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REVIEW

Applications of phytochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural poor

Viswambharan Sarasan · Geoffrey C. Kite · Gudeta W. Sileshi · Philip C. Stevenson

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Abstract Plants provide medicine and pest control resources for millions of poor people world-wide. Widespread harvesting of medicinal and pesticidal plants puts pressure on natural populations, thus severely compromising their contribution to the income and well-being of traders and consumers. The development of in vitro propagation techniques appropriate for developing countries will provide a robust platform for effective propagation and cultivation of endangered plants. This review focuses on advances in the application of phytochemical and in vitro tools to identify and rapidly propagate medicinal and pesticidal plants. Problems of over-harvesting can be alleviated and ex situ cultivation in agroforestry systems can be facilitated through improving seed germination, in vitro cloning and the use of mycorrhizal fungi. We also present a case for effective use of phytochemical analyses for the accurate identification of elite materials from wild stands and validation of the desired quality in order to counter loss of efficacy in the long run through selection, propagation or ex situ management in agroforestry systems. Future prospects are discussed in the context of medicinal activity screening, sustainable propagation, on-farm planting, management and utilization.

Keywords Agroforestry · Herbal medicine · Botanical insecticide · Poverty alleviation · Sustainable uses of plants

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V. Sarasan (✉) · G. C. Kite · P. C. Stevenson
Royal Botanic Gardens, Kew, Richmond,
Surrey TW9 3AB, UK
e-mail: v.sarasan@kew.org

G. W. Sileshi
World Agroforestry Centre (ICRAF), Southern Africa Programme, Chitedze Agricultural Research Station,
P.O. Box 30798, Lilongwe, Malawi

P. C. Stevenson
Natural Resources Institute,
University of Greenwich, Kent ME4 4TB, UK

Introduction

Herbal medicine consumption is widespread, where 80% of people in developing countries rely on plants as their primary source of healthcare (Tilburt and Kaptchuk 2008; Alves and Rosa 2005) such as the treatment of diseases including HIV/AIDS and malaria (Mills et al. 2005; Titanji et al. 2008). A recent review (Titanji et al. 2008) reported that 217 plant species were used as antimalarials in Cameroon alone. Over a hundred phytochemicals have been isolated from 26 of these species among which some are potential leads for the development of new antimalarial drugs (Titanji et al. 2008). The use of pesticidal plants as alternatives to expensive and harmful synthetic pesticides is also extensive; particularly among resource poor farmers in developing countries (Kamanula et al. 2011; Rother 2010), where their value is greatest and where users have poor access or insufficient resources to acquire commercial products (Isman 2008). Unregulated use of plants invariably revolves around collection from the wild; thus, the development of technologies to increase their availability and reduce pressure on natural stands will benefit poor people directly. While the collection and sale of plant products provide additional income for rural poor, it also raises concerns about over-harvesting of endangered species (Wynberg 2002).

Wild plant harvesting is often destructive. For example, Augustino and Gillah (2005) reported the frequent use of

roots for medicines among African herbalists and suggested these practitioners be trained in sustainable harvesting as a priority. Even harvesting of flowers has been demonstrated to impact on the regeneration of wild populations of *Protea* (Witkowski et al. 1994), although wild harvesting is not necessarily unsustainable (van Andel and Havinga 2008).

The management of medicinal and pesticidal plants is critical to the achievement of most of the United Nations' Millennium Development Goals (MDGs), which represent the human needs and basic rights that all individuals are entitled to freedom from extreme poverty and hunger, a quality education and good health. This is particularly so for MDG 7 which aims to ensure environmental sustainability by reversing the loss of environmental resources and reduce the loss of biodiversity since as many as 1.6 billion people, invariably the poorest, rely on forest resources for all or part of their livelihoods. In developing countries, a deficit of skilled human resources and basic infrastructure limit the achievement of MDG goals (Brink et al. 1998). In this paper, we review the conservation and sustainable utilization of medicinal and pesticidal plants through phytochemical and in vitro techniques with additional emphasis on the use of arbuscular mycorrhizae (AM) and growth promoting bacteria.

