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In vitro Propagation Protocol Development for *Oxytenanthera abyssinica* A. Rich. Munro

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ABSTRACT

Oxytenanthera abyssinica A. Rich. Munro has various economic and ecological importances in Ethiopia. Nowadays, the need for planting the species across the wider landscape, as plantation forest, is increasing. However, conventional propagation methods of *O. abyssinica* are found generally to be inefficient because they have low multiplication rate, are time consuming, labor intensive and too costly. Accordingly, tissue culture protocol developed at Holeta biotechnology lab by the collaboration of the Forest Directorate of EIAR and Holeta biotechnology laboratory in 2017 for the species was later optimized at Bahir Dar-ORDA tissue culture laboratory in 2020 and 2021 so as to produce sufficient number of lowland bamboo seedling through seed culture. Murashige and Skoog (MS) medium augmented with 6-Benzylaminopurine (BAP) was used for shoot initiation and multiplication. MS medium supplemented with 3-Indole-Butric Acid (IBA) was used for *in vitro* rooting. In shoot initiation experiment all viable seeds were proliferated in 5-7 days of culturing. Shoot was successfully multiplied at 0.004 g/L BAP and best rooting response was found at 0.005 g/l IBA. The present optimized protocol enables actors who need large numbers of low land bamboo seedling for a forestation/reforestation programs.

Keywords: *Oxytenanthera abyssinica*; 6-Benzylaminopurine; 3-Indole-Butric Acid; Forestation/ reforestation programs

INTRODUCTION

Bamboo is a perennial grass belonging to the Poaceae (Gramineae) family and Bambuseae subfamily. Bamboos have a long history as an exceptionally versatile and widely used resource in the world. Nowadays, it is becoming so increasingly important in the world's forest economy, because it is: A superior wood substitute, cheap and efficient to produce and utilize, environmentally friendly and the world forest is shrinking hence requiring alternative sources [1,2].

There are two indigenous bamboo species in Ethiopia namely lowland bamboo (*Oxytenanthera abyssinica* A. Rich. Munro) and Ethiopian highland bamboo. These two species are found restricted in limited agroecological regions, *i.e.* highland bamboo in highland areas of altitude 2200-3500 m.a.s.l. and lowland bamboo from 500-1800 m.a.s.l. Ethiopian lowland bamboo is used for housing, handicrafts, pulp and paper industries, energy source, and food; it fits ISO standards for the production of an array of lumber-based and stick-based products [3]. It has also high value in carbon sequestration. Medical use of *O. abyssinica* is documented in different

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countries including Ethiopia [4]. *O. abyssinica* has also important phytochemicals with a resultant antioxidant property. Furthermore, investigation on bamboo shoots showed that *O. abyssinica* shoot is rich in nutrients.

O. abyssinica is a perennial monocarpic or once flowering plant. Conventionally, it is propagated through seeds, rhizome-based and clump division techniques. However, gregarious flowering at long intervals followed by the death of clumps, short viability of seeds presence of diseases and some pests are limiting factors to use seeds as valuable source of propagation. The rhizome-based offset method, is inefficient as propagules are difficult to extract, bulky to transport, have low multiplication rate, labor intensive and too costly and planting materials are insufficient in number for large-scale plantation [5].

Considering problems encountered in both sexual and asexual conventional propagation of *O. abyssinica*, innovative method that brings about rapid large scale production is highly desirable. In this regard micro propagation is an excellent means. Kahsay et al., developed protocol for mass propagation of *O. abyssinica* at Holeta biotechnology lab by using 3-BAP, NAA and IBA hormone at different concentration and reproducible protocol that can enable the *in vitro* rapid multiplication of *O. abyssinica* from seed culture [6].

The main objective of this developmental research was, therefore, to further optimize the protocol for *in vitro* multiplication of *O. abyssinica*, from seed culture using 3-BAP and IBA hormone for better improvements at Bahir Dar-ORDA tissue culture laboratory. The main targets of this study were to determine, identify an appropriate cytokinin and determine its optimal concentration for shoot proliferation and multiplication; identify an appropriate auxin and determine its optimal concentration for root induction [7].

MATERIALS AND METHODS

Source of Experimental Material

The seeds for this study were obtained from Assosa, Benishangul Gumuz Region, Ethiopia. Healthy seeds were selected carefully and they were stored in plastic bag at +4°C in refrigerator. Seeds were stored for more than a year in Bahradr tissue culture laboratory [8].

