimatization.

ASPHODELACEAE

Kniphofia leucocephala which belongs to the family *Asphodelaceae,* is a highly endangered medicinal species. It is extant at only one location, Langepan, KwaZulu-Natal in South Africa, where the population is threatened by human activities. McCartan and van Staden (2003) established a continuous culture system for this species as part of a program for the propagation and re-introduction of the species into the wild. The efficiency of the system in terms of shoot multiplication and, especially, the frequency and rate of root initiation was strongly influenced by the concentration of BA in the shoot multiplication medium. The optimum shoot multiplication medium for subsequent root initiation contained 2 mg l^{-1} BA alone. The shoots successfully rooted and acclimatized. More than 200 shoots can be produced from one shoot after 4-5 week cycles.

HYACINTHACEAE

The family *Hycinthaceae* consists of bulbous plants with thick and sometimes contractile roots. They are highly valued for their medicinal properties. They constitute about 14% of the medicinal plant materials traded in South Africa (Mander, 1998) and majority of the micro propagation works reported in South Africa are on the members of the family (van Staden, 1998; McCartan and van Staden, 1999**).** Hannweg and Watt (1996) developed an organogenenic protocol for regeneration from inflorescence pieces of *Bowiea volubilis*. The protocol involved the culture of 10 mm long pieces of inflorescence on MS basal nutrient medium containing 30 g l^{-1}, sucrose and 10 g l^{-1} agar. A combination of 1 mg l^{-1}, 2, 4-Dichlorophenoxyl acetic acid (2,4-D) and 1 mg l^{-1} 6-Benzylaminopurine (BAP) were used. Cultures were initially maintained in the dark for 6–8 weeks. The explants were thereafter, transferred to fresh medium without growth regulators, where bulblet development, shoot elongation and rooting occured within 4–5 weeks. This protocol produces more than 4 plantlets per explant and thousands of plantlets can potentially be produced from a single inflorescence. The number of plantlets that can be obtained, together with the simplicity of the approach, make this protocol a cost-effective and attractive option for the propagation of *B. volubilis*. Protocols for this species have also been developed using other explants like bulb scales and shoots (Cook et al., 1988), twin scales and split shoots (van Staden et al., 1991). The manipulations involved can be performed by semi-skilled personnel and do not require sophisticated equipment.

Continuous culture systems at 8-week sub-culture interval were also successfully established for *Scilla kraussii* and *S. dracomontana* using leaf explants (McCartan and van Staden, 2002). In both species, the

best result for shoot initiation and number of shoots was obtained using only kinetin at a concentration of 1 mgl^{-1}. This growth regulator played a very significant role in the efficiency of the system. The protocol can give 700-1500 shoots of *S. kraussii* and 30-60 shoots of *S. dracomontana* from a single shoot with five sub-cultures at 8-weeks intervals. The continuous culture systems can be used to produce large quantities of plantlets, especially where conventional propagation is limited either by the small size of the bulb or due to the limited number of seed as in *S. dracomontana*. For *Scilla natalensis* micropropagation protocol was established by McCartan and van Staden (1998) using explants from the bulbs, bulb scales, shoots and leaf. A modified MS medium with a combination of 1-2 mg l^{-1} kinetin and 1-2 mg l^{-1} indole-3-acetic acid (IAA) was found to give a satisfactory result. Protocol for *Scilla hyacinthiana* developed by Nair (1989) used modified MS with a combination of kinetin (5 mg l^{-1}) and NAA (1 mg l^{-1}).

Eucomis species are deciduous bulbous geophytes also belonging to the family Hyacinthaceae. The bulbs are greatly valued in traditional medicine for the treatment of a variety of ailments, and are thus, heavily harvested for trade in South Africa. The *in vitro* propagation of the genus was developed by Taylor and van Staden (2001). Leaf explants were used to initiate multiple shoot production in all the species studied. The MS medium, supplemented with 100 mg l $^{-1}$ myo-inositol, 20 mg l^{-1}sucrose, and solidified with 2 mg l^{-1} gel rite was used. The optimal hormone combination for shoot initiation in the majority of species was 1 mg l^{-1} NAA and 1 mg l^{-1} BA, while optimal root initiation was achieved on media supplemented with 1 mg l^{-1} IBA or NAA, depending on the species. A continuous culture system using this protocol produced 25–30 plantlets per culture.