Sustainable utilization and production of plants: problems and solutions

Currently, medicinal and pesticidal plants are harvested in an unregulated manner from natural stands, which are often regarded as common property resources. Bark and subterranean plant parts are commonly harvested (Titanji et al. 2008; Zschocke et al. 2000). Roots of *Mondia whitei* (Cunningham 1993; Lampiao et al. 2008) are popular; bark, stem and roots of endangered *Warburgia salutaris* and *Ocotea bullata* (Botha et al. 2004; Kowalski and van Staden 2001) and *Prunus africana* are exploited, while the rhizome of *Alepidea amatymbica* is used (Cunningham 1993). In a recent survey in South Africa, 57% of the *O. bullata*, 60% of the *Curtisia dentata*, 70% of the *P. africana* and 39% of the *Rapanea melanophloeos* stems were bark-stripped (Geldenhuys (2004). Harvesting of *P. africana* bark for European pharmaceutical companies has severely impacted on natural populations in Cameroon (Hall et al. 2000). The dramatic increase in the use of corms of the African potato (*Hypoxis hemerocallidea*) for the treatment of HIV/AIDS (Mills et al. 2005) has endangered this medicinal plant (Ndong et al. 2006). Similarly, the tubers of Devil's Claw (*Harpagophytum procumbens*) and *Dioscorea dregeana* are in high demand in Africa (Dinda et al. 2007; Dharani et al. 2010). However, propagation of Devil's Claw has now been developed and has reduced harvesting from the wild (Stewart and Cole 2005).

The decision to propagate medicinal plants using tissue culture depends on the plant part collected, level of threat in the wild, market demand and the quality of transplants for propagation (Table 1). While different methods of propagation are required, some plants need simple and low-cost methods. From a conservation viewpoint, there are two plant categories of concern: slow growing species with limited distribution where demand exceeds supply, e.g. *W. salutaris* (Botha et al. 2004), and species harvested unsustainably, e.g. *Securidaca longepedunculata* root bark for insect control (Belmain and Stevenson 2001; Stevenson et al. 2009).

Sustainable utilization and production may be ensured by linking in vitro technology with agroforestry development (Vicient and Martínez 1998), which can effectively replace the collection from wild stands while providing other ecosystem benefits such as carbon sequestration and biodiversity conservation on farm land. Of special interest in this case is the use of somatic embryos as synthetic seeds (Vicient and Martínez 1998) for the conservation and utilization of threatened species in agroforestry. Somatic embryogenesis also has other practical applications in agroforestry, including germplasm cryopreservation, virus elimination, in vitro metabolite production, and in vitro mycorrhizal initiation (Vicient and Martínez 1998). The possibility of combining the conservation potential of cryopreservation with the amplification potential of somatic embryogenesis makes this technique very practical in maintenance of germplasm of agroforestry species. Plant cell in vitro culture technology (e.g. embryogenic calli or cell-suspension cultures) provides a potential solution for species whose seeds cannot be stored at low temperatures or those that can only be propagated through vegetative parts. In addition, in vitro propagated plants managed on farmland will provide a more reliable source of medicinal and pesticidal materials thus overcoming variable quality (Canter et al. 2005).

Micropropagation of plants of medicinal and pesticide importance

Micropropagation techniques have improved propagation of medicinal and pesticidal plants (Table 2). Considerable progress has been made in the in vitro propagation of medicinal plants with few seeds and/or low germination rate (Sarasan et al. 2006; Singh et al. 2009). The use of controlled environments can provide propagules for planting at seasonally appropriate times, while in vitro tools can generate plants with useful chemical variation of

Table 1 Selected African medicinal and pesticidal plants that can benefit from in vitro propagation for both long-term conservation and income generation projects

