Micropropagation of members of the Hyacinthaceae with medicinal and ornamental potential - A review

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The Hyacinthaceae comprises many genera, which are characterized by bulbs, thick roots, basal leaves and simple or more rarely branched racemes. These genera are widely exploited for their medicinal, pharmaceutical and ornamental potential. In South Africa, these plants are harvested without permits from wild populations, processed and then sold as traditional medicine. This is having a negative impact on wild populations. Although the active ingredients of many of the bulbs have not been identified, several bufadienolides such as prosclaren A have been isolated from members of this family. These bufadienolides have pharmaceutical potential as cardiotonic. Thus, many members of the Hyacinthaceae, have potential as alternative horticultural/agricultural bulb and/or flower crops for medicinal, pharmaceutical and ornamental purposes. Conventional propagation of these plants, however, is usually fairly slow. Micropropagation provides a rapid means to propagate selected chemotypes or cultivars, serving both conservation and commercial interests. Many members of Hyacinthaceae have been micropropagated. This review summarizes this information, highlighting the potential and problems surrounding this family of flowering plants.

Keywords: adventitious shoots, axillary shoots, bufadienolides, conservation, in vitro propagation, somatic embryos, tissue culture.

Abbreviations: 2,4-D = 2,4-dichlorophenoxyacetic acid; AC = activated charcoal; BA = benzyladenine, BDS = modified B5 medium; CM = coconut milk; DC = dawn County; DM = tobacco medium; EM = endosperm medium; F = filter; GC = growth chamber; H = horseradish; IBA = indole-3-butyric acid; IP = 6-[3,5-dimethyl-2,4-x-naphthylenediamin]-purine; IPR = 6-[3,5-dimethyl-2,4-x-naphthylenediamin]-purine; LDL = liquid diluent; MS = Murashige & Skoog medium; NM = naphthalene-1-acetic acid; PAC = paclobutrazol; TDZ = thidiazuron; YE = yeast extract; [7G]BA, [8G]BA = 3,7,9 glucosides of benzyladenine.

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Introduction

The Asparagales forms a large and fairly homogenous complex of families. It is believed to be a monophyletic group, which evolved in parallel with the Liliales and Dioscoreales. The Asparagales consists of 31 families including several new families. These families include the Alliaceae, Asparagaceae, Asphodelaceae, Dracennaceae, Eriocarpaceae and Hyacinthaceae, comprise genera previously placed within the Liliales sensu lato (Dahlgren et al. 1985; Perry 1985). The Hyacinthaceae comprises several genera including Bowiea, Eucomis, Lachenalia, Ledebooria, Scilla and Urginea. These genera are characterised by bulbs with thick and sometimes contractile roots, basal leaves and simple or more rarely branched racemes. The inflorescences comprise actinomorphic, bisexual flowers, which range from white, blue, violet, and more rarely yellow, red, brown to nearly black. The Hyacinthaceae are widely distributed, occurring in southern Africa and in a region from the Mediterranean to SW Asia (Dahlgren et al. 1985; Duncan & du Plessis 1989).

Medicinal and pharmaceutical potential

In South Africa, approximately 20 000 tons of plant material, worth about R270 million, is harvested, processed and sold as traditional medicine annually (Gosling 1998). Approximately 14% of this plant material comprises bulbs (Mander 1997), which are destructively harvested, processed and sold for the treatment of various ailments (Table 1). These bulbs, which are sold whole, sliced or chopped, are usually administered as decoctions, emetics and enemas (Watt & Breyer-Brandwijk 1962; Jacot Guillamod 1971; Hutchings 1989; Mander et al. 1995; van Wyk et al. 1997).

Scilla natalensis is one of the top-ten most popular medicinal plants in South Africa (Cunningham 1988; Williams 1996; Mander 1997). Despite its specially protected conservation status, approximately 95 tons of these bulbs are sold illegally in Durban annually. The price of these bulbs is relatively low ranging from R1.89 to R6.80 per kilogram. This is mainly due to the occurrence of large populations of these bulbs locally. The price of another popular medicinal plant, Bowiera volubilis, is substantially higher ranging from R11.74 to R27.80 per kilogram (Mander 1997). The price of these bulbs has increased steadily with the decline in their availability and size (Cunningham 1988).

