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Review

Role of biotechnology in the conservation of rare, threatened and endangered medicinal plant species in the Kingdom of Eswatini (Swaziland)

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ABSTRACT

The use of indigenous medicinal plants by people is widespread in the Kingdom of Eswatini and the Southern Africa region as a whole. However, due to over exploitation, and for some other reasons like climate change, some indigenous medicinal plants have become endangered and are threatened with extinction. Subsequent loss of biodiversity is at stake. Urgent intervention is therefore, required to conserve them. The purpose of this study was to document plant biotechnology techniques which can be used in conservation of rare, endangered and potentially threatened medicinal plants of crucial importance in the country and region. Various ways of conservation through plant biotechnology are discussed.

Keywords: Indigenous medicinal plants, endangered, biodiversity conservation, plant biotechnology.

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INTRODUCTION

Traditional use of medicinal plants

Since ancient times, mankind has been dependent on plants for food, flavours, medicinal and many other uses (Sidhu, 2010). Secondary metabolites of plants are economically important as drugs, fragrances, pigments, food additives and pesticides (Khan et al., 2009). Medicinal plants are the most important source of life saving drugs for the majority of the world's population (Tripathi and Tripathi, 2003; Debnath et al., 2006; Khan et al., 2009). Medicinal properties may be present in one or all of their parts: root, stem, bark, leaf, flower, fruit or seed (Jitendra et al., 1996). Medicinal plants and traditional medicine play an important role in the health care system of most developing countries. The traditional health care practice is mainly dependent on medicinal plants collected from the wild (Kasagana and Karumuri, 2011).

Africa is a rich source of medicinal plants, perhaps, the best known species is *Phytolacca dodecandra* (endod plant) (Hoareau and DaSilva, 1999). Other notable examples are *Catharanthus roseus*, which yields anti-tumour agents such as vinblastine and vincristine; and *Ricinus communis*, which yields the laxative-castor oil (Hoareau and DaSilva, 1999).

Medicinal plants threat

Genetic biodiversity of traditional medicinal herbs and plants is continuously under the threat of extinction as a result of growth-exploitation, unsustainable harvesting techniques, loss of growth habitats, and unmonitored trade of medicinal plants (Hoareau and DaSilva, 1999). Harvesting from the wild, the main source of raw material, is causing loss of genetic diversity and habitat destruction (Canter et al., 2005). The rich resource is decreasing at an alarming rate as a result of over exploitation (Afolayan and Adebola, 2004). Kasagana and Karumuri (2011) reported that the medicinal plant biodiversity is being depleted due to both man-made and natural calamities.

There is a concern that many medicinal plants, not to

mention the knowledge about their use, are on the verge of extinction (Jitendra et al., 1996). The indigenous knowledge associated with the conservation and use of medicinal plants is also disappearing at an alarming rate (Kasagana and Karumuri, 2011). This is so, because most of the indigenous knowledge is not properly or not documented at all, at least in Eswatini. The indigenous knowledge (IK) holders are the elderly who often pass such knowledge from generation to generation without any documentation. It seems today's generation is no longer interested in indigenous knowledge systems (IKS). Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds (Balunas and Kinghorn, 2005).

The need to protect rare medicinal plants seems to be urgent. Plant samples collected today may, in the future, be found to combat dreaded diseases, but there is no guarantee that the plant will then still exist (Jitendra et al., 1996). This could be unfortunate not only for the patients but for the countries that could develop lucrative industries out of the budding resource (Jitendra et al., 1996). The rapid erosion of the genetic diversity of both wild and cultivated plants has attracted more and more international concern (Villalobos et al., 1991). As a matter of priority, any strategy must address the plight of the increasing number of wild medicinal plants threatened with extinction (Jitendra et al., 1996). As a consequence, germplasm conservation techniques have become accessible to protect the third world's germplasm diversity (Villalobos et al., 1991).

Objective

This paper reviews the different techniques of plant biotechnology, which could also be used in Eswatini, in the conservation of rare, threatened and endangered medicinal plant species. It discusses the need for conservation of medicinal plant species, and the impact of biotechnology in the conservation measures.