Source material	Use in conservation, requirements and applications	Applications in income generation projects
Seed-derived juvenile stock		
Bobgunnia madagascariensis (r), *Dicoma kirkii* (wp), *Diplorhyncus condylocarpon* (fr), *Griffonia simplicifolia* (sd), *Harpagophytum procumbens*[a] (tb), *Helichrysum kraussii* (r), *Kigelia africana*[a] (fr), *Kedrostis foetidissima* (r), *Lentinus tuber-regium* (tb), *Mondia whitei* (r), *Oncoba spinosa* (fr), *Othonna natalensis* (r), *Pterocarpus angolensis* (fr) NT, *Securidaca longepedunculata*[a] (rb) VU, *Stangeria eriopus* (r) VU, *Voacanga africana* (sd)	Best practice available for taxa where whole plants, roots, tubers, bulbs, fruits and seeds are collected (a) Basic infrastructure and training required for applying in vitro methods (b) Genetically diverse propagules are essential as collection methods leads to deterioration of genetic diversity	Propagation protocols are easier to establish to grow genetically diverse propagules. AM fungi and growth promoting bacteria may be used during weaning stages of propagation (a) AM: *Gigaspora* spp., *Glomus* spp., *Scutellospora* sp., *Entrophospora* spp., *Acaulospora* spp. (b) Growth promoting bacteria: *Acetobacter* spp, *Pseudomonas* spp.
Mature-phase explants from elite plants (woody shrubs and trees)		
Cassia abbreviata (bk), *Curtisia dentate* (bk), *Erythrophleum lasianthum* (bk), *Erythrophleum suaveolens* (bk), *Erythrina abyssinica* (bk), *Ocotea bullata*[a] (bk), *Prunus africana*[a] (bk) VU, *Warburgia salutaris*[a] (bk) EN	Not ideal for widespread planting for conservation (a) Potential for integrated joint projects between phytochemists, ethnobotanists and conservation biotechnologists (b) Ideal for developing elite germplasm collection for commercial exploitation	Best option to produce uniform plants year around. As taxa may be recalcitrant in culture, advanced training and basic infrastructure required (a) Direct rooting of shoots in shade houses or poly tunnels (ideal for capacity building in small holdings and co-operatives) (b) In vitro rooting of shoots and supplying the propagules to small holdings and co-operatives to acclimatize in shade houses or poly tunnels (c) AM and growth promoting bacteria may be used during weaning stages of propagation as above

Species listed shows part of the plant collected for use and the latest conservation rating

EN, endangered; VU, vulnerable; NT, near threatened (IUCN 2010)

[a] Medicinal plants of current interest in commercial product development and elsewhere (van Wyk 2008); *bk* bark, *fr* fruit, *r* root, *rb* root bark, *sd* seed, *tb* tuber, *wp* whole plant (based on Cunningham 1993 and Dharani et al. 2010)

Table 2 In vitro culture methods applied for propagation and utilization of selected species of pesticidal and medicinal plants

Plant species	Study	Method/response	Reference
Aloe polyphylla	Effect of different sugars	Shoot multiplication using glucose as carbon source without plant growth regulators	Nandha and Sarasan (2007)
Spilanthes acmella	Thin cell layer culture	Shoot regeneration	Singh et al. (2009)
Harpagophytum procumbens	Assessment of tubers of plants in the wild and in vitro raised plants for secondary metabolites	Better effectiveness of tubers from the wild compared to in vitro raised plants	Bairu et al. (2010)
Cephalis ipecacuanha	Seedling versus in vitro raised plantlets	Improved alkaloid content in in vitro raised plants	Yoshimatsu (2008)
Azadirachta indica	Limonoids levels in suspension cultures	Importance of two stage suspension culture method	Raval et al. (2003)
Kniphofia leucocephala	Micropropagation	Continuous culture system for propagule development for re-introduction	McCartan and van Staden (2003)
Bowiea volubilis	Micropropagation	Twin scales and split shoot culture	van Staden et al. (1991)
Mondia whitei	Juvenile material multiplication	2,000 propagules over 6 weeks	McCartan and Crouch (1998)

active components (Canter et al. 2005). In addition, micropropagation ensures disease-free propagules facilitating exchange of materials across borders (Hartmann et al. 1997) and maintaining germplasm and source material in pathogen-free condition and in the production of foundation clone source (Vicient and Martínez 1998).