Although the active ingredients of many of these bulbs have not yet been identified, several bufadienolides have been isolated from members of the Hyacinthaceae including Bowiera volubilis (Jha 1988; Finnie et al. 1994), Drimia robusta (van Wyk et al. 1997; Luyt et al. 1999), Urginea alissina (Hutchings et al. 1996), U. indica (Jha & Sen 1981, 1983; Jha 1988) and U. maritim (Shyr & Staba 1976; Verbiscar et al. 1986; Gentry et al. 1987; Jha 1988). Bufadienolides such as prosclarinidin A are valuable since they can be administered to digitalin sensitive/intolerant patients (Jha 1988). These bufadienolides, however, are usually not used as cardiotics due to their low therapeutic indices (Jäger & van Staden 1995). However, some bufadienolides identified in certain organs of Bowiera volubilis are thirty to sixty times more active than those from Digitalis (Hutchings et al. 1996). Prosclarinidin A, which is derived by enzyme hydrolysis from scillaren A, is produced under several trade names including Caradin®, Cardion®, Prosclarin®, Sandoscill®, Scillacrist®, Talusin® and Urgilan® (Budavari 1996). These cardiac
Table 1 Medicinal uses of members of the Hyacinthaceae in South Africa

| Plant name | Medicinal uses | References |
|------------|----------------|------------|
| *Allium canadensis* (L.) Leighton syn. *A. major* | As an anthelmintic, thirst quencher and to treat venereal diseases | Watt & Breyer-Brandwijk 1962; Hutchings 1989 |
| *Allium cooperi* Bak. | As an anthelmintic, lotion for washing wounds and to treat venereal diseases | Watt & Breyer-Brandwijk 1962; Hutchings 1989 |
| *Allium fuscopurpureum* (L.f.) Dryand. | To treat illnesses caused by poisons | Hutchings et al 1996 |
| *Allium scariosum* Jacq. | As an anthelmintic, lotion for washing wounds in animals and to treat venereal diseases | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971; Hutchings 1989 |
| *Allium shawii* Bak. syn. *A. trichophyllum* | As an anthelmintic and to treat constipation and gonorrhoea | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971; Hutchings 1989 |
| *Bowenia vihobila* Harv. | To treat dropsy, barrenness in women and headaches | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Dipcadi brevifolium* (Thumb.) Fourr. | To treat heart pains and breathlessness | Hutchings 1989; Hutchings et al 1996 |
| *Dipcadi gracillimum* Bak. syn. *D. polyphyllum* | To treat gonorrhoea and pimplies | Watt & Breyer-Brandwijk 1962 |
| *Dipcadi viride* (L.) Moench | To treat gonorrhoea and flatulence | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Drimia ciliaris* Jacq. | As an emetic, expectorant and diuretic | Watt & Breyer-Brandwijk 1962 |
| *Drimia ciliata* Jacq. | To treat high blood pressure and stabbing pains | Hutchings et al 1996 |
| *Drimia nervifloris* Bak. | To treat external tumours that have been lanced | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971 |
| *Drimia robusta* Bak. syn. *D. alta* | As an expectorant, emetic and enema to treat feverish colds | Hutchings 1989; Hutchings et al 1996 |
| *Enaconis autumnalis* (Mill.) Chitt. | To treat colic, flatulence, syphilis and hangovers | Hutchings et al 1996 |
| *Enaconis bicolor* Bak. | For colic and as a purgative | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Enaconis comosa* (Houtt.) var. *comosa* syn *E. punctata* | To treat rheumatism and teething infants | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Enaconis polyanthos* N.E. Br. | For people suffering from mental disease | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Enaconis regia* (L.) L’Herit | To treat venereal disease, lumbago, diarrhoea, respiratory conditions especially cough and biliousness and to prevent premature childbirth | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Ledebouria cooperi* (Hook.f.) Jessop syn. *Scilla cooperi*, *S. inodora*, *S. zonata* | Used in the initiation ceremony of boys; to treat gastro-intestinal, gynecological and psychological ailments | Watt & Breyer-Brandwijk 1962; Batten & Bokelmann 1966; Hutchings 1989; Hutchings et al 1996 |
| *Ledebouria ovatifolia* (Bak.) Jessop syn. *Scalla ovatifolia* | To treat gastro-intestinal and gynecological ailments, buckache and influenza | Hutchings 1989; Hutchings et al 1996 |
| *Ledebouria revoluta* (L.) Jessop syn. *Scilla laneacea* | For treating lumbago and gall sickness in animals; to bathe skin eruptions and as an ointment for wounds and sores | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971 |
| *Massonia echinata* L.f. syn *M. bowkeri* | For ophthalmic applications, sterility and toothache | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971 |
| *Ornithogalum longibracteatum* Jacq. | To treat swellings or growths | Hutchings 1989; Hutchings et al 1996 |
| *Ornithogalum dubium* Houtt. syn *O. minatun* | As an anthelmintic | Buton & Bokelmann 1966 |
| *Ornithogalum temsfootsia* Delarorce subsp. *tenafolium* syn. *O. ecklonii* & *O. virans* | As a rat poison | Watt & Breyer-Brandwijk 1962; Jacot Guillamond 1971 |
| *Ornithogalum thyrooides* Jacq. | To treat diabetes mellitus | Watt & Breyer-Brandwijk 1962 |
| *Schizobasis intricata* | To treat chest complaints | Hutchings et al 1996 |
| *Scilla nutans* Planch. syn. *S. kraussii* & *S. drecomontana* | As a purgative and to treat sprains, fractures, boils, veldsore and lung sickness in cattle | Hutchings et al 1996 |
| *Scilla nervosa* (Burch.) Jessop syn. *S. rigidifolia* | To treat fevers, gastro-intestinal ailments and insanity | Bryant 1966; Hutchings 1989; Hutchings et al 1996 |
glycosides increase the force of the systolic contraction, inhibit the atrioventricular conduction, and enhance the ‘automacy’ of the cardiac tissue and, therefore, are used to treat heart failure and certain arrhythmias (Walton et al. 1994).

