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STANDARDIZATION OF MICROPROPAGATION TECHNIQUE OF MULBERRY (*MORUS ALBA* L.)

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Abstract– A standardization of protocol for rapid multiplication of mulberry (*Morus alba L.)* was established *in vitro* using nodal explant excised from hydroponically grown mature mulberry plants. Shoot initiation was induced on Murashige and Skoog (MS) medium supplemented with different concentrations of (BAP) 6- Benzyl amino purine. Effect of different concentration of 6- Benzyl amino purine was studied on multiplication of shoot, revealed that elongated shoots (2-3 cm) were cultured on half strength MS medium supplemented with (IBA) indole-3-butyric acid. Higher percentage of shoot initiation (90%) was observed on MS medium supplemented with 2 mg/l 6-bezyl amino purine. Initiated shoots gave a higher average number of shoots and elongation was observed when it was subcultured on MS medium supplemented with 1.4 mg/l 6-bezyl amino purine. A maximum percentage of root formation (70%) was observed when elongated shoots were subcultured on half strength MS medium containing 1.3 mg/l indole-3-butyric acid. Rooted plants transferred to pots containing sterile FYM, garden soil and sand (1:1:1) for acclimatization in green house showed (75%) survival capacity.

INTRODUCTION

Morus alba is a fast-growing shrub or moderate - sized tree, belongs to *Morus* genus and to family *Moraceae* (Orwa *et al.,* 2009). Mulberry is generally distributed in tropical, subtropical, temperate and sub-artic areas **(**Chowdhuri *et al.,* 2006). It's cultivated for the tasteful fruits, for its potential pharmaceutical and cosmetic use and for its economic importance in silk industry for its foliage (Chiancone *et al.,* 2007).

Mulberry is an indispensable crop for the sericulture industry as it is the exclusive source of feed for silk – worms **(**Rohela *et al.,* 2020). The main focus of mulberry breeding for improve leaf productivity as it alone contributes more than 38.2 percent to the sericulture productivity (Banejee, 1998). However, perennial nature of the plant coupled with prolonged juvenile period slows down the process of mulberry improvement (Kavyashree *et al.,* 2001).

Nowadays, its cultivation has greatly decreased and it is mainly used as ornamental plant in gardens. Particularly, black mulberry is cultivated for the tastefulfruits, but also for its potential pharmaceutical and cosmetic use (Chiancone *et al.,* 2007).

Abbreviations
BAP : 6-benzyl amino purine
IBA: indole-3-butyric acid
MS : Murashige and Skoog medium.

Using biotechnology methods for targeted crop improvement, Attempts have recently been made to complement conventional breeding with modern biotechnological tools such as plant tissue culture, recombinant DNA technology and molecular markers tofacilitate mulberry genetic improvement (Vijayan *et al.,* 2014). Using different explants such as shoot tip and nodal segment (Yadav *et al*., 1990), axillary bud (Vijayan *et al*., 2000), hypocotyl and cotyledon (Bhatnagar *et al*., 2001), leaf (Vijaya Chitra and Padmaja, 2005). *In vitro* regeneration has been attempted with various degrees of success. Since there are variations in regeneration among mulberry varieties (Bhau and Wakhlu, 2003; Rao *et al*., 2010).

The purpose of this work was to create a procedure for rapid multiplication in order to

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produce micro propagated seedlings of mulberry (*Morus* sp.) through in vitro culture utilising nodal explants, which will aid in the resolution of the poor rooting ability of stem cutting through conventional breeding.

MATERIALS AND METHODS

Plant material

The nodal explant required for the study were collected from hydroponic-grown plus trees identified in CSIR-IHBT, Palampur (HP). The explants were washed under running water for 15-20 min, then washed serially in Tween 20/80 (2 -3 drops) for 15 min, Streptomycin and Bavistin 1% each mixed in autoclaved distilled water for 3 min thoroughly and later immersed in 0.1% $HgCl_2$ and 70 percent ethanol for 1:30 min and 40 sec. this will be done under laminar air flow cabinet.

Cultural condition

The sterilized explants inoculated in a jar containing media. The laminar air flow cabinet surface sterilized with ethanol. Then the autoclaved required material such as spirit lamp, media, forceps, culture plates, blade holder, lighter etc., will be kept on to clean laminar air flow. The UV light switched on for 45 minutes to maintain the aseptic environment inside the cabinet. Before inoculation heat sterilized environment should be maintained. The cut ends of explants kept in such a way that it comes in maximum contact with media. While transferring the explants heat sterilization given to forceps and blade holder to avoid the chance of contaminations on each transfer of explants. All the aseptic manipulations such as surface disinfection of explants, preparation and inoculation of explants carried out in the laminar air flow cabinet. Acclimatization stage, good rooted plantlets (5-8 roots of length 4-6 cm) was carefully washed with H_2O and bavistin to remove adhered agar and traces of medium; then they were transplanted to plastic pots (diameter: 10 cm) containing sterile soil, FYM and sand (1:1:1). The top of the pots was covered with transparent plastic.

