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In vitro-Propagation of Threatened African Sandalwood (*Osyris lanceolata*) Using Indole-3-Butyric Acid Hormone (IBA)

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ABSTRACT

Osyris lanceolata is a threatened shrub endemic to East Africa and South African regions, which is being severely affected by fungi, uprooting for oil extraction, poor natural regeneration, phenological structures (dioecious), medicinal values, lack of sexual recruitment and habitat loss. It has been found a challenge through application of *in situ* conservation of natural trees as such since rapid human population growth and the available natural strands of valuable plants of African sandalwood have not been able to meet the demands of the people in world. However, this study *via* using the advances of plant propagation method it provided new options for conserving and multiplication of *Osyris lanceolata* species using *in vitro* culture techniques of by using IBA hormone. Propagation of *Osyris lanceolata* by IBA in non-mist poly-propagator was investigated by taking young stem of *Osyris lanceoleta* from Bazawit Hill and we provided an alternative propagation technique to the use of seeds. New leaf was initiated on the young stems of the *Osyris* after six weeks of the experiment. The influence of IBA as rooting promoter at three concentrations (50, 100 and 150 ppm) were recorded. Unexpectedly, from collected data we observed that the success 83.3% can be achieved from young stem of untreated stem by hormone and 50% were achieved in the treated plant, this propagation technique is a viable alternative to seed. The success may influenced by application of the origin stem cutting from a mother plant with a type of soil.

Keywords: African sandalwood; IBA; Propagation; *Osyris lanceolata;* Fungi

INTRODUCTION

Osyris lanceolata is a shrub or small deciduous tree that grows to 1-7 m in height depending on the soil type, climatic conditions and genetic variation, and has a wide geographic distribution in Africa from Algeria to Ethiopia and south to South Africa, Europe (Iberian peninsula and Balearic Islands), Asia (India to China), and Socotra [1-3]. *Osyris lanceolata* is distributed in African countries such as Tanzania, Ethiopia and Kenya frequently found in arid to semiarid areas, primarily on stony and rocky soils [4] or occasionally in rocky sites and along the margins of dry forests, evergreen bushland, grassland, and thickets at an altitude range of 900-2250 m above sea level.

In East African countries, *Osyris lanceolata* constituted an important source of medicine [5]. A decoction of the bark and root is considered to be useful for treating diarrhoea, gonorrhea, chronic mucus infections, and urinary diseases a decoction of the bark in boiling water is used to treat candidiasis and related fungal infections while the essential oil

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extracted from the bark is used to treat diarrhea, chest problems, and joint pains [6]. Fibers from the roots are used in basket making while the strong red dye from the bark and roots is used in skin tanning [7]. Since *Osyris lanceolata* is an evergreen tree with long flowering periods, it is a good forage plant [8]. The utilization of *Osyris lanceolata* in the perfumery and fragrance industries in the early 1900's followed a decline in the resource base of Indian sandalwood (*Santalum album* L.). Several communities in Kenya also use *Osyris lanceolata* to produce dyes, to treat various ailments, and to brew herbal tea [3].

However, *Osyris lanceolata* is critically endangred since propagation of by seeds is difficult due to a limited supply and availability of seed at the right time (being a dioecious species, the spatial distribution of trees affects the reproductive outcome, storage difficulties and thus poor germination [8]. Consequently, several interventional measures are required to conserve *Osyris lanceolata*. A study by on the storage and pre-sowing treatments on seed germination demonstrated that the test a covering the embryo plays a significant role in limiting germination by restricting gas and water entry and also acts as a mechanical barrier to embryo growth. However, complete removal of the test a and soaking the zygotic embryo in hot water enhanced seed germination by 66.5%, shortened the time to seedling emergence and promoted early seedling growth [9]. Stem cuttings (8-10 cm long with 3-4 leaves from young trees or seedlings) could be induced to root with a maximum of 15% rooting when dipped first in a fungicide (Bavistin) for 5 min, then in 1% Indole-3-Butyric Acid (IBA) for 6 h, but "this concentration could be increased to 32.5% when 75% of the original leaves were left intact" [10]. In the same study, the choice of substrate was shown to affect the rooting ability of cuttings, with 30% of cuttings rooting in vermiculite, which was superior to sand, vermiculite+sand (1:1), activated coconut peat and peat. To enhance root production in young stem cuttings collected in early spring by air layers that were left on parent trees for eight weeks and watered every two days to allow root initiation with the help of three concentrations (50, 100 and 150 mg/L) of IBA during February, June, September, and December were effected. Fortunately, 50 mg/L IBA was optimum for root initiation and from conducting research seasons June to September was best for air layering with about 80% rooting success after potting plants in sand, forest soil and animal manure (2:1:1) and fertilizing with 5 g/container of NPK (Nitrogen: Phosphorus, Potassium) fertilizer achieved 60% rooting success through air layering [11].