Micropropagation techniques have been developed for medicinal plants, e.g. *Lippia sidiodes* and *L. filifolia*, due to their medicinal value and scarcity (da Costa et al. 2007; Peixoto et al. 2006), and the technique can be applied to related pesticidal plants, e.g. *L. javanica*, reported to control ticks on livestock in southern Africa (Madzimure et al. 2011). Large-scale micropropagation of pesticidal plants has been achieved, e.g. with *Spilanthes acmella* using thin layer culture methods (Singh et al. 2009) and seedling leaf explants (Pandey and Agrawal 2009; Saritha and Naidu 2008). This species is toxic to larvae of the malaria vector *Anopheles gambiae*.

In species where whole plants, bark, fruits and rhizomes are collected, availability of quality seeds will be limited. In some cases, either poor germination rate or lengthy period required for germination can be impediments for exploitation as income generating species. Therefore, improving seed germination by in vitro methods and/or micropropagation from juvenile material is important for rapid in vitro propagation of genetically diverse stocks. Cloning of mature-phase individuals is the best option to produce uniform plants as reported for neem (Arora et al. 2010), where no variations in active compounds occurred. The case of 'arogyapacha' (*Trichopus zeylanicus* ssp. *travancoricus*) in Kerala (India) illustrates how indigenous knowledge can be used to identify target species (Pushpangadan et al. 1988). Krishnan et al. (1995) developed a method to propagate this species to produce nearly 80,000 shoot buds from a single bud over 2 years in culture but this has not yet been commercially exploited.

Somaclonal variations may develop after long-term culture and complex culture regimes. Improvement in germination methods through in vitro methods can raise genetically diverse propagules and preserve genetic diversity. Traditionally, seed-derived cultures were used for mass propagation to exploit the medicinally active constituent, e.g. α-bisabolol, from *Salvia stenophylla*. However, in vitro raised plants were higher in α-bisabolol (Musarurwa et al. 2010). Similarly, Devil's Claw tubers from culture-derived stock had higher iridoid content than those of wild plants (Bairu et al. 2010). Micropropagation has the potential to produce planting stock for restoring locally threatened *Hydrastis canadensis* populations and for commercial cultivation (Obae and West 2010). If tissue-cultured superior plants can be cloned in large numbers, they can relieve pressure on plants in the wild. For example, *Cephaelis ipecacuanha* propagation was improved by tissue culture, and more significantly the production of established plants in the field produced more emetic alkaloids than seed-derived plants (Yoshimatsu 2008).

However, this is not always the case. Callus and root cultures of *Scrophularia nodosa* a medicinal plant with wound healing activity associated with iridoids (Stevenson et al. 2002) was not able to synthesize these compounds and quantities differed in regenerated plants from wild material (Sesterhenn et al. 2007). Additionally, micropropagation is difficult in some species owing to recalcitrance and phenolic production as reported for *O. bullata* (Kowalski and van Staden 2001).

Prospects in adopting in vitro tools in resource poor areas

While biotechnology can play a significant role in commercializing plants, creating jobs and foreign exchange, the high operating cost and the serious deficit of skills and infrastructure have been limiting research and development (Brink et al. 1998). Recently, methods have been developed to propagate plants cost effectively. According to Pence (2011), the cost of in vitro methods is greater than conventional propagation, but they may be critical for species under higher threat. The least expensive method is conventional seed propagation followed by cuttings and micropropagation. Low-cost methods for cassava micropropagation have been used successfully in China, Cameroon, and Congo (Zok 1993; Mabanza et al. 1995), Nigeria and in several other countries in Africa (Ng et al. 1994). Growth rooms without electricity and air conditioning (Thro et al. 1999) and commercial fertilizer in place of tissue culture formulation (Santana et al. 2009) have been successfully used for cassava tissue culture and similar models might be adopted elsewhere with only basic facilities. Low-cost facilities have also been developed for banana tissue culture in Kenya.

