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Prospects of Biotechnological Approaches for Propagation and Improvement of Threatened African Sandalwood (*Osyris lanceolata* Hochst. & Steud.)

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Abstract

The African Sandalwood plant (*Osyris lanceolata*) is a threatened shrub or a small hemi-parasitic tree endemic to East Africa and South African regions, which is being severely affected by uprooting for oil extraction, poor natural regeneration, phenological structures (dioecious), medicinal values, lack of sexual recruitment, habitat loss, anthropogenic and climate factors. It has been found that through application of *in situ* conservation of natural trees with respect to rapid human population growth, the available natural strands of valuable plants such as African sandalwood have not been able to meet the demands of the people in world specifically developing countries. However, advances in plant biotechnology provide new options for collection, multiplication and short- to long-term conservation of *Osyris lanceolata* species, using *in vitro* culture techniques. Different aspects of biotechnological applications can be extensively used to reduce the risk of extinction of this valuable plant species and to improve the quality and quantity of essential oils produced by it. Therefore, tissue culture appears to be a promising approach for the propagation and conservation of African sandalwood plant.

Keywords

African Sandalwood, Auxin, Cytokinin, MS Media, Micropropagation

1. Introduction

The African sandalwood (*Osyris lanceolata*) belongs to Santalaceae family, a shrub or a small hemi-parasitic

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tree growing to a height of six meters, always found associated with their host plants [1]. The tree occurs on rocky ridges and mountain slopes with an altitude between 900 m to 2550 m above sea level. The plant is indigenous to East and South African regions.

The tree is highly valued for its fragrant heartwood, which contains sandal oil used in perfumes and cosmetics industries and at the same time it is very expensive [2]. It has also other uses including as sources of food, medicine, timber, firewood, ornament and preservative [1]. The African sandalwood plant is threatened due to uprooting for oil extraction, poor natural regeneration, phenological structures (dioecious), medicinal values, anthropogenic and climate factors [3].

The production of sandalwood around the world has fallen sharply over the past decades due to the mushrooming of illegal sandalwood processing units [2]. Therefore, in order to restore the previous stocks of sandalwood species in its natural stands, conventional breeding of sandalwood for introgression of new genetic information can be used. However, it is an expensive and difficult task because of its long generation time, sexual incompatibility and heterozygous nature [4]. Alternatively, biotechnological approaches including *in vitro* propagation and regeneration techniques are proposed to overcome difficulties of propagating African sandalwood plant. Therefore, efforts are currently ongoing to cultivate African sandalwood plant at commercial level through the use of clonal propagation of superior trees that could yield plantlets in large numbers for commercial planting purposes. It has been proved from scientific research that micropropagation of plants is a tool for mass production and generation of disease-free trees [5]-[7].

The values and services obtained from the African sandalwood tree have resulted in the plant being smuggled out of forests reserve, game reserves, nature reserves and general land in East African region for sandalwood oil extraction [1]. The combination of uprooting the whole tree for oil extraction, poor natural regeneration through seeds and root suckers, high demand and markets of sandalwood oils, human population growth, change of human life style and the species being dioecious threatens the plant existence and forces its extinction [8] [9].

This review briefly presents on the prospects of biotechnological approaches for propagation and improvement of threatened African Sandalwood (*Osyris lanceolata* Hochst. & Steud.).

1.1. Tissue Culture of African Sandalwood (*Osyris lanceolata*)

Worldwide, many plant species are threatened to extinction because of the gradual disappearance of the terrestrial natural ecosystems as a result of human activities. These activities include habitat destruction specifically clearing of indigenous vegetation for agriculture, overutilization, invasion of aliens species, non optimal management strategies and climate change [10] [11]. From various reports currently it is estimated that more than 50% of the world's plant species are endemic to 34 global biodiversity hotspots (GBH), that once covered 15.7% of the earth's land surface and which are now reduced to 2.3% due to an increasing threats of extinction as Red List of Threatened Plants [10].