Bufadienolides such as scilliroside and scillaren A also are effective rodenticides with the French Pharmacopoeia Codex describing a ratitica paste composed of powdered bulb of Red Squill (Urginea maritima), flour and sugar (Balbaa et al. 1979). The efficiency of these strongly emetic rodenticides is largely due to the inability of rodents to regurgitate. This renders these products extremely safe and specific since humans and domestic animals including chickens, cats and dogs readily regurgitate accidentally-ingested baits (Crabtree 1947). In the 1940s, several tons of powdered bulb of Red Squill (Urginea maritima) were imported into the USA. This ceased with the introduction of warfarin and other anticoagulant-type rodenticides. The development and subsequent evaluation of hybrids for commercialisation has been established over the past thirty years (De Hertogh et al. 1981, 1983) to select high-yielding strains suitable for commercial exploitation.

### Ornamental potential

Approximately 16 000 ha are allotted to ornamental bulb production in the Netherlands, representing 55% of the world’s total production area, with significantly smaller areas allotted to ornamental bulb production in the USA (4 449 ha), the UK (4 300 ha), Japan (1 622 ha), France (1 285 ha) and South Africa (425 ha) (De Hertogh & Le Nard 1993a). Despite the ornamental potential of the Hyacinthaceae, this is generally too slow for commercial propagation. Several artificial techniques, which include scaling, basal cuttage (scooping and scoring) and bulb cuttings, are used to increase the rate of natural multiplication. These, however, are usually restricted by the size of the mother bulb.

