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Develop Micro Clonal Propagation Protocol For *Oxytenanthera Abyssinica* a Rich Munro to Large Scale Micro Propagation

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Abstract

In Ethiopia, *Oxytenanthera abyssinica* A.Rich. Munro has varies economic and environment importance. However, conventional propagation methods of *O. abyssinica* are generally inefficient due to their low multiplication rate, time consuming, labor intensive, and too costly. The objective of this study was to develop a protocol for mass micropropagation of *O. abyssinica* through seed culture. Murashige and Skoog (MS) medium augmented with 6-Benzylaminopurine (BAP) was used for shoot initiation and multiplication. For in vitro rooting, MS medium supplemented with 3-Indole-butyric acid (IBA) was used. In shoot initiation experiment all viable seeds were proliferated in 5-7 days of culturing. In shoot multiplication at 0.004 g/L BAP was Successfully shoot multiplied, also best root responding were found at 0.005 g/l IBA. The present optimized protocol enables for any actors who needs large numbers of low land bamboo seedling for industry, small and micro enterprise and for reforestation programs.

Keywords: IBA; BAP; micropropagation; rooting.

Introduction

Bamboo is hardened and fastest-growing perennial grass species [1] and it is a woody culms and gregarious, monocarpic flowering plant [2]. They belong to the subfamily Bambusoideae and family Poaceae (sometimes called Gramineae), in the same family with cereal crops such as rice and wheat and sugar cane [3]. The term bamboo comprises more than 1,500 species that are widely distributed in the tropical, subtropical and temperate regions of all continents except Antarctica and Europe, between 46°N and 47°S. Geographically bamboo distribution can be classified into three zones: the Asian Pacific zone, the American zone and the African zone [4]. The highest diversity and area coverage of bamboo is recorded from the Asian continent, followed by America and Africa [5]. The sizes of bamboos vary from small annuals to giant perennial timber bamboo species [6]. Dwarf bamboos may be as little as 10cm in height, but stands of tall species may attain 15–20m, and the largest known (e.g. *Dendrocalamus giganteus* and *Dendrocalamus brandisii*) grow up to 40m in height and 30cm in culm (stem) diameter [7; 8; 9]. 43 species of bamboo in 11 genera can be found in Africa, covering an estimated area of 3.6 million ha [10]. Out of these African bamboo species, Ethiopia has only two endemic species, namely the high land bamboo (*Yushania alpina* K. Schumacher) and low land bamboo (*Oxytenanthera abyssinica* A.Rich. Munro). These two species are restricted in limited agro-ecological regions, i.e. in highland areas of altitude 2400–3500 m.a.s.l. and in lowland areas from 500–1800 m.a.s.l. [11]. For adaptation, Ethiopia were imported different bamboo species and they are under field trial in different locations those are: *Dendrocalamus asper*, *Dendrocalamus hamiltonii*, *Dendrocalamus giganteus*, *Dendrocalamus membranaceus* Munro, *Bambusa vulgaris* Var. green, *Bambusa vulgaris* Var. Vitata, *Guadua amplexifolia* [12].

How ever, *Osyris lanceolata* is critically endangered 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 (Mwang'ingo et al.,2006), storage difficulties and thus poor germination (MBUYA et al.,1994). Consequently, several interventional measures are required to conserve *Osyris lanceolata*. A study by (Kokwaro ,2009) on the storage and pre-sowing treatments on seed germination demonstrated that the testa 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 testa and soaking the zygotic embryo in hot water enhanced seed germination by.