This situation has forced the establishment of various initiatives and approaches by governments, group of people, non-governmental organizations and International agencies for the integrated conservation of rare and threatened plant species both *in situ* and *ex situ* including Botanical gardens which are charged with collecting and maintaining endangered species. For instance, the Global Strategy for Plant Conservation aims to stop this loss by setting targets for understanding and conserving plant diversity, promoting sustainable use, providing education and building capacity to support plant conservation [10].

However, current approach of *in situ* conservation alone is not sufficient to meet the challenges of saving threatened species such as African sandalwood plant. As a complementary techniques to current plant conservation strategies through the use of *in situ* and *ex situ* preservation in botanical gardens and seed banks, additional approaches are needed for some of the endangered/threatened species including African sandalwood tree. Therefore, complementary strategies of *in situ* conservation combined with *ex situ* conservation and *in vitro* propagation are important techniques for preserving and conserving the biodiversity of many endangered tree species including African sandalwood [10].

It is suggested that seed banking may be utilized for *ex situ* conservation of the majority of endangered species. Although there are significant number of species for which seed banking is not an option including *Osyris lanceolata* of which its natural regeneration is less than 50% from seeds [9]. As from this fact conservation of African sandalwood species and its populations is highly important through micropropagation and regeneration that can offer a large number of the species propagules from a cross-section of the genetic diversity [12].

One of the plant tissue culture techniques, micropropagation, is a practice used to propagate plants under sterile conditions or in a controlled environment, often to produce clones of a plant [13]-[15]. This technique relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency), but plant cell diferentiation depend much on plant growth regulators for promoting shoot and root formation and multiplication. In fact, research reports show that single cell, protoplasts, pieces of leaves, or roots can often be used to generate a new plant on culture media given the required nutrients, optimal external environment and plant growth regulators [13]. This tissue culture technology is primarily based on micropropagation in which rapid proliferation is achieved from tiny stem cuttings, leaves, axillary buds and from somatic embryos, cell clumps in suspension cultures and bioreactors. Therefore, tissue culture is proved to be an excellent technology option for the propagation and conservation of threatened or endangered species because small amount of tissues are used for mass propagation without damage to the donor and producing a large number of plants in a given time with little space compared to other methods [16]. Furthermore, it allows the maintainance of large genotypic libraries of selected species for plant conservation.

Scientific researches have proved micropropagation of plant as an important tool for rapid plant proliferation, cultivation of trees and breeding with many advantages which are rapid multiplication of species under threat, offer an alternative means for trees domestication and generation of disease-free planting materials [5] [14]. Therefore, *in vitro* techniques using tissue culture offer a great possibility to overcome the threats to threatened plant species which consider a number of factors such as genotype, culture medium composition, growth regulators and their combinations, physical environment, aseptic environment and explant developmental stage [17].

The *in vitro* propagation of sandalwood species was attempted as early as 1963 where by induction of callus from mature endosperm on modified White's medium was reported, but the callus did not proliferate further [18]. In addition, the callus induction, differentiation of embryoids and subsequent development into plantlets from endosperm have been reported by [19], that can be used to produce economically superior sandalwood plants. Furthermore, various explants such as nodal segment [20]-[22], leaf disc [21], with varying degree of success, have been used for rapid multiplication of sandalwood trees. However, there is only one published report on shoot bud formation directly from *in vitro* cultured leaves for sandalwood by [21]. It has been proved that leaves obtained from naturally growing sandalwood plants provide a useful source of explants that eliminate the risk of contamination and reduces the possibilities of genetic variation common in plants regenerated from cultured cells or tissues [21]. Therefore, shoots of *Santalum album* from its leaves and bud formation on Murashige and Skoog (MS) basal media supplied with 6-Benzyl-aminopurine (BAP) at low concentrations from leaves was reported [21]. However, a systematic study on the effects of media composition, combinations of growth regulators on morphogenesis is still insufficient, which may overlook the potential media composition, combinations of certain plant growth regulators that are more suitable for propagation of the sandalwood species.