SOME IMPORTANT MEDICINAL PLANTS IN ESWATINI

The Kingdom of Eswatini (Swaziland) is one of the African countries with richly diversified flora (Fuggle and Rabie, 1992). This could be attributed to the fact that Eswatini has a range of ecological zones which encompass almost every feature of the African continent except for the deserts (Fuggle and Rabie, 1992). Some of the flora are species endemic to Swaziland while some are indigenous to the Southern Africa region. These plants are used as source of food and medicine especially in the rural population, where the majority of the country's population come from. Five important and threatened indigenous medicinal plants which are used in traditional medicine in the Kingdom of Eswatini are presented in this paper.

Warburgia salutaris (siSwati name: Sibhaha, Common name: Pepper-bark tree)

This is a tropical forest tree which extends southwards as far as KwaZulu-Natal, eastern and northern Gauteng and across Swaziland (Giles, 2004). Its growth habitat is forests and kloofs. Dludlu et al. (2017) reported eighteen localities where *Warburgia salutaris* is found in Eswatini, across a wide range of habitats, physiographic zones, geology, and vegetation type. The *Warburgia salutaris* plant (Figure 1) is listed as endangered in the International Union of Conservation of Nature (IUCN) red data list and as critically endangered in the Swaziland National Trust Commission (SNTC) (2016) list. The bark, leaves, stems and roots are used to treat numerous health complaints, including abdominal pains, backache, blood disorders, chest complaints, colds, coughs, febrile complaints, fever, headache, inflammations, influenza, malaria, respiratory complaints, rheumatism, sores, stomach ulcers, toothache and venereal diseases (Maroyi, 2013).

Siphonochilus aethiopicus (siSwati name: Sidvungule, Common name: Wild ginger)

Siphonochilus aethiopicus is a perennial herb with large, erect, hairless leaves which develop annually from small cone-shaped rhizomes (Light, 2002) and spectacular purple or pink flowers (van Wyk, 2008) (Figure 2). The fresh rhizomes and roots are very popular in traditional medicine in southern Africa (Fouche et al., 2013). The main use of *Siphonochilus aethiopicus*, as identified by traditional healers, is for the treatment of coughs, colds, flu, malaria and menstrual disorders (Manzini, 2005). However, the plant is listed as endangered by SNTC.

Ozoroa sphaerocarpa (siSwati name: imfuce-lemnyama, Common name: Currant resin tree), ***Syzygium cordatum*** (siSwati name: Umncozi, Common name: Waterberry) **and** ***Breonadia salicina*** (siSwati name: Umhlume, Common name: African teak)

The bark of these three plant species are used in combination in traditional medicine for the treatment of diarrhoea (Sibandze et al., 2010). *Syzygium cordatum* and *Ozoroa sphaerocarpa* (Figure 3) possibly possess anti-diarrhoeal activity mediated by inhibiting the growth of *Escherichia coli*, while *Breonadia salicina* lacks anti-*Escherichia coli* activity but its anti-diarrhoeal activity may

Figure 1. *Warburgia salutaris* tree growing at Mafutseni area in Eswatini.

Figure 2. *Siphonochilus aethiopicus* plant with its flowers growing at Ekwakheni area in Eswatini.

be due to some other mechanisms. However, there is dearth of information on distribution and conservation status of these three plants. Since the bark of the plants are used in traditional medicine, the plants are at risk of extinction if unsustainable harvesting methods are employed.

CONSERVATION OF MEDICINAL PLANTS

The goal of conservation is to support sustainable development by protecting and using biological resources

Figure 3. *Ozoroa sphaerocarpa* tree at Mphini area in Eswatini.

in ways that do not diminish the world's variety of genes and species or destroy important habitats and ecosystems (Kasagana and Karumuri, 2011). Plant materials procured from naturally occurring stands are being rapidly depleted because of the use of parts like roots, bark (Figure 4), wood, stem and the whole plant in the case of herbs (Malik et al., 2012).