Materials and Methods

Surveying Diseased Faba bean leaves (showing browning, wilting, yellowing, spots, blights, or combinations and its roots were collected randomly from 3 districts at 20 kebeles using plastic bags (Ambagyrorgise, Dabat and Debarke around Gondar and healthy water hyacinth was collected in Lake Tana for treatment .The fungus was isolated using potato dextrose agar medium from infected leaf and root. Fungal pathogens are able to infect various plant parts such as roots, stems, leaves, flowers and fruits, inducing characteristic visible symptoms like spots, blights, anthracite and wilts. Collected infected parts of Faba bean was cut into small pieces. After washing the tissues thoroughly in sterile water, the causal fungi are isolated from plant tissues exhibiting clear symptoms. The infected tissues along with adjacent small unaffected tissue are cut into small pieces (2–5 mm squares) and by using flame-sterilized forceps, they are transferred to sterile petridishes containing 97% ethanol used for surface sterilization of plant tissues. The plant parts were transferred to PDA plates and incubated for 5-7 d for the complete growth of fungi. The fungi were identified according to cultural characters.

water samples were collected for various physicochemical and biological parameters, analyses has been carried out by following standard methods. All nutrient including nitrate,nitrite, ammoniumnitrate, phosphate were analysedcolorimetrically Using UV-Vis spectrophotometer.Estimation of chlorophyll a and phyophytin was carried Out by Strickland &Parson method(1972).Phytoplankton samples. Thermal and atomic power plants. The collected samples were immediately transferred to laboratory. The animals were washed, sorted and examined fresh with a dissecting microscope, preserved in 5% seawater formalin. Identification of were collected from the surface water during low tide and high tide using plankton net(mesh size 20mm), the samples were subjected to qualitative and quantitative analyses. Fresh sample were collected on a month intervals to periodically record the macro fouling fauna in the coastal region along Dahanu and Tarapur coast. The samples were collected from the piers, jetty, boats, floating ropes, stones, shells, outboard motors and boats in the coastal zone near macrofoulers were done by following standard monograph and research papers.The identified macrofoulers were categorized according to their phylum and class [5-10].

Area of Study

The air layers experments were left on the parent trees for 20 weeks to allow root initiation. During this period, air layer experment were watered every four days and inspected every four week for showing weather it respond root or not . Each air layer treatment were replicated three times in each 50,100, 150 ppm IBA concentration and for control 0ppm or distilled water were applied at Bzawit hill ,Bahrdar.

Results

New root success were achieved in air layring approches that were conducted in Novmbere, 2019 at Bazawit Hill. The firist root responding time were at 12 weeks or 4 monthes of the experment and root responding time of each treatment were differed. However, 88.8 % of all the treatead plants by IBA hormone were formed root after 16 weeks of the experment.Among all treatments only at 50 ppm experment one stem plants not responding rooting after 16 weeks of the experment .

The combined effect between the experment conducted season,soil composition which wre use for wraped the grild area of the steams and IBA concentration showed a good root initiation in the present study of air layer propagation technology .The treated stems of osyris lanceoleta with IBA 150 ppm initially showed root respond other than other treatmeants at 12 weeks of experiment.

areas. The muddy and rocky area found along Dahanu and Tarapur coast are rich diversity of flora and fauna, most of which are fouling (sedentary) in nature. A lot of motile forms like crabs and amphipods are also found in concurrence with

the macro fouling species. The biodiversity of macro biofoulers varies according to the certain physico-chemical factors like, temp, pH, O, BOD, nutrients, salinity etc. The present study showed significant variation in the physicochemical and biological parameters of the selected sites along Dahanu and Tarapur coast near Thermal and Atomic power plant. (Table1) [10-13].

Table1: Variation in different physico-chemical and biological parameters of surface and bottom water at Dahanu and Tarapur coast