Any aspect of plant tissue culture on one of sandalwood species (*Osyris lanceolata*) has not been done. This provides the opportunity to undertake research so as to develop a potential system of tissue culture propagation protocol using leaf and nodal segment explants leading to clear understanding of the possible role of media composition and plant growth regulators in deciding morphogenic pathway of African sandalwood which further paves the way for its *in vitro* genetic improvement including genetic transformation.

1.2. Effects of Various Media Compositions on the *in Vitro* Propagation of African Sandalwood

The basic nutritional requirements of cultured plants are similar to those of natrually growing normal plants. However, growth and morphogenesis of plant tissues *in vitro* are largely affected by the composition of the culture media [23]. In practice, nutritional components promoting optimal growth of a tissue under laboratory may vary with respect to the particular plant species. Thus media composition is formulated considering the specific requirements of a given plant species to be cultured [24]. [24], argue that most woody plants can not tolerate relatively high salts and chloride levels of MS medium, thus it is recommended to use Woody Plant Medium while culturing woody and shrub trees. However, there are several reports where MS medium performs by far better than Woody Plant Medium in many woody plants [15] [21] [25]-[28].

MS basal medium is the most widely used medium. It is composed of salts that supply the needed macronutrients and micronutrients to the explant initiated for tissue culture experiments [29]. However, modification of MS medium according to the nutritional requirements of a plant and its developmental stage is essential to attain the best proliferation percentages and morphological characteristics [30]. In practice, the media composition is not the only factor for plantlets proliferation. There are other parameters such as explant sources, maturity stage and genetic variation [17]. To achieve an optimum plant growth differentiation, media composition, culture media conditions, explant sources and sterilization procedures must be considered to meet the requirements of the plant cells or tissues [31]. MS basal medium supplemented with α-naphthalene acetic acid (NAA), benzyladenine (BA), sucrose and agar at the pH ranging from 5.6 to 5.8 has been reported to provide good results of propagating the sandalwood species from leaves and nodal segment [32].

1.3. Possible Effects of Auxin and Cytokinin Concentrations on Inducing Multiple Shoots and Roots of African Sandalwood

Multiple shoot induction involves the application of plant growth regulators in stimulating the development of axillary buds, which are usually present in the axil of each leaf [33]. Therefore, the auxins and cytokinins are of particular significance in an *in vitro* culture [32]. It has been reported that most of the plant cultures require a combination and variations of auxins and cytokinins concentration for growth regulation [30]. Thus to improve the success of axillary bud proliferation and root formation for African sandalwood (*Osyris lanceolata*), the optimal balance between auxins and cytokinins must be determined.

2. Conclusions

The African sandalwood (*Osyris lanceolata*) is a woody plant of significant commercial value. Despite high demands, it is not widely spread due to the constraints on its natural regeneration and human pressure especially overharvesting and unsustainable utilization through uprooting for oil extraction. Its commercial success is hindered by the slow conventional propagation through root suckers or seeds. It is suggested that an alternative propagation via *in vitro* regeneration method is required for the large scale production of African sandalwood to exploit its potential as commercial plant.

The use of plant biotechnological methods such as *in vitro* regeneration of African sandalwood through the culture of seeds, leaf explants and multiple shoot formation gives the promise for its sustainability. Therefore, advances in plant tissue culture of African sandalwood will enable its rapid multiplication and sustainable use of this plant for future generations.

References

[1] Machua, J., *et al.* (2009) Propagation of *Osyris lanceolata* (*East African Sandalwood*) in Recent Advances in Forestry Research for Environmental Conservation, Improved Livelihood and Economic Development. *Proceedings of the 4th KEFRI Scientific Conference*, KEFRI Headquarters, Muguga, 6-9 October 2008.