There are two methods for the conservation of plant genetic resources, namely *in situ* and *ex situ* conservation (Kasagana and Karumuri, 2011). *In situ* conservation can be described as conservation of natural or semi-natural ecosystems in various types of protected area, with various management aims such as; maintaining ecosystem diversity, biodiversity in general or special landscapes, and providing habitat for target species such as mega-vertebrates, birds, forest species, medicinal plants, or for concentrations of endemic species (Heywood and Dulloo, 2005). For species threatened with extinction, *in situ* conservation may be the best option; however, this alone may not always be sufficient to ensure the survival of a species (Reed et al., 2011). In addition to standard methods such as seed banking and development of living collections, *in vitro* tools can provide additional backup collections and provide alternative propagation and conservation of species (Reed et al., 2011).

Ex situ conservation involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Collecting cuttings of plants (Figure 5) and seeds is generally the most cost-effective method for providing material for *ex situ* conservation (Reed et al., 2011).

Figure 4. Debarked *Ozoroa sphaerocarpa* tree from its naturally occurring stand at Ndinda area in Eswatini, for traditional medicine use.

Figure 5. Stem propagation of *Ozoroa sphaerocarpa* in a nursery of the University of Swaziland Research Farm, Mafutseni.

UTILISATION OF BIOTECHNOLOGY IN MEDICINAL PLANTS

Biotechnology

Medicinal plants are one of the most important groups of plant genetic resources. Their use in biotechnology has assumed considerable significance because of over exploitation of these plants to meet the increasing demand (Bajaj et al., 1988; Dixit et al., 2005). Plant biotechnology offers new means of improving biodiversity conservation rather than threatening biodiversity in various ways (Pathak and Abido, 2014; Wieczorek, 2003). According to Chebet et al. (2003) plant biotechnology involves three major areas which are:

- *in vitro* propagation and tissue culture for production of disease-free plants,
- use of molecular markers for improved selection in plant breeding, and
- genetic engineering.

Micropropagation

Micropropagation can be defined as a rapid method of reproducing true-to-type multiples of plants from plant organs in aseptic culture *in vitro* under controlled conditions. The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants (Debnath et al., 2006; Tripathi and Tripathi, 2003). Plant tissue culture techniques offer an integrated approach for the production of standardized quality phyto-pharmaceutical through mass production of consistent plant material for physiological characterization and analysis of active ingredients (Debnath et al., 2006).

In vitro methods provide tools that can be used in a variety of ways, depending on the need of the species (Reed et al., 2011). The biotechnological tools are important to select, multiply, improve and analyze medicinal plants (Khan et al., 2009). By the use of tissue cultures, various problems in plant biotechnology, such as micropropagation, biosynthesis and biotransformation of biologically active compounds, storage of plant cells and organs, and genetic engineering of higher plants can be solved (Bajaj et al., 1988). *In vitro* propagation of plants possesses huge potential in production of high quality based medicines at the same time conservation of medicinal plants (Pathak and Abido, 2014).

Micropropagation can facilitate regeneration, multiplication and international distribution of useful germplasm in a disease-free condition (Drew, 1997; Afolayan and Adebola, 2004). Specific techniques include *in vitro* mass propagation, the production of disease-free plants as well as regeneration systems for plant transformation (Brink et al., 1998). *In vitro* regeneration holds tremendous potential for the production of high quality plant based medicine (Tripathi and Tripathi, 2003). *In vitro* methods provide opportunities for propagating

and preserving endangered plant species when seed-based methods are not adequate (Pence, 2010).

According to Sidhu (2010) the advantages of *In vitro* micro propagation of medicinal plants are the following:

- Higher rate of multiplication.
- Environment can be controlled or altered to meet specific needs of the plant.
- Plant available all year round (independent of regional or seasonal variation).
- Identification and production of clones with desired characteristics.
- Production of secondary metabolites.
- New and improved genetically engineered plants can be produced.
- Conservation of threatened plant species.
- Preservation of genetic material by cryopreservation.