Drimia robusta is another member of the family. It is a bulbous species of tremendous medicinal importance in South Africa. Hot water infusion from pulverized bulbs or leaves of this plant is used by the Zulus as an enema for feverish colds. The bulbs are also used in protective charm mixes, while the leaves are said to be diuretic and are used to clean the bladder and to treat diseases of the uterus (Pujol, 1990). Micro propagation protocol was developed for this plant by Ngugi et al. (1998). Bulb scales explants were used to initiate *in vitro* bulblet formation using MS medium supplemented with 100 mg l^{-1} myo-inositol, 30 g l^{-1}, 1 mg l^{-1} NAA and 2 mg l^{-1} BA. Bulblets were rooted in the medium containing 1 mg l^{-} NAA or without hormones.

Another important medicinal plant of the family *Hyacinthaceae* is *Schizobasis* intricate. Micro propagation protocol for this plant was developed by Drewes et al. (1993). Bud formation was initiated on bulb scale explants using MS medium supplemented with a combination of BA (2 mg l^{-1}) and NAA (2 mg l^{-1}). An average of 14 shoots per explant was achieved from this protocol. Rooting medium of halve-strength MS with 1 mg L-1 NAA gave the best results.

LILIACEAE

Among the indigenous members of *Liliaceae* in South Africa that are considered highly endangered is *Aloe polyphylla* (Emanoil, 1994). The plant is highly valued for its ornamental and medicinal properties. Abrei and van Staden (2001) developed a micro propagation protocol for this species using basic medium supplemented with 1mg l^{-1} BA with an average of 15 regenerated shoots per culture. Chukwujekwu et al. (2002) also demonstrated that, at concentration of 1-2 mg l^{-1}, other growth regulators such as zeatin, IBA, NAA and kinetin used either singly or in combination, gave satisfactory results. This protocol was established using young shoot explants of *in vitro* grown plants. The basal medium was MS supplemented with 100 mg l^{-1} myo-inositol, and 30 g l^{-1} sucrose. Temperature and sucrose also influenced shoot proliferation. The optimal temperature was 25°C, while 30 g l^{-1} was the optimal concentration of sucrose.

The genus *Haworthia* is native to South Africa and Namibia but largely restricted to southern Cape Province. *Haworthia* species are used intensively in South Africa in traditional medicine (Cunningham, 1988). They are sought after by local traditional healers because of their alleged powerful healing properties in the treatment of constipation, diarrhea, dysentery, hemorrhoids, indigestion, nausea, stomach ache and worms (Hutchings, 1989). *H. limifolia and H. koelmaniorum* are particularly threatened due to their over-exploitation. Micro propagation protocols for these two species was developed by Mycock et al. (1997) through somatic embryo genesis using leaf explants. The somatic embryo induction medium was MS with 20 g l^{-1} sucrose and 10 g l^{-1} agar. Direct somatic embryo genesis was best in leaf explants exposed to 2 mg l^{-1} 2, 4-D. For *H. koelmaniorum,* inclusion of abscisic acid (ABA) (0.2-0.3 mg l^{-1}) in the medium was found to enhance embryoid induction.

Thuranthos basuticum is a bulbous geophyte endemic to the eastern parts of South Africa. The bulb is used in traditional native medicine for making powerful charms believed to make people invisible (Phillips, 1917). As a result of over-exploitation and the decline in the natural population, a protocol for *in vitro* regeneration was developed for the plant by Jones et al. (1992). Bulb explants were used for the initiation and elongation of bulblets on MS medium supplemented with a combination of NAA and BA. The most favorable shooting responses were obtained using high NAA (5 mg l^{-1}) and BA (5 mg l^{-1}) concentrations. Best rooting was obtained with a combination of NAA (5mg l^{-1}) and BA (1 mg l^{-1}). The protocol is suitable for mass propagation of the species.