### Conventional propagation

Several members of the Hyacinthaceae, therefore, have potential as alternative horticultural/agricultural crops, providing crops of both bulbs and inflorescences. Seed, however, is seldom used for commercial propagation, except where the species or cultivars produce large quantities of reliable seed with short juvenile periods (Rees 1992). Some members of the Hyacinthaceae including Scilla siberica, S. siberica cv ‘Alba’, and S. bifolia, however, are only propagated by seed, while others including S. siberica cv ‘Spring Beauty’ and S. tubergentiana (= S. mitschischenkoana) (Bryan 1989) are propagated vegetatively (Le Nard & De Hertogh 1993). Although offsets are used to propagate many members of the Hyacinthaceae, this is generally too slow for commercial propagation. Several artificial techniques, which include scaling, basal cuttage (scooping and scoring) and bulb cuttings, are used to increase the rate of natural multiplication.

### Micropropagation

Several members of the Hyacinthaceae have been micropropagated using various techniques. These include the proliferation of axillary shoots, the initiation of adventitious shoots and the induction of somatic embryos, which are discussed in greater detail (Table 2). Some details, however, such as the frequency of shoot initiation, the average number of shoots initiated, the frequency of fasciated, deformed or mutated shoots, the frequency of plantlets successfully acclimatized, and the propagation potential within a specified time-frame are seldom reported. These details, which should be included routinely in micropropagation reports, are essential for commercial facilities.

### Axillary shoots

In *Eucomis*, axillary shoots were proliferated from twin-scale explants, which were cultured on medium containing combinations of BA and NAA. These shoots, which were then sub-cultured onto the same medium, proliferated more shoots resulting in a continuous culture system. After 8–10 weeks, the shoots were then rooted on medium with or without NAA (Ault 1995b).

Although axillary shoots are genetically-stable, the limited availability of these axillary shoots restricts the potential of this technique (Hussey 1980). Furthermore, ‘mixed’ cultures comprised of axillary and adventitious shoots do occur occasionally (Hussey 1976a). The origin of these axillary shoots is seldom confirmed, and thus, the frequency of ‘mixed cultures’ is rarely reported.

### Direct adventitious shoots

In several members of the Hyacinthaceae, adventitious shoots were initiated directly on, or along the periphery of, various

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**Table 1 Continued**

| Plant name and synonyms | Description | Reference |
|-------------------------|-------------|-----------|
| *Urginea altenrina* (L.f.) Bak. | To treat rheumatic swellings, gastro-intestinal and respiratory ailments | Watt & Breyer-Brandwijk 1962; Hutchings 1989; Hutchings et al 1996 |
| *Urginea epigea* R.A. Dyer | As a treatment for colds and headaches | Watt & Breyer-Brandwijk 1962 |
| *Urginea macrocentra* Bak. | As an anthelmintic | Hutchings 1996 |
| *Urginea physodes* (Jacq.) Bak. | To treat gynaecological ailments and to facilitate delivery during birth | Hutchings 1989; Hutchings et al 1996 |
| *Urginea rubella* Bak. | For the treatment of colic | Watt & Breyer-Brandwijk 1962; Jacot Guillemot 1971 |
| *Urginea suaveolens Schinz.* syn. *U. burkei* | As an abortifacient and to treat paralysis, circulatory diseases and rheumatism pains | Watt & Breyer-Brandwijk 1962 |

¹Plant name and synonyms (Arnold & De Wet 1993).
Table 2  Micropropagation of some members of the Hyacinthaceae

| Plant name | 1st and 2nd Explants | Medium and supplements | Growth response | References |
|------------|-----------------------|-----------------------|-----------------|------------|
| *Tulipa palustris* | Bulb scales | MS. Sucrose (30000), BA (1), NAA (1), Agar (8000) | Shoots | Cook et al. 1988 |
| | | | Plantlets | |
| *Ornithogalum dubium* | Bulb-scales | MS. Sucrose (30000), BA (1), 2,4-D (1), Agar (10000) | Shoots | Staden 1995a |
| | | | | |
| *Hyacinthus orientalis* cv. Delir’s Blue | Shoots | MS. Sucrose (30000), BA (1), NAA (1), Agar (8000) | Shoots | Cook et al. 1988 |
| | | | | |
| *Hyacinthus orientalis* cv. Pink Pearl | Shoots | MS. Sucrose (30000), BA (1), NAA (1), Agar (8000) | Shoots | Cook et al. 1988 |
| | | | | |
| *Lachenalia arthabanae*, *L. bulbifera*, *L. purpurascens* | Leaves | MS. Sucrose (30000), BA (2), NAA (0.1), Agar (10000) | Shoots | Cook et al. 1988 |
| | | | | |
| *Lachenalia* spp. | Leaves | MS. Sucrose (30000), BA (1), NAA (1), Agar (8000) | Shoots | Cook et al. 1988 |