Parameters	Dahanu Coast		Tarapur Coast	
	D1	D2	T1	T2
Temperature °C)	23.5-33.00(aver.28.70)	24.5-32.5(aver.27.79)	22.5-35.5(aver.29.54)	21.0-34.5 (aver.28.90)
pH	6.70-8.60 (aver.7.86)	6.09-8.02(aver.7.30)	5.70-9.45(aver.8.42)	6.40-10.69(aver.8.90)
Salinity %	29.4-32.8(aver.30.20)	23.65 –35.70(aver.27.5)	21.50-39.62(aver.24.20)	22.9-37.60(26.42)
CO ₂ (mg/l)	2.30-17.60(aver.9.25)	1.69-15.60 (Aver.7.85)	1.89-14.30(aver.6.92)	2.50-18.30(aver.8.62)
DO(mg/l)	2.50-6.58(aver.4.56)	1.49-5.67 (aver.3.42)	1.90-3.52(aver.2.60)	2.13-4.96 (aver.3.41)
BOD(mg/l)	0.35-4.70(aver.2.73)	0.89-5.61(aver.2.89)	0.70-5.79(aver.2.42)	0.90-4.90(aver.2.78)
Nitrite(μmol/L)	0.09-2.72(aver.0.94)	0.03- 3.12 (aver. 1.69)	0.04-1.90(aver.0.76)	0.02-2.86 (aver.0.89)
Nitrate(μmol/L)	16.20-51.66(aver.34.50)	14.17-54.24	12.41-54.70(aver.28.60)	15.82-49.3(aver.26.8)
Ammonia (μmol/L)	0.20-21.5(aver.7.9)	0.4- 23.60 (aver.8.13)	0.43-19.73(aver.6.41)	0.19-21.62 (aver.7.81)
TN(μmol/L)	40.20-142.6(aver.81.75)	36.60-90.13(aver.76.30)	21.60-91.40 (aver.54.60)	32.41-96.30(aver.66.81)
Phosphate (μmol/L)	1.20-8.70(aver.4.90))	0.90-9.12(aver.3.71)	0.69-6.71(aver.2.89)	0.89-5.69(aver.2.81)
TP(μmol/L)	2.70-18.66 (aver.9.26)	3.62-20.12(aver.10.69)	1.90-16.30 (aver.8.42)	1.49-21.86(aver.9.70)
Chlorophyll a(mg m ⁻³)	2.45-7.50(aver.4.65)	1.60-6.19(aver.3.44)	1.86-5.61 (aver.3.90)	1.67-4.93(aver.2.69)
Phyophytin (mg m ⁻³)	0.69-2.15(aver.1.49)	0.4-1.69 (aver.0.46)	0.29-1.63(aver.0.67)	0.92-1.29(aver.0.63)
Phytoplankton cell count (no × 10 ³ /l)	8.6-11412.6(aver.1250.7)	7.9-12732.3(aver.1345.8)	6.09-10416.7(aver.945.9)	5.70-9724.3(aver.820.8)

Control Measures Of Biofouling

The simplest method for treatment of biofouling is simply to remove by mechanical cleaning eg, by treatment of the fouled surface with high-pressure water jets. TBT, Copper, UV irradiation, Chlorination, Titanium alloy (2m/sec) and Silicone elastomers (for fast vessels) .Several kinds of natural antifouling agents that inhibit growth of fouling organisms have been isolated from marine organisms like bacteria, marine Algae.

Conclusion

Several coastal ecosystems along the west coast of India are now thus highly disturbed and threatened, encountering problems like pollution, siltation and erosion, flooding, saltwater intrusion, storm surges and other hazards. Marine biofouling is one of the major unsolved problems currently affecting the shipping industry and industrial aquatic processes in Maharashtra. It is commonly refers to the adverse growth of marine organisms on immersed artificial structures such as ship hulls, jetty pilings, navigational instruments, aquaculture net cages and seawater in taking pipes etc. Hence. Appropriate management strategies are needed to ensure the sustainable development and management of coastal areas and their resources. Land-based industrial and domestic effluents further impact the abundance and composition of marine communities in coastal areas. Very little work has been carried out in India on macro-biofouling communities. Therefore, the present study has been carried out along Dahanu and Tarapur coast near thermal and atomic power plants to assess the macro-fouling pattern, monthly settlement and species dominance between two coastal areas of Palghar, Maharashtra.

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