Explants Surface Disinfection

Selected healthy seeds were sterilized to get ride off all micro-organisms. Also, the seeds were washed with tape water to remove debris. Then, to get clean seeds it soaked in distilled water for 2 hrs by shaking and washed by Double Distilled Water (DDW) with liquid soap with 2-3 drops of Tween-20 for 20 minutes. Then, treated by antifungal of mancozine 20 g/l for 20 minute and washed the seeds with DDW three times. By following that procedure, seeds were treated by 2% of NaOCl for 20 minute and washed the seeds three times by 2-3 drop of Tween-20 for five minute. After pre-treatment, the seeds were treated with 1% NaOCl and washed three times by DDW. Finally, it treated with 70% ethanol for 30 seconds under laminar air flow cabinet. After sterilization of the MS medium, for shoot initiation three jars for each treatments (0.003, 0.004 and 0.005 g/L BAP) five seeds were placed randomly in Completely Randomized Design (CRD) arrangement [9].

Culture Media Preparation

For all experiments the pH of the above mentioned prepared nutrient medium was adjusted to 5.80 before adding 0.4% agar. Full-strength MS medium with 3% sucrose was used for culture initiation and multiplication experiments. About 50 ml of the medium were dispensed to 300 ml jar for initiation experiment and also 50 ml of the medium were dispensed to 300 ml jar for multiplication and rooting experiments. The media were autoclaved at 121°C with 15 PSi pressure for 20 minutes and kept under room temperature for four days before used [10].

Establishment of Culture Shoots

Disinfected seeds were cultured in 9 jars that contained the above mentioned nutrient 50 ml of MS medium with BAP and 3 jars PGRs free medium for shoot initiation study. Then the cultured sample were brought to growth room shelf with a photoperiod of 16/8 h light/dark using cool-white fluorescent lamps (photon flux density, 40 µmol $m^{-2}s^{-1}$ irradiance) at 25°C± 2°C. After seven days, all of the cultured samples were initiated shoot [11].

DISCUSSION

Shoot Multiplication

To avoid the carry over effect of shoot initiation media during shoot multiplication, initiated propagules consisting of three shoots each were subculture on PGRs free MS medium for two weeks. Each propagule was placed vertically and lightly pressed into the culture medium supplemented with of 0.003-0.005 g/litre of BAP with each activated charkol for inhibition of oxidants of the cells mostly for phenol exudation. MS medium without PGRs was used as control. 12 jars each with three propagules were used and kept under light conditions [12]. Then, after two weeks multiplication of new leaf were best at 0.004 g/litre of BAP as it showed in **Figure 1**.

Figure 1: Multiplication of new leaf from initiated seed at 0.004 g/L BAP hormone.

Rooting of Shoots

The *in vitro* regenerated three shoots in bunch were used for rooting studies after sub-cultured on PGRs free MS medium for 2 weeks. The rooting response of these shoots was studied on different concentrations of IBA (0.004, 0.005 and 0.006 g/L) and with for each treatments used 0.1 g/L activated charcoal for inhibition of oxidants of the cells mostly for phenol exudation on MS medium and without hormone was used as control [13]. For each treatment three jars, each with three clumps were used. All shoots were incubated on rooting medium for 4 weeks and kept under light conditions in culture room in CRD arrangement. Among all treatment, 0.005 g/L of IBA solution experiment were best for root formation as it shown in **Figures 2 and 3**.

Figure 2: Root formation of Low land bamboo *via* IBA hormone after one month.

Figure 3: Acclimatizing low land bamboo seedling in nursery.

CONCLUSION

2% of NaOCl solution for 25 min, 2-3 drops of Tween-20 for 20 minutes and antifungal of mancozine of 20 g/l for 20 minute were effective for disinfection of low land bamboo seed 0.004 g/L BAP supplemented with MS medium showed best shoot proliferation, better shoot number and requires 5-7 days to induce shoot. Similarly, for the shoot multiplication experiment, the tested cytokinin at 0.004 g/L BAP gave the efficient shoot number and shoot multiplication. In the root induction, IBA was also best at 0.005 g/L supplemented with MS medium gave best root number. Finally, this study recommends using this protocol for mass propagation of low land bamboo for reforestation of degraded land which is highly exposed for drought and for industry purpose.

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