Farmers who adopt in vitro propagated material may benefit more from income increases through reduced pest control costs and higher effective yields (Muyanga 2009). For example, bananas propagated using in vitro tools have been shown to have increased vigour and suffer lower yield loss from weevils, nematodes, and fungal diseases in Kenya (Dubois et al. 2006; Muyanga 2009). Generally, there are indications that in vitro technology can be commercially sustainable. The few studies on economic analysis of in vitro tools specific to medicinal plants also provide a strong case for sustainability. In India, Das et al. (2010) conducted cost-benefit analysis on the propagation of the medicinal plant *Stevia rebaudiana* by stem cutting and in vitro tools. According to their analysis although

cost-benefit ratios were comparable for in vitro and cuttings propagated in the field, more income was generated using in vitro propagation than propagation by cuttings. Similarly, Rao et al. (2000) reported five times higher returns to in vitro propagated woody plants in India.

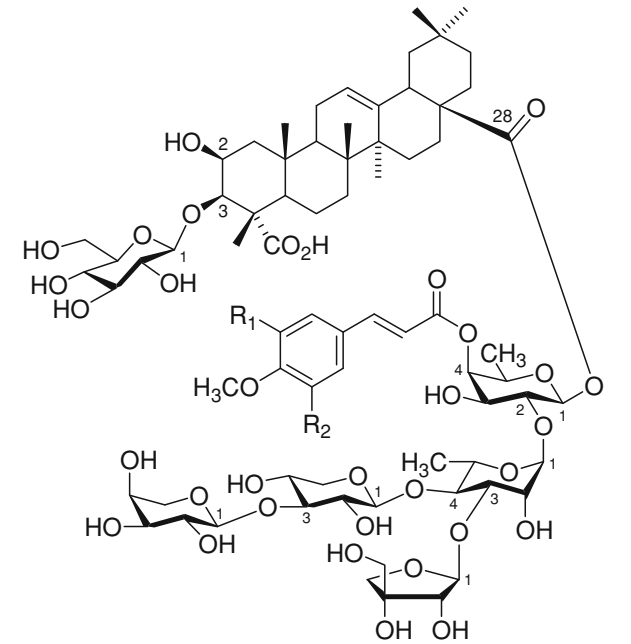
Since farmers are the managers of in vitro propagated materials and ultimate benefactors, farmer participatory methods need to be emphasized in the selection, development and commercialization. For example, participatory methods have facilitated the domestication and cultivation of indigenous fruit trees of southern Africa. Together with villagers, the superior trees were identified on the basis of farmers' criteria and propagation and management practices were developed (Akinnifesi et al. 2008). Then farmers can generate income by embarking on agribusiness initiatives such as selling planting materials and production of raw materials for use as medicinal or pesticidal products. In the case of selling planting materials, farmers may follow successful business models like the banana tissue culture nursery operators in Kenya and Uganda (Dubois et al. 2006). A nursery operator buys acclimatized tissue culture seedlings and later sells at a premium price to other farmers. In Kenya, a central tissue culture laboratory at Jomo Kenyata University and Technology is linked to village nursery operators. Such public–private partnership models may be envisaged for medicinal and pesticidal plant business initiatives. In addition, individual farmers or co-operatives may generate income through sales of raw materials or involvement in agroforestry and social forestry initiatives that link farmers to emerging carbon markets.

Phytochemical studies

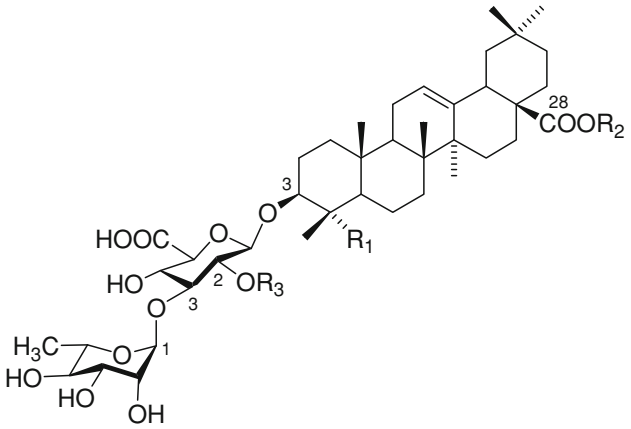
Variation in morphological, genetic and chemical characters of medicinal plants in developing countries is not well studied, although van Wyk (2008) reports of variations in the natural stands. This is crucial to guide commercialization of medicinal plants, selection of elite clones, and the standardization of raw materials. For example, Devil's Claw reportedly produces 57 different medicines (Kathe et al. 2003). Phytochemical screening (including molecular markers) will facilitate the accurate identification of elite materials (species, provenances, single plants or plant parts), where plant chemistry varies between different specimens. Once optimal source genotypes have been identified, in vitro tools may be applied to mass propagate from seeds or vegetative materials. Phytochemical tools can also facilitate validation of the desired quality in order to counter loss of efficacy in the long run through selection, propagation or ex situ management, e.g., in agroforestry. Unless traded plant materials have consistent high quality, trade may be lost (and hence incomes for those who depend upon the plants) and users may lose interest altogether in plants for pest control or medicine.