References:
- Cook, B. W., Van Staden, J., & Frenkel, S. E. (1988). Micropropagation of some members of the Hyacinthaceae. *S Afr J Bot.*, 65(5 & 6), 364-374.
| Table 2 Continued |
|-------------------|
| Muscari armeniacum cv. Fairly Giant | Bulb scales | Modified MS, Sucrose (30000), BA (5), NAA (2), AC (1000), Gelrite (2000) | Bulblets | Peck & Cumming 1986 |
| Ornithogalum cv. Rolow | Leaves | Modified MS, Sucrose (30000), BA (2), NAA (0.1), Agar (7000) | Shoots | Landby & Niederwieser 1992 |
| Ornithogalum cv. Regel | Leaves | Modified MS, Sucrose (30000), BA (2), NAA (0.1), Gelrite (2000) | Bud | Vcelar et al. 1992 |
| | Meristems from buds | Modified MS, Sucrose (30000), BA (1), NAA (1), adenine arabinose (1), Gelrite (2000) | Virus-indexed plants | |
| Ornithogalum hybrid | Leaves | Modified MS, Sucrose (30000), BA (2), NAA (0.1), Agar (7000) | Shoots | Niel 1981 |
| | Shoots | Modified MS, Sucrose (30000), Agar (7000) | Plantlets | |
| | Leaves | Modified MS, Sucrose (30000), BA (2), NAA (0.1), Agar (7000) | Callus & shoots | Van Rensburg et al. 1989 |
| Ornithogalum maculanum | Leaves | Modified MS, Sucrose (30000), BA (2), NAA (0.1), Agar (7000) | Plantlets | Hussy 1976b |
| Ornithogalum thyrsoides | Stem, Leaves, Ovary, Sepals, Bulb scales | MS, Sucrose (20000), Agar (7000) | Plantlets | |
| Ornithogalum umbellatum | Twin-scales of bulbs | MS, Sucrose (30000), NAA (8), Agar (5000) | Callus | Nayak & Sen 1995 |
| | Callus | MS, Sucrose (30000), BA (0.5), NAA (2), Agar (5000) | Shoots | |
| | Shoots | MS (5%), Sucrose (30000), Agar (5000) | Plantlets | |
| Schizobasis striata | Bulb Scales | MS, Sucrose (30000), BA (2), NAA (2), Agar (8000) | Shoots and callus | Drewes et al. 1993 |
| | Shoots | MS (5%), Sucrose (30000), NAA (1), Agar (8000) | Plantlets | |
| Scilla hyacinthina | Leaves | MS, Sucrose (30000), Agar (10000) | Shoots | Nair 1989 |
| | Shoots | MS, Sucrose (30000), Kinetin (5), NAA (1), Agar (10000) | Plantlets | |
| | MS, Sucrose (20000) | MS, Sucrose (30000), IAA (1), Gelrite (2000) | Plantlets | |
| Scilla natalensis | Bulbs | Modified MS, Sucrose (20000), Gelrite (2000) | Shoots | McCartan & Van Staden 1998 |
| | Leaf and bulb scales of shoots | Modified MS, Sucrose (20000), Kinetin (1-2), IAA (1-2), Gelrite (2000) | Shoots (Figures 1D & E) | Plantlets | |
| | Shoots | Modified MS, Sucrose (20000), IAA (1), Gelrite (2000) | Plantlets | |
| Thunbergia beatuicola | Bulb scales | MS, Sucrose (30000), BA (0.1), NAA (5), Agar (8000) | Plantlets | Jones et al. 1992 |
| Urginea indica | Twin-scales of bulbs | Modified MS, Sucrose (30000), Kinetin (1-2), 2,4-D (1-4), ± NAA (2), ± CM (150), ± YE (1000), Agar (6000) | Callus depending on ploidy | Jha et al. 1991 |
| (2n, 3n 7 4n) | Callus | Modified MS, Sucrose (30000), Kinetin (2), 2,4-D (0.5), ± NAA (0.5-1), ± CM (150), ± YE (1000), Agar (6000) | Shoots depending on ploidy | |
| | Shoots | Modified MS, Sucrose (30000), Kinetin (1) or 2,4-D (1), Agar (6000) | Somatic embryos depending on ploidy | |
| Urginea indica | Twin-scales of bulbs | Modified MS, Sucrose (30000), 2,4-D (2), CM (150), Agar (6000) | Callus and somatic embryos | Jha et al. 1986 |
| | Somatic embryos | Modified MS, Sucrose (30000), BA (0.1), CM (100), Agar (6000) | Plantlets | |
| | Plantlets | Modified MS, Sucrose (30000), Kinetin (0.05), NAA (0.01), Agar (6000) | Bulbous plantlets | |
| Urginea indica | Twin-scales of bulbs | Modified MS, Sucrose (30000), 2,4-D (2), CM (150) OR, Kinetin (2), NAA (2), YE (1000), Agar (6000) | Callus and shoots | Jha et al. 1984 |
| | Shoots | MS (5%), Sucrose (5000), Agar (6000) | Plantlets | |
| Urginea maritima | Bulb scales | Modified MS, Sucrose (40000), BA (0.1-0.3), NAA (0.1-0.3), Agar (7500) | Plantlets | El Grari & Backhaus 1987 |
| | Bulblets | Modified MS, Sucrose (40000), NAA (0.1-0.3), Agar (7500) | Plantlets | |
| Velltheinia bracteata cv. Lemon Flame & Rosalba & V. capensis | Leaves and floral stems | MS, Sucrose (30000), BA (2), NAA (0.1), Agar (7000) | Shoots | Ault 1996 |
| Shoots | MS, Sucrose (30000), K-NAA (0-2), Agar (7000) | Plantlets | |
| Velltheinia bracteata (Figure 1F) | Leaves and bulb-scales | MS, Sucrose (30000), BA (2), NAA (0.1), Agar (8000) | Shoots (Figure 1G) | Taylor & Van Plantlets (Figure 1H) Staden 1997 |
| Shoots | MS, Sucrose (30000), IBA (2), Agar (8000) | Plantlets | |