[2] Subasinghe, S. (2013) Sandalwood Research: A Global Perspective. *Journal of Tropical Forestry and Environment*, **3**.

[3] Mwang'ingo, P., *et al*. (2008) Sex Distribution, Reproductive Biology and Regeneration in the Dioecious Species *Osyris lanceolata* (African Sandalwood) in Tanzania. *Tanzania Journal of Forestry and Nature Conservation*, **76**, 118-133.

[4] Rugkhla, A. and Jones, M. (1998) Somatic Embryogenesis and Plantlet Formation in *Santalum album* and *S. spicatum. Journal of Experimental Botany*, **49**, 563-571. http://dx.doi.org/10.1093/jxb/49.320.563

[5] Ezekiel, A. (2010) Low Cost Vegetative Propagation of Tropical Trees. *International Journal of Botany*, **6**, 187-193. http://dx.doi.org/10.3923/ijb.2010.187.193

[6] Hartmann, H., *et al.* (1997) The Biology of Propagation by Cuttings. *Plant Propagation*: *Principles and Practices*, **6**, 276-328.

[7] Daud, N.H., Jayaraman, S. and Mohamed, R. (2012) Methods Paper: An Improved Surface Sterilization Technique for Introducing Leaf, Nodal and Seed Explants of *Aquilaria malaccensis* from Field Sources into Tissue Culture.

[8] Teklehaimanot, Z., *et al.* (2004) Influence of the Origin of Stem Cutting, Season of Collection and Auxin Application on the Vegetative Propagation of African Sandalwood (*Osyris lanceolata*) in Tanzania. *The Southern African Forestry Journal*, **201**, 13-24. http://dx.doi.org/10.1080/20702620.2004.10431770

[9] Mwang'ingo, P., *et al.* (2006) Propagating *Osyris lanceolata* (African Sandalwood) through Air Layering: Its Potential and Limitation in Tanzania. *Southern African Forestry Journal*, **207**, 7-13. http://dx.doi.org/10.2989/10295920609505247

[10] Reed, B.M., *et al*. (2011) Biodiversity Conservation and Conservation Biotechnology Tools*. In Vitro Cellular & De-*

velopmental Biology-Plant, **47**, 1-4. http://dx.doi.org/10.1007/s11627-010-9337-0

[11] Diffenbaugh, N.S. and Scherer, M. (2011) Observational and Model Evidence of Global Emergence of Permanent, Unprecedented Heat in the 20th and 21st Centuries. *Climatic Change*, **107**, 615-624. http://dx.doi.org/10.1007/s10584-011-0112-y

[12] Rogers, S.M. (2003) Tissue Culture and Wetland Establishment of the Freshwater Monocots *Carex*, *Juncus*, *Scirpus*, and *Typha*. *In Vitro Cellular & Developmental Biology-Plant*, **39**, 1-5. http://dx.doi.org/10.1079/IVP2002358

[13] Idowu, P., Ibitoye, D. and Ademoyegun, O. (2009) Tissue Culture as a Plant Production Technique for Horticultural Crops. *African Journal of Biotechnology*, **8**, 3782-3788.

[14] Yadav, K., Singh, N. and Verma, S. (2012) Plant Tissue Culture: A Biotechnological Tool for Solving the Problem of Propagation of Multipurpose Endangered Medicinal Plants in India. *Journal of Agricultural Technology*, **8**, 305-318.

[15] Sidhu, Y. (2011) *In Vitro* Micropropagation of Medicinal Plants by Tissue Culture. *The Plymouth Student Scientist*, **4**, 432-449.

[16] Woo, S.M. and Wetzstein, H.Y. (2008) An Efficient Tissue Culture Regeneration System for Georgia Plume, *Elliottia racemosa*, a Threatened Georgia Endemic. *HortScience*, **43**, 447-453.