PEDALIACEAE

Harpagophytum procumbens DC (*Pedaliaceae*), also known as the Kalahari devil's claw, is an important medicinal plant in southern Africa. It is indigenous to the Kalahari Desert in Botswana and Namibia and used for the treatment of rheumatism, arthritis, liver problems, stomach ailments, hypertension, urinary tracts infections, diabetes and malaria (Watt and Breyer-Brandwijk, 1962; Kgathi, 1989). The plant is of great interest in pharmaceutical and homeopathic industries. An *in vitro* regeneration protocol for the plant has been achieved via shoot tip cultures (Shushu, 2001). Single node segments and shoot tips were cultured on MS medium supplemented with various BA concentrations (0.1-0.5 mg l^{-1}) in combination with NAA (1 mg l^{-1}). All the media used induced axillary buds, shoot tip growth and root formation. Shoot length decreased with increase in BA concentration. Maximal shoot length was obtained in MS medium with no hormone supplement, and average shoot number ranged between 1.5 and 2.2 shoots/explant. Addition of 1 mg l^{-1} NAA improved the growth of the cultures but also induced callus formation.

PERIPLOCACEAE

The over-exploitation of *Mondia whitei (Periplocaceae)* by the Zulus in South Africa was reported as early as the 19th century (Hooker, 1871). The plant is a liana and endemic to south-central and east Africa. It is very popular due to its pleasant aromatic character and taste of the rootstock. Medicinally, it is used as a tonic for the stomach, easing of flatulence, abdominal pains, constipation and treatment of bilharzia. The roots are also reported to be used as aphrodisiac (Oryem-Origa et al., 1995). McCartan and Crouch (1998) developed a micro propagation protocol for *M. whitei* using single-node explants from *in vitro* grown seedlings. The basic medium was MS supplemented with 1 mg l^{-1} BA. The protocol is capable of producing 2000 plantlets from a single seed in 7-8 subcultures at 4- 6 week intervals. Addition of charcoal to the medium was found to significantly reduce vitrification. This protocol is very suitable for producing large quantities of the plant.

SOLANACEAE

Withania somnifera is widely used in the Indian and African traditional medicine. It is generally used as an abortifacient, amoebicide, bactericide and contraceptive (Asthana and Raina, 1989). The plant is reported to have anti-stress, anti-inflammatory, anti-arthritic and anti-tumor properties. The species occurs mainly in KwaZulu-Natal, Free State and the Eastern Cape provinces of South Africa where the local inhabitants have been using it since time immemorial as a remedy for a number of diseases (Van Wyk et al., 1997). Protocols for *in vitro* regeneration in *Withania somnifera* were developed by Rani et al. (2003). Explants were taken from the leaves, hypocotyls, roots and cotyledonary leaf segments. Callus was induced in MS medium supplemented with various concentrations and combinations of 2,4-D and kinetin. Maximum callusing (100%) was obtained from root and cotyledonary leaf segments grown on the medium supplemented with a combination of 2 mg l^{-1} 2, 4-D and 0.2 mg l^{-1} kinetin. Maximum shoot multiplication was observed after 60 days of the second subculture on the medium containing 2 mg l^{-1} BA. These shoots were rooted best on the medium containing 2 mg l^{-1} (IBA). The plantlets were transferred to the field after acclimatization and showed 60% survival.

CONCLUSION

There is no doubt that the booming trade in medicinal plants in South Africa is having a negative impact on the natural populations of these valuable resources and is threatening the survival of many species. Enforcement of legislations has failed to stop the indiscriminate harvesting of the plants as the trade is the major source of livelihood for an estimated one million people, mostly the rural poor. The provision of alternative sources of medicinal plants by encouraging their cultivation will go a long way in reducing their heavy dependence on the wild populations. Conventional propagation methods have proved to be inadequate to meet this challenge. Large scale production through plant *in vitro* regeneration will provide a means of putting the plant onto the market at lower prices. In addition, the technique is cost effective, relatively simple and can be performed by semi-skilled persons. Micro propagated plants can be used to supplement the natural stock of plants in wild populations as well as provide a ready supply to the herbal medicinal trade.

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