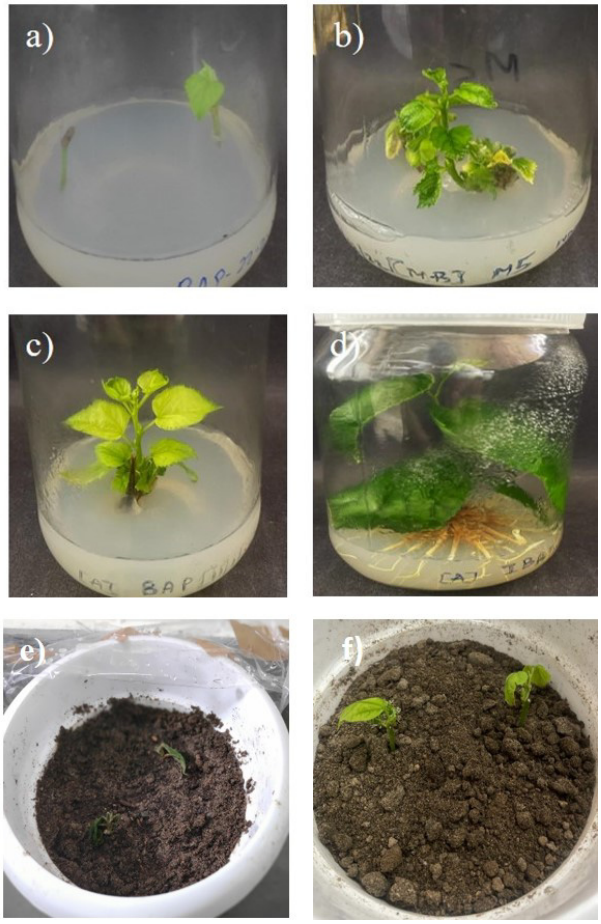

Fig. 1. Micro Propagation Stages of Mulberry Plant (*Morus sp.*): (A) Shoot Induction of nodal explants on MS Medium Supplemented with BAP 2 Mg/l (B) Multiplication of Induced Shoots on MS Medium Containing 2 Mg/L BAP (C) Elongation supplemented with 1.4 Mg/l BAP (D) Rooted Plantlets on MS Medium with 1.3 Mg/l IBA supplemented with strength MS media (E) Acclimatization stage in green house (F) Micro propagated plants after 15 days of acclimatization.

Table 1. Effect of IBA on Rooting from shoots of Mulberry Plant (*Morus sp.*)

IBA(1.3MG/L)	7 days	14 days	21 days	28 days
No. of leaf	1.66	3.33	3.33	3.33
No. of shoot	1.33	2.33	3	4
No. of root	0	17.66	17.66	32.66
Width of leaf	0.93cm	2.33cm	2.56cm	3.266cm
Leaf length	1.33cm	3.33cm	3.66cm	3.16cm
Root length	0	2.83cm	2.16cm	2.83cm
Shoot length	1.66cm	3.33cm	2.83cm	3.66cm

Experimental Design

All experiments were carried out in three replicates. Data were collected from different experiments four weeks after culture. For statistical analysis of data, analysis of variance and mean separation were carried out using operational statistics (SPSS) software.

RESULTS AND DISCUSSION

Nodal explant started to initiate after one week of culture on shoot initiation media and were kept for 4 weeks. Growth of induced shoots varied when nodal explant was cultured on MS medium without and with different concentration of BAP (0.5, 1, 1.5, 2, 3, 4 mg/l). MS media supplemented with 2.0 mg/l BAP showed a maximum shoot induction, this treatment was more effective than other treatments (Zaki *et al.,* 2011) studied same results from the nodal explant of *Morus nigra* L.

The effect of plant growth regulator on shoot multiplication increased, when induced shoots were again subculture on MS Media containing same concentration of BAP 2 mg/l.

Higher number of shoot elongation was noticed when MS media supplemented with 1.4 mg/l of BAP. Similar results were found in the *in vitro* regeneration protocol for Mulberry (*Morus alba* L.) through Tissue culture techniques when MS media supplemented with 1.35 mg/l or 0.6 μM BAP (Rana, Amin and Azad, 2022). The elongated shoot (3-4 cm) shown the best root growth after with 35 days from subculture on rooting medium supplemented with ½ MS media containing 1.3 mg/l IBA. On the contrast to has been reported before that healthy elongated shoot of (*Morus alba* L.) cv. Al-Taify subcultured on (MS + 2 mg/l IBA) showed 100 percent initiation of roots. (Attia and Sdessoky, 2014). It was concluded that IBA is a potential auxin that induces rooting in *in vitro* regenerated shoots.

CONCLUSION

An effective *in vitro* micro propagation system was established using nodal explants of hydroponics grown mulberry plant (*Morus sp.),* which consists of shoot initiation and multiplication of nodal explant in the presence of 2 mg/l BAP, Elongation supplemented with 1.4mg/l BAP and finally a rooting stage with 1.3 mg/l IBA. Among all the bioregulators IBA stands best overall. This protocol will help for mass production for horticulture, pharmaceutical industries and *in vitro* germplasm conservation of mulberry (*Morus sp.).*

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Table 2. Effect of Different Concentrations of BAP on Shoot Initiation and elongation from Nodal Explants of Mulberry Plant (*Morus sp.*)

BAP(2.0MG/L)	7 days	14 days	21 days	28 days
No. of leaf	2.66	5.1	6.3	8
No. of shoot	1.16	2	2.5	2.6
No. of root	0	0	0	0
Width of leaf	0.85cm	1cm	0.9cm	1.3 cm
Leaf length	1.18cm	1cm	1.4cm	1.78 cm
Root length	0 cm	0 cm	0 cm	0 cm
Shoot length	4.2cm	3cm	2.16cm	2.83cm
BAP(1.4MG/L)	7 days	14 days	21 days	28 days
No. of leaf	1.66	4.66	5	6
No. of shoot	1.33	2.33	3.3	6
No. of root	0	0	0	0
Width of leaf	0.86 cm	1.33 cm	1.46 cm	6.5 cm
Leaf length	1.43 cm	2.33 cm	2.33 cm	2.5 cm
Root length	0 cm	0 cm	0 cm	0 cm
Shoot length	1.43 cm	2 cm	1.93cm	2.6 cm

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