S. longepedunculata is reported to treat various medical conditions and in Nigeria is known as the mother of all drugs being used to heal numerous ailments (Daper et al. 2007). For example, Maiga et al. (2005) reported securinine to be active against the malaria parasite (*Plasmodium falciparum*) and the root extract has trypanocidal properties (Atawodi 2005). Root bark of the tree is used as a pesticide in stored grain (Belmain and Stevenson 2001) and has been validated with the activity attributed to the methylsalicylate and saponins (Jayasekera et al. 2005; Stevenson et al. 2009). The pest control activity is restricted to the root bark which can be explained by the differing chemistries between the roots and the stems (Fig. 1). Two saponins (**1** and **2**) were identified previously in the root bark of *S. longepedunculata* and shown to be biologically active against storage pests (Stevenson et al. 2009). Recent analyses demonstrate a chemical homogeneity of the population across Africa from Ghana to Zambia (Stevenson, unpublished). The saponin fraction as a whole was also shown to be biologically active (Stevenson et al. 2009) and was highly variable between the specimens and may be important in selecting the best genotypes for micropropagation depending on variations in biological efficacy of these components. Field trials with the root bark and stem bark indicate the latter lacks efficacy in storage pest management whereas the root bark is effective (Stevenson et al. 2010b). Thus, the qualitative differences in the saponins are likely to account for the difference in efficacy, while the occurrence of methylsalicylate as an active component of the root is also an important chemical marker for activity (Jayasekera et al. 2005).

Bobgunnia madagascariensis, a leguminous tree, is used in traditional medicine and pest control. In the laboratory, extracts of the root bark had very high activity against a chloroquine-resistant strain of the malaria parasite *P. falciparum* (Ouattara et al. 2006). The superior antifungal activity against *Candida* species has also prompted the deposition of a patent (Hostettmann and Schaller 2000) and preclinical testing with the aim to introduce new antifungal drugs useful for the treatment of systemic mycoses associated with HIV infections (Hostettmann et al. 2000). *B. madagascariensis* has also been exploited in medical, veterinary and agricultural pest control (Schaller et al. 2000; Sileshi et al. 2008). The powdered pods are reportedly insecticidal against mosquito larvae (Maiga et al. 2005), while farmers in Zambia and Malawi use the pods for control of termites (Kamanula et al. 2011). The widespread commercial harvesting and sale of roots of this species are significant and they are traded widely (Cunningham 1993). While cultivation would increase availability, *B. madagascariensis* has low seed viability and



1 $R_1 = R_2 = H$
2 $R_1 = R_2 = OCH_3$



3 $R_1 = CH_3$, $R_2 = \beta$-Glc, $R_3 = \beta$-Xyl
4 $R_1 = CH_3$, $R_2 = \beta$-Glc, $R_3 = H$
5 $R_1 = CH_3$, $R_2 = R_3 = H$
6 $R_1 = CHO$, $R_2 = R_3 = H$
7 $R_1 = CH_3$, $R_2 = H$, $R_3 = \beta$-Glc
8 $R_1 = CHO$, $R_2 = H$, $R_3 = \beta$-Glc
9 $R_1 = CH_3$, $R_2 = R_3 = \beta$-Glc