[17] Qu, L., Polashock, J. and Vorsa, N. (2000) A Highly Efficient *in Vitro* Cranberry Regeneration System Using Leaf Explants. *HortScience*, **35**, 948-952.

[18] Rangaswamy, N. and Rao, P. (1963) Experimental Studies on *Santalum album* L. Establishment of Tissue Culture of Endosperm. *Phytomorph*, **13**, 450-454.

[19] Lakshmisita, G., Shobha, J. and Vaidyanathan, C. (1980) Regeneration of Whole Plants by Embryogenesis from Cell Suspension Cultures of Sandalwood. *Current Science* (*Bangalore*), **49**, 196-198.

[20] Sarangi, B., Golait, A. and Thakre, R. (2000) High Frequency *in Vitro* Shoot Regeneration of Sandalwood. Central Institute of Medicinal and Aromatic Plants, *Journal of Medicinal and Aromatic Plant Sciences*, **22**, 322-329.

[21] Mujib, A. (2005) *In Vitro* Regeneration of Sandal (*Santalum album* L.) from Leaves. *Turkish Journal of Botany*, **29**, 63-67.

[22] Sanghamitra, S. and Chandni, U. (2010) Methodological Studies and Research on Micropropagation of Chandan (*Santalum album* L.): An Endangered Plant. *International Journal on Science and Technology* (*IJSAT*), **1**, 10-18.

[23] North, J., Ndakidemi, P. and Laubscher, C. (2010) The Potential of Developing an *in Vitro* Method for Propagating *Strelitziaceae*. *African Journal of Biotechnology*, **9**, 7583-7588.

[24] Smith Roberta, H. (2000) Plant Tissue Culture. Techniques and Experiments Second Edition. *Academic Press*, **37**, 43-58.

[25] Feyissa, T., Welander, M. and Negash, L. (2005) Micropropagation of *Hagenia abyssinica*: A Multipurpose Tree. *Plant Cell*, *Tissue and Organ Culture*, **80**, 119-127. http://dx.doi.org/10.1007/s11240-004-9157-1

[26] Muthan, B., Rathore, T.S. and Rai, V.R. (2006) Micropropagation of an Endangered Indian Sandalwood (*Santalum album* L.). *Journal of Forest Research*, **11**, 203-209. http://dx.doi.org/10.1007/s10310-006-0207-x

[27] Ravi, M., Ramanjaneyulu, P. and Rao, A.V.B. (2014) Micropropagation of *Terminalia Arjuna* Roxb., from Nursery Plant Material. *International Journal of Emerging Trends in Science and Technology*, **1**, 997-1004.

[28] Rahman, A.Z., Othman, A.N., Kamaruddin, F.L.I. and Ahmad, A.B. (2015) Direct Shoot Regeneration from Callus of *Melicope lunu-ankenda*. *Natural Science*, **7**, 81-87. http://dx.doi.org/10.4236/ns.2015.72009

[29] Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, **15**, 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

[30] North, J., Ndakidemi, P. and Laubscher, C. (2011) Effects of Various Media Compositions on the *in Vitro* Germination and Discoloration of Immature Embryos of Bird of Paradise (*Strelitzia reginae*).

[31] Nayanakantha, N., Singh, B. and Kumar, A. (2010) Improved Culture Medium for Micropropagation of *Aloe vera* L. *Tropical Agricultural Research and Extension*, **13**, 87-93.

[32] Muthan, B., Rathore, T.S. and Rai, V.R. (2006) Factors Influencing *in Vivo and in Vitro* Micrografting of Sandalwood (*Santalum album* L.): An Endangered Tree Species. *Journal of Forest Research*, **11**, 147-151. http://dx.doi.org/10.1007/s10310-005-0208-1

[33] Chawla, H. (2004) Plant Biotechnology Laboratory Manual for Plant Biotechnology. Oxford and IBH Publishing Co. Pvt. Ltd.