1Figures in parentheses indicate mg l⁻¹ or ml l⁻¹.

Explants. The explant source, orientation, position, size, however, influenced shoot initiation (Niederwieser & Van Staden 1990a & 1992; Niederwieser & Vcelar 1990; Landby & Niederwieser 1992). In Scilla, the explant source influenced shoot initiation with leaf explants producing more shoots than bulb explants (McCartan & Van Staden 1998). In Lachenalia, the age of the
Figure 1  A: Inflorescences of *Eucomis* hybrids and species; B: Adventitious shoot initiated along the periphery of a leaf explant of *E. poleevansii*; C: Inflorescence of *Scilla natalensis*; D: Adventitious buds initiated on the adaxial surface of a leaf explant of *S. natalensis*; E: Adventitious shoots initiated on a leaf explant of *S. natalensis*; F: Inflorescence of *Veltheimia bracteata* cv. Lemon Flame; G: Plantlets of *V. bracteata* cv. Lemon Flame; H: Acclimatized plants of *V. bracteata* cv. Lemon Flame.
explants induced shoot initiation, with young explants producing more shoots than old explants. This may have been linked to endogenous cytokinin levels particularly in the intermediate and old explants (Niederwieser & Van Staden 1990a). In Ornithogalum, the orientation of the explants also influenced shoot initiation with apolar explants producing more shoots than polar explants (Landby & Niederwieser 1992), while in Lachenalia, callus and deformed shoots were formed when explants were cultured with the adaxial surface down (Niederwieser & Vcelar 1990). In Lachenalia, the explant size influenced shoot initiation with small explants producing more shoots than large explants. The optimum size explant was 3.3 x 15 mm (Niederwieser & Vcelar 1990). This may have been related to the wound surface, which is proportionally larger in smaller explants. In Ornithogalum, however, additional wounding on the surface of the explant inhibited shoot initiation (Landby & Niederwieser 1992). In Hyacinthus, the explant size also influenced shoot initiation, which decreased linearly with a decrease in explant size (Pierik & Post 1975).