Fig. 1 Chemical structures of saponins reported in *Securidaca longepedunculata* and *Bobgunnia madagascariensis* the occurrence of which has implications for their pesticidal effect (Marston et al. 1993; Stevenson et al. 2009, 2010a, b)

poor germination. Plants propagated from seed also exhibit a high degree of genetic variability (Berger and Shaffner 1995). Seven saponins (Fig. 1) were identified in extracts of *B. madagascariensis* pods by Marston et al. (1993) and Stevenson et al. (2010a, b). Compounds **5**–**7** were molluscicidal against *Biomphlaria giabrala* and *Bulinus globosus*, intermediate hosts for schistosomiasis, whereas **8** and **9** were only weakly effective or not active. The activity of **5** was higher than the other compounds, which might be attributable to its lower polarity and ability to cross cell membranes. The presence of specific saponins in the pods has significant implications on the biological activity of the plant material and its value. The occurrence of **5** and to some extent **6** and **7** is therefore important in the selection of material for propagation and demonstrates that it is insufficient to produce plant material but that the most appropriate genetic material is used and highlights the need for more phytochemical research to provide information on the judicious use of herbal remedies and pesticidal plants. This may reduce harm through failed expectations and pharmacologic adverse effects. Collaboration with local scientists on aspects of tissue culture and molecular markers will ensure sustainability as it builds research capacity (Bothma and Thompson 2004).

Photoautotrophic micropropagation, mycorrhizal fungi and growth promoting bacteria

Photoautotrophic micropropagation involves growing shoot cultures on low sugar or sugar-free media, supplying the plantlets with carbon dioxide and sufficient light with either diffusive or forced ventilation. By replacing agar with vermiculite, cellulose fibres, perlite or coir as the support leads to improved rooting and transplantation success (Marriott et al. 2010; Sarasan 2010). Rooting of jojoba plants was improved using perlite and cellulose filters as acclimatization of plantlets was improved by enhancing ventilation (Mills et al. 2009). Using a photoautotrophic system, tuberization was achieved from the lateral roots of Devil's Claw (Levieille and Wilson 2002). Improvement in survival and quality of the propagules has also been achieved by increased air exchange through diffusive ventilation (Kozai and Kubota 2001; Sarasan et al. 2006; Sarasan 2010). Thus, there is a potential for the propagules to be rooted and weaned by direct planting by unskilled farmers or small rural enterprises for further growth and income generation.

'Biotization' or the induced tolerance to abiotic and biotic stresses to propagules as a result microbial inoculation is used in many crop plants commercially and increasingly so in other groups as well (Nowak 1998). Applications of mycorrhizal fungi and growth promoting bacteria for improving the transplant quality have been reviewed in detail (Rai 2001; Kapoor et al. 2008). Several reports have highlighted the positive effects of both mycorrhizal fungi and growth promoting bacteria on the

propagation of medicinal plants (Table 3). Substantial improvement in weaning was achieved in *Baptisia tinctoria* by the mycorrhizal fungus *Glomus etunicatum* (Table 3). Increase in percentage of rooting in mycorrhizal (90%) compared to the non-inoculated controls (60%) and reduction of weaning stress have been reported by Grotkass et al. (2000). Tang et al. (2000) reported an increase in phenolic compounds in the bark of the micropropagated plants and resistance to canker disease of poplar inoculated with *Glomus mosseae*. Elmeskaoui et al. (1995) developed a system combining a photoautotrophic environment, mycorrhizae and 5,000 ppm CO_2. After 3 weeks, plantlets placed in contact with the primary symbiosis were colonized by the mycorrhizal fungus, and colonized plantlets had better root systems and shoot growth than control plants. Abu-Zeyad et al. (1999) found a positive effect of mycorrhizal fungi on the accumulation of castanospermine in the leaves of *Castanospermum australe* plants in the field. This and the review of the literature by Liu and Yang (2008) clearly indicate that combining mycorrhization with photoautotrophic systems can alleviate the problems plantlets face in vitro and ex vitro.