In order to restore the previous stocks of sandalwood species in its natural stands, conventional breeding of sandalwood for introgression of new genetic in-formation can be used. However, it is an expensive and difficult task because of its long generation time, sexual incompatibility and heterozygous nature [12]. Therefore, this research was attempted to address the endangered sandalwood *via* the approaches of using rooting hormone propagation techniques in non-mist poly-propagator.

MATERIALS AND METHODS

Study Site Description

Stem cuttings were collected in Novmber, 2019 at Bezawit Hill, Bahrdar town. It is far two-and-a-half kilometres South of from the Martyrs Memorial, Bahrdar town, Northen Ethiopia. It was palace of Haile Selassie, in the out flow edge of Blue Nile from Lake Tana. The experiment were conducted at Bahrdaer plant tissue culture laboratory. It is near to Bazawit hill. The Bazawit hill vegetation cover is predominantly grass (**Figure 1**).

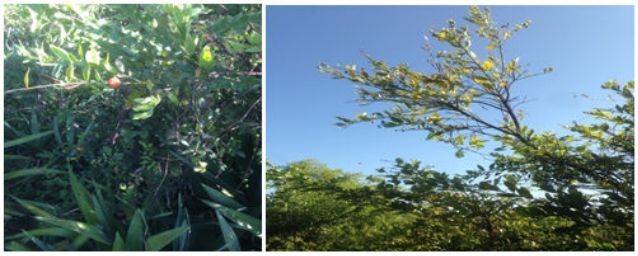

Figure 1: *Osyris lanceoleta* in Bazawit hill.

Experimental Design and Treatments

Three factors in relation to a new leaf formation success were investigated. The effect of the season at which cuttings were collected in November, the effect of origin of the stem cutting within a shoot portions (*i.e.* terminal); and the effect of rooting hormone (Indole-3-Butyric Acid) application at concentrations of 0, 50, 100 and 150 ppm. The control set (0 ppm) was treated with distilled water. For stem cuttings, the terminal portion was taken from the tip of the shoot down to 15 cm and each stem cutting contained above two nodes. Auxin concentration was applied by dipping the trimming ends of the cuttings (2-3 cm) into IBA solution for 8 hrs. The IBA concentrations were prepared by dissolving IBA powder diluting with distilled water to make a desirable concentration. Each treatment was replicated four times and for controls eight replication was conducted. We make a total of 20 for the whole experiment.

Experimental Establishment and Management

Stem cuttings were collected from sprouts of the mature trees at Bzawit hill, Bahrdar as observed in **Figure 1**. Cuttings were collected in the morning and transported to the experimental site in Bahrdar tissue culture laboratory where further processing and preparation took place. Before planting, stem cuttings were treated with IBA concentrations by dipping the trimming ends for 8 hrs by 0 ppm, 50 ppm, 100 ppm and 150 ppm of IBA concentration and planted in a none mist poly-propagator as shown in **Figures 2 and 3**. Cuttings were initially raised new buds after 5 weeks from planting date as shown in **Figure 4** below. As indicated in **Figure 5** the original leaves of the cutting were trimming after 3 weeks of the experiment to facilitate the bud initiation. Non mist propagator (Length=196 cm, width=37 cm and height=15 cm) were filled with sand (30%), redish soil (25%) and forest soil (45%).

The propagator were perforated at the bottom to allow easy drainage of water and covered by white plastic. Watering of the cuttings was done every days in the after none and after 6 weeks of the experiment 50% of the treatment formed new leaf as clearly observed in **Figure 6** and the untreated by hormone were 83.3% successes.