These adventitious shoots originated from single cells or from groups of cells. In Lachenalia, the shoots were initiated mainly from single epidermal cells primarily derivatives of the stomatal mother cells, although a few shoots were initiated from groups of cells (Niederwieser & Van Staden 1990b). The frequency of mutations in shoots initiated from single cells is usually fairly high, resulting in 'solid mutations' (Hussey 1980). The adventitious shoots also have been restricted to certain surfaces of explants. In Bonivia, the shoots were initiated on the adaxial surfaces of bulb-scales. This may be associated with anatomical differences between these surfaces, which included thicker cuticles, more chloroplasts and stomata on the abaxial surfaces, as well as the presence of unidentified globular bodies on the adaxial surfaces (Van Staden et al. 1991).

Some adventitious shoots have been initiated on medium containing no plant growth regulators, although most adventitious shoots have been initiated on medium containing various cytokinins and auxins. In Lachenalia, the addition of BA had no influence on shoot initiation in hybrids with high cytokinin-like activity, but increased shoot initiation in hybrids with low cytokinin-like activity (Niederwieser & Van Staden 1992). Furthermore, the substitution of cytokinin glucosides [3G]BA, [7G]BA & [9G]BA for BA inhibited shoot initiation (Van Staden & Drewes 1994). In Hyacinthus, the addition of cytokinins had no influence on shoot initiation, although the addition of auxins, particularly IAA and IBA, increased shoot initiation (Pierik & Steegmans 1975; Bach & Cecot 1988, 1989). In Scilla, the addition of NAA inhibited shoot initiation (McCcartan & Van Staden 1998), while in Hyacinthus, the addition of NAA promoted callus formation (Pierik & Steegmans 1975). In Hyacinthus, the carbohydrate source also influenced shoot initiation with glucose and sucrose producing more shoots than fructose (Bach et al. 1992). In Muscari, the addition of charcoal promoted bulbil formation and inhibited callus formation (Peck & Cumming 1986), while in Hyacinthus, the addition of paclobutrazol promoted bulbil formation (Bach et al. 1992). Some adventitious shoots produced roots and/or bulbs spontaneously. Other adventitious shoots have been rooted on medium containing no plant growth regulators (Cook et al. 1988), or various auxins, usually IBA (Nel 1981, 1983; Taylor & Van Staden 1997) or NAA (Drewes et al. 1993; Drewes & Van Staden 1993). Although adventitious shoots are genetically less stable than axillary shoots, particularly when they originate from single cells, (Hussey 1980) this technique is frequently used to clone medicinal and ornamental plants rapidly and economically.

Callus and indirect adventitious shoots

In Bonivia, callus was initiated on medium containing 2,4-D and coconut milk, and then transferred to medium containing 2,4-D and casein hydrolysate. This callus was comprised of green nodules, which produced adventitious shoots, when transferred to medium containing low concentrations of 2,4-D (Jha & Sen 1985). In Ornithogalum, callus was initiated on medium containing NAA alone (Hussey 1976b; Nayak & Sen 1995) or in combination with BA (Van Reensburg et al. 1989). The frequency and size of the callus decreased with increasing sucrose concentration (Van Reensburg et al. 1989). Hussey (1976b) found that the callus was comprised of a mixture of diploid and tetraploid cells. He also found that the frequency of tetraploid cells increased with callus age (Hussey 1976b). In contrast, Nayak and Sen (1995) found that the callus was genetically-stable for several years. The callus produced adventitious shoots when transferred to medium containing no plant growth regulators (Hussey 1976b) or medium containing combinations of BA and NAA (Nayak & Sen 1995). In Urvinea, callus was initiated on medium containing 2,4-D, NAA and kinetin. The frequency of callus initiation was increased by the addition of yeast extract. This callus produced small adventitious shoots and roots when transferred to medium containing low concentrations of auxins and vitamins (Jha & Sen 1984).