In some cases, a mixture of fungi and/or bacteria would be more effective than a single species (Rai 2001). Mycorrhization of tissue-cultured propagules has the potential to produce plants with increased levels of biologically active secondary metabolites (Rai 2001; Kapoor et al. 2008). The use of mycorrhizal fungi can also be applied for the conservation of rare and threatened plants and especially medicinal plants. As this can be done at relatively low cost and is easy to incorporate into the ex vitro rooting stage or weaning stage the implementation will be straightforward. In marginal and phosphorous deficient soils this has greater implications and would help improve plant growth as reported in a micropropagated medicinal plant *Scutellaria integrifolia* (Joshee et al. 2007). By incorporating the best available method for rooting, the use of mycorrhizal fungi, growth promoting bacteria and photoautotrophic micropropagation systems, custom made in vitro propagation packages can be developed for medicinal and pesticidal plants, and implemented in developing countries. As transplant quality and establishment of propagules in the field are two very important aspects of in vitro propagation technology, transfer for income generation needs intensive research in the coming years.

Models for income generation projects exploiting in vitro methods

In this section, we discuss two possible implementation pathways for income generation projects. The first is where funding is adequate and an operation requires the establishment of the requisite micropropagation facilities. Multiplied plantlets can then be given to individual farmers or co-operatives (Table 1). However, training of farmers is crucial for successful transplanting and management of propagated plants. Investment in farmer training particularly rural women (or women's groups) can translate into viable projects that generate income and employment. Less well-funded projects require the replacement of costly equipment (autoclaves, laminar flows) but may result in microbial contamination of cultures. This can be managed effectively by starting in vitro culture in media with low sucrose and plant safe antimicrobials.

Table 3 Use of arbuscular mycorrhizal (AM) fungi and growth promoting bacteria to improve transplant quality of tissue cultured plants of medicinal importance

Inoculant	Plant species	Response	Reference
Glomus mosseae, *Gigaspora ramisporophora*, *Scutellospora fulgida*, *Entrophospora columbiana*	*Echinacea pallida*	Improved survival and growth rate	Lata et al. (2003)
G. mosseae, *Gigaspora ramisporophora*, *Scutellospora fulgida*	*Podophyllum peltatum*	Improved acclimatization rate and lignan content	Moraes et al. (2004)
Glomus etunicatum	*Curcuma zedoaria*	Improved acclimatization and dry weight	Miachir et al. (2004)
G. etunicatum, *Glomus intraradices*, *Gigaspora margarita*	*Scutellaria integrifolia*	Improved plant and seed fresh weight from micropropagated plants	Joshee et al. (2007)
Glomus deserticola	*Prunus avium*	Improved weaning success	Lovato et al. (2006)
Mixed species	*Curculigo orchioides*	Better acclimatization by mixture rather than monoculture	Sharma et al. (2008)
Mycorrhiza and bacteria mixture	*Chlorophytum borivilianum*	Improved establishment in field conditions	Mathur et al. (2008)
G. etunicatum	*Baptisia tinctoria*	Improvement of rooting and reduction of weaning stress	Grotkass et al. (2000)

Micropropagation in hot countries requires substantial capital for cooling owing to heat generated by lighting but new low heat lighting systems are now available and their installation can help reduce running costs and has been done effectively for banana micropropagation using tubular Sky Lights as a source of maintenance-free natural light while positioning shelves carefully to receive maximum lighting (Kodym et al. 2001). Replacing agar with potato or corn starch would also cut costs and was applied successfully for banana micropropagation (Kodym and Zapata-Arias 2001). Lavanya et al. (2009) reported that rooting ex vitro and application of the bio-control agent *Trichoderma viride* could make micropropagation of neem feasible and reduce the production cost, and could be adopted as a rural enterprise. The twin scales and split shoots method for the in vitro propagation of *Bowiea volubilis* is simple (van Staden et al. 1991) and the manipulations involved can be performed by semi-skilled workers and do not require sophisticated equipment.

Conclusions

Widespread harvesting of medicinal and pesticidal plants will lead to loss of genetic diversity and income to poor people especially women who benefit greatly from the collection and trade in plants. Conventional tree breeding is slow and horticultural tools may not be sufficient to meet the future demand for these plants. From the review of the literature, we conclude that development of appropriate in vitro micropropagation techniques combining mycorrhizal fungi and growth promoting bacteria can ensure availability of uniform and disease-free propagules for cultivation and management of threatened plants on farmland. This requires investment in capacity building and the requisite infrastructure in developing countries especially in Africa.

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