The use of *in vitro* propagated plants for reintroduction or restoration of rare species is also finding application, and this relies on the development of successful methods for acclimatizing plants from culture to *in situ* conditions (Reed et al., 2011). For instance, micropropagation protocols for cloning of some medicinal plants such as *Catharanthus roseus* (Apocynaceae), *Chlorophytum borivilianum* (Liliaceae), *Datura metel* (Solanaceae), and *Bacopa monnieri* (Scrophulariaceae) have been developed (Debnath et al., 2006). A protocol has been developed for plant regeneration from encapsulated nodal segments of *Tylophora indica* (Faisal and Anis, 2015). An efficient protocol providing a dual regeneration pathway via direct shoot organogenesis and somatic embryogenesis for an endangered species, *Metabriggsia ovalifolia*, was established from leaf explants (Ouyang et al., 2016). As we learn more about the metabolic requirements of diverse plants, design of better growing conditions and nutrient media to fit the specific needs of rare plants should ease the difficulty of growing threatened and endangered species (Reed et al., 2011).

Cryopreservation

There are many instances in which seeds are sterile or not available, or so few plants remain that collecting whole plants would negatively impact the population (Reed et al., 2011). In addition, Chin (1996) reported that many tropical species have seeds that die when dried or frozen (recalcitrant seeds), and thus they cannot be stored using conventional seed-banking technologies (as cited in Reed et al., 2011). Although the most economical means of germplasm storage for seed propagated species is in the form of seeds, this is not always feasible because of the following reasons:

- Some crops do not produce viable seeds,
- Some seeds remain viable for a limited duration only and are recalcitrant to storage,
- Seeds of certain species deteriorate rapidly; due to seed borne pathogen, and
- Some seeds are very heterozygous not suitable and for maintaining true to type genotypes.

Seeds or vegetative material from genetically diverse wild populations can be difficult to obtain, and methods should be in place for the propagation, storage, sustainable utilization, and storage of these genetic resources in liquid nitrogen (Reed et al., 2011). An effective approach to circumvent the above problems may be application of cryopreservation technology (Kasagana and Karumuri, 2011). Cryopreservation is defined as the viable freezing of biological material and their subsequent storage at ultra-low temperatures (-196°C) using liquid nitrogen (Drew, 1997; Kasagana and Karumuri, 2011). Cryopreservation was achieved initially using cryo-protectants and controlled cooling. This method shows potential with organised tissue, including embryos and meristems (Drew, 1997). According to Kasagana and Karumuri (2011), the use of liquid nitrogen, either by itself or as a source of nitrogen gas, is based on the following unique combination of features:

- chemically inert,
- relatively low cost,
- non-toxic,
- non-flammable, and
- readily available.

Tripathi and Tripathi (2003) pointed out that cryopreservation is long-term conservation method in liquid nitrogen and provides an opportunity for conservation of endangered medicinal plants. *In vitro* collecting of tissues is less invasive than removing whole plants and allows for an efficient sampling of a large number of plants when seeds are not available (Reed et al., 2011). Cryopreservation represents the only safe and cost effective option for long term conservation of plant germplasm (Rajasekharan and Prakashkumar, 2010), and management of *in vitro* produced materials for biotechnological applications (Dixit et al., 2005). The *in vitro* production of secondary metabolites in plant cell suspension cultures has been reported from various medicinal plants (Tripathi and Tripathi, 2003).

In vitro techniques provide the option of cryopreserving embryos or vegetative tissues for long-term storage as an alternative to seed banking (Reed et al., 2011). Various explants have been used for cryopreservation of medicinally important plants (Rajasekharan and Prakashkumar, 2010). Established cultures can comprise a medium term *ex situ* collection, but these cultures can also provide tissues for cryopreservation and long term storage (Reed et al., 2011). For many tropical species that cannot support dehydration, tissue culture and cryopreservation are the best alternatives (Villalobos et al., 1991).