Although vast quantities of adventitious shoots can be produced from callus, the frequency of genetically-aberrant shoots may be relatively high. Thus, propagation via indirect adventitious shoots is usually avoided. In certain breeding programmes, however, genetically-aberrant plantlets could be a useful source of somaclonal variation.

Direct and indirect somatic embryos

In Scilla, embryogenic callus was initiated on medium containing NAA and coconut milk. The embryogenic callus, which was comprised of small thin-walled cells with large nuclei, formed shoots and roots when transferred to medium containing no plant growth regulators (Chakravarty & Sen 1989). In Urvinea, embryogenic and non-embryogenic callus was initiated on medium containing 2,4-D and coconut milk. The embryogenic callus, which was comprised of large, vacuolated parenchyma cells, formed greenish zones of small cells with large nuclei when exposed to 2,4-D for prolonged periods. These greenish zones produced globular embryos, which elongated to form banana-shaped bipolar embryos when transferred to medium containing low concentrations of BA with or without coconut milk. The bipolar embryos produced bulbous plantlets when transferred to medium containing kinetin and NAA. In Urvinea, the age of the callus, the frequency of sub-culturing and the cytological state of the callus influenced the initiation and subsequent development of the callus (Jha & Sen 1986). The ploidy of the callus (diploid & tetraploid) also influenced medium requirements (Jha et al. 1991). Despite the potential of this technique, somatic embryos have not been induced in many members of the Hyacinthaceae.

Conclusions

The Hyacinthaceae comprises several genera, which are widely exploited for their medicinal, pharmaceutical and ornamental potential. In South Africa, several members of the Hyacinthaceae are harvested without permits from wild populations, processed and then sold as traditional medicine. This is reducing the density, distribution and genetic diversity of wild populations. Furthermore, the enforcement of existing legislation has proved ineffective with vast quantities of plants being traded locally and internationally. Consequently, it has been suggested that ex situ
conservation through cultivation may alleviate pressures on natural resources, whilst meeting the demand for these plants. Conventional vegetative propagation, however, is usually fairly slow. Micropropagation, therefore, provides a rapid and economical means to propagate these endangered plants, whilst supplying an alternative source of superior quality plants for the consumer market.

Many bufadienolides have been isolated from members of the Hyacinthaceae. These bufadienolides are used as cardiotonics and, therefore, have pharmaceutical potential. This has led to the identification and quantification of bufadienolides in various clones and cytotypes in vitro and in situ to select high yielding strains suitable for commercial exploitation. In *Bowenia*, the same bufadienolides were accumulated at similar concentrations for both in vitro and in situ plants (Finnie et al. 1994). In *Urginea*, however, the accumulation of bufadienolides was linked to the formation of specific organs in vitro, but was independent of the genotype, explant source and regeneration system (Jha et al. 1991). Micropropagation, therefore, not only provides a rapid and economical means to propagate selected chemotypes, but also a means to produce these bufadienolides in vitro. This is important economically since it obviates the need to cultivate these plants *ex vitro*.

Several members of the Hyacinthaceae are cultivated as ornamentals, although the total area allotted to the production of these bulbs is relatively small. Breeding programmes, which have been established for certain genera, may increase the popularity of these plants. Micropropagation, therefore, not only provides a rapid and economical means to propagate newly-developed cultivars, but also a means to eliminate viruses such as *Hyacinthus* Mosaic Virus (Blom-Barnhoudt 1986) and Ornithogalum Mosaic Virus (Vcelar et al. 1992), thus reducing crop losses and facilitating the export of these plants. Micropropagation also provides a means to conserve valuable germplasm (Louw 1995), therefore retaining useful character traits for future breeding programmes.

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