Figure 2: Inserting stem cutting in IBA hormone solution.

Figure 3: Planting the stem cutting in non-mis poly-propagatore.

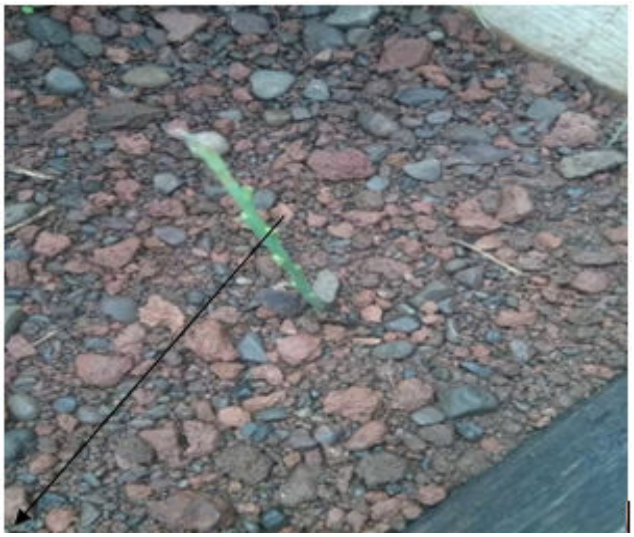

Figure 4: New buds in a non-mist poly propagatore in stem cutting after 5 weeks of planting.

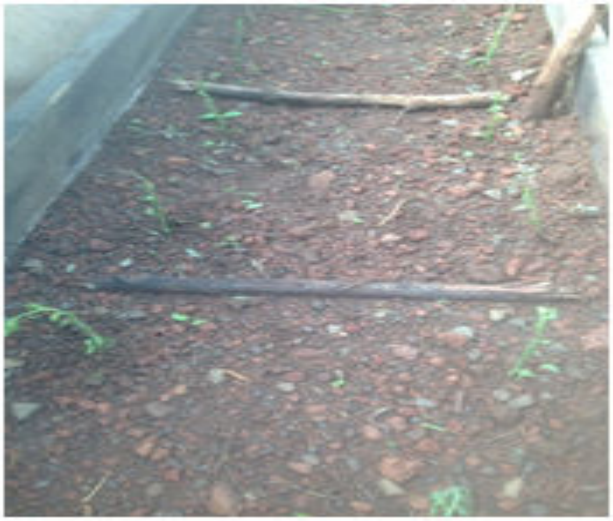

Figure 5: After 3 weeks removed the initial leaf in the stem.

Figure 6: New leaf of *Osyris lanceoleta* in the stem in non-mist polypropagatore after 6 weeks.

RESULTS AND DISCUSSION

New leaf success (the number of cuttings that leafed) was achieved in stem cuttings that were collected in November, 2019. The number of cuttings by showing a new leaf from its bud was not differed between hormone concentrations.

However, 83.3 % of the un treated cutting by hormone or it treated by distilled water were best new leaf formation as clearly indicated in **Table 1** better than the treated cutting by hormone.

Table 1: The effects of stem cutting treated by IBA hormone and distilled water.

IBA concentration	Numbers of stem cutting	Numbers of responded new shots after 6 weeks	New shots formation in %
50 ppm	4	2	0.5
100 ppm	4	2	0.5
150 ppm	4	2	0.5
0 ppm (Distilled water)	6	5	0.833

CONCLUSION

Due to *Osyris lanceoleta* seed is very limited and have germination problem, it is very difficult to propagate by seed. However, this research finding *via* using the advances of plant propagation method it provide new options for conserving and multiplication of *Osyris lanceolata* species using IBA hormone at different concentration and distilled water by taking stem plant material of *Osyris lanceoleta* in non-mist poly propagatore.

AUTHOR CONTRIBUTIONS

Adugnaw Admas, Smegnew Melese, Amare Genetu, Zewdu Yilma, Berhane Kidane and Melaku Admasu in this study they participated by initiated the research idea, designed and revised the manuscript. All authors had read and approved the final manuscript.

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