For each species and tissue type, a cryopreservation

protocol needs to be developed/adapted to the natural, cold, freezing and desiccation of the species, explant size and type (Benson, 2009). Classical cryopreservation techniques, which are based on freeze-induced dehydration, are suitable for freezing undifferentiated cultures and apices of cold-tolerant species (Rajasekharan and Prakashkumar, 2010). Feedback of fundamental knowledge can assist the cryopreservation of storage recalcitrant species and germplasm types (Benson, 2009). The development of cryopreservation protocols is significantly more advanced for vegetatively-propagated species than for recalcitrant seed species (Rajasekharan and Prakashkumar, 2010).

In vitro techniques are well developed for the collection, propagation and cryopreservation of many species (Reed et al., 2011). New cryopreservation techniques, which are based on vitrification, are successfully employed with all explants, including cell suspensions and calluses, apices, and somatic and zygotic embryos of temperate and tropical species of medicinal plants (Rajasekharan and Prakashkumar, 2010).

Embryo culture

Conservation of endangered species can also be attained by practicing embryo culture technique. Embryo culture is a type of plant tissue culture that is used to grow embryos from seeds and ovules in a nutrient medium (Hussain et al., 2012). Embryo culture involves isolating and growing an immature or mature zygotic embryo under sterile conditions on an aseptic nutrient medium with the goal of obtaining a viable plant (Bridgen, 1994). In embryo culture, the plant develops directly from the embryo or indirectly through the formation of callus and then subsequent formation of shoots and roots. The technique depends on isolating the embryo without injury, formulating a suitable nutrient medium, and inducing continued embryogenic growth and seedling formation (Bridgen, 1994). A successful protocol has been developed for the *in vitro* propagation of *Khaya grandifoliola* by excising embryos from mature seeds (Hussain et al., 2012). This process is however, difficult due to the tedious dissection necessary and the complex nutrient medium requirements (Bridgen, 1994).

Integration approach

As extinction pressure is increasing, it is important that highly threatened species are identified, and that integrated conservation measures are undertaken, utilizing all the tools available, including the *in vitro* methods (Bradford and Alston, 2004; Reed et al., 2011; Yao et al., 2016). The integrated approaches of our culture systems will provide the basis for the future development of novel, safe, effective, and high quality products for consumers (Debnath et al., 2006). All efforts to conserve and use genetic resources will contribute to the benefit of future human generations (Villalobos et al., 1991).

By focusing on tissue culture, the skills necessary to maintain and manage a biotechnology laboratory can be developed. The second phase is the application of biotechnological tools, which can improve the efficiency of selection and breeding of varieties/cultivars (Brink et al., 1998). The successful production of transgenic plants requires an adequate infrastructure, expertise in tissue culture and molecular biology, and sustainable funding to cover the high cost of such research (Brink et al., 1998). Research in plant biotechnology is playing a crucial role in the production and conservation of plant-based resources globally (Moyo et al., 2011). Germplasm conservation and cryopreservation ensure genetic stability and retention of biosynthetic potential (Rajasekharan and Prakashkumar, 2010).

CONCLUSION AND RECOMMENDATIONS

Due to increasing genetic loss of medicinal plant species from naturally occurring stands, conservation of these species is of paramount importance. The role of biotechnology in conserving rare, threatened and endangered medicinal plants is highly significant. Micropropagation techniques are essential for plants like *Warburgia salutaris* and *Siphonochilus aethiopicus,* which have been nationally declared endangered, for continuous mass propagation. For plants whose barks are most sought after, for traditional medicine, like *Breonadia salicina*, *Syzigium cordatum* and *Ozoroa sphaerocarpa*, are at risk of extinction and therefore, the biotechnological tools can be used to propagate and conserve them. It is therefore, necessary to develop micropropagation protocols for all the plant species which are endangered and threatened with extinction in Eswatini. The integration approach of sustainable harvesting, conventional propagation methods and plant biotechnology should be employed to mitigate the erosion of the medicinal plant genetic resource in Eswatini and Southern Africa as a whole.

It is recommended that Institutions involved in plant research and conservation in the Kingdom of Eswatini should put their efforts together in ensuring that conservation measures also include application of the plant biotechnological tools.

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