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Effect of different type of media on *in vitro* regeneration of mulberry (*Morus indica):* An economically important tree

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ABSTRACT

Mulberry (Morus spp.) is a crop of economic importance in the sericulture industry. In present investigation, simple protocol for shoot induction via micropropagation of Morus indica in different types of tissue culture media was developed. All four media were tested with different growth hormones for multiplication stage. MS was found to be the best medium for shoot induction, high frequency of induced shoots were obtained on MS+ BAP (3.0 mg/l). Multiple shoots were also achieved from in-vitro raised shoots on all the four different medium supplemented with BAP (1.0-1.5 mg/l), NAA (0.25-0.5 mg/l) and GA_3 (0.5 mg/l). The highest shoot length (2.12 ± 1.01) with maximum no. of leaves were obtained in MS medium, followed by shoot length (2.0±1.37) in WPM media.

Key words: *Morus indica*, Micropropagation, Germplasm, Schenk and Hildebrant (SH), Murashige and Skoog media (MS), Woody plant media (WPM), Gamborg medium (B5) GA_3.

INTRODUCTION

In vitro micropropagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations (Nehra and Kartha, 1994). With *in-vitro* micropropagation, the multiplication rate can be greatly increased and also permits the production of pathogen-free material (Mehbooba Zaki *et al*.,2012). Mulberry (Morus spp.) is a crop of economic importance in the sericulture industry. Its foliage forms the sole source of food for the domesticated silkworm, Bombyx mori L. Mulberry belongs to the Moraceae family and to the genus Morus. It is a perennial tree or shrub, dioecious, heterozygous and perennial tree (Mehbooba Zaki *et al.*,2012). Mulberry (*Morus*) is a fast growing deciduous woody tree with alternate leaves, unisexual to bisexual flowers in the leaf axils, and fleshy fruits. It is a monoecious or dioecious plant up to 10 - 12 m high. (Das *et al.*, 1994).

The economic importance of mulberry is primarily due to its leaf, which is being used for feeding the silk producing insect Bombyx mori L. Man's interest in mulberry cultivation originated with the growth of civilization and his fascination with quality fabric that led him search for silk. It has been estimated that nearly 60% of the production cost of silkworm cocoon is incurred by mulberry leaf production (Das and Krishnaswami 1965). Mulberry leaves containing more water, total sugar and soluble carbohydrate and less mineral are best relished by silkworms. The fruits of mulberry has a tonic effect on kidney energy and thus, it is used as an antiphlogistic, a diuretic and an expectorant, (Koyuncu F., 2004). Morus fruit has good source of several phytonutrients and contain high amounts of total phenolics, total flavonoids and ascorbic acid (Ercisli & orhan, 2007) and (Koyuncu, 2004), and hence has good demand in local and international market.

Mulberry is a cross pollinated crop and hence heterozygosity prevails. Therefore, propagation through seeds does not conserve stable genetic makeup, which limits the genetic improvement through conventional propagation techniques such as grafting, air layering, cuttings and by seed sowing (R Kavyashree 2007). Vegetative propagation

of this plant is unsuccessful due to long time taken for adventitious shoot development and low rooting potential that might be due to several factors including physiological and environmental ones (Narayan *et al.*, 1989). During the last three decades, micropropagation techniques have been extensively utilized as a valuable and viable tool for overcoming such constraints in mulberry**.** (Pattnaik *et al.*, 1996 & Ohyama K, 1970).

Most of the initial studies on mulberry tissue culture concentrated on the regeneration of complete plantlets from various explants like shoot tip, axillary bud, winter bud, leaf, cotyledon, hypocotyls etc. The present investigation was designed to study the effect of different type of media on micropropagation of mulberry.

MATERIALS AND METHODS

2.1. Collection of plant material:

The young nodal segments and shoot tips were excised from field grown matured plants of mulberry, used as explants for tissue culture protocol. The explants were collected from the nature care located in the Devleela Biotechs premises.

2.2. Standardization of Surface Sterilization of explants:

Sterilization is a procedure used for elimination of microorganisms and other sources of contamination. Explants were first washed with running tap water and dipped in 1% bavistin solution (fungicide) for 10 minutes and were then dipped in lukewarm water for 10 minutes. Then the explants were dipped in 70% ethanol in the laminar air flow for 2 minutes and then washed with double distilled water for 3 times. Next the explants were dipped in 0.1% $HgCl_2$ and consecutively washed with double distilled water for 3 times.

2.4. Standardization of media

2.4.1 Culture initiation:

The sterilized nodes were inoculated on basal Murashige and Skoog medium (MS) (Murashige, Skoog 1962), woody plant medium (WPM) (Lloyd, McCown 1981), Gamborg medium (B5) (Gamborg et al. 1968) and Schenk, Hildebrandt medium (SH) (Schenk, Hildebrandt 1972) supplemented with 30 gm/lit sucrose (HiMedia Laboratories Pvt. Ltd., Mumbai, India), respectively. 0.8% (w/v) agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used as the gelling agent on the respective media. The pH of all media was adjusted between 5.5- 5.8 using 0.1N NaOH or 0.1N HCl before solidification. The media were autoclaved at 121°C for 15 min for sterilization. All cultures were incubated in a controlled environment at a temperature of 25 ± 2°C. Naphthalene acetic acid (NAA), gibberellic acid (GA_3), and 6-benzylaminopurine (BAP) were different hormone sources used in the present study. The cultures were incubated at 25 ± 2°C under fluorescent light with a 16/8 light/dark photoperiod. Shoot initiation was studied in MS medium with different hormone concentrations of BAP (1.0, 2.0, 3.0 mg/l).When optimum growth occurred sub culturing was done after a period of two weeks in the same media. After sub culturing the explants were subjected to shoot multiplication.

The nodal segment of length 0.5-1.0 cm were inoculated on MS media. Different concentrations and combinations of hormones including BAP 0.5–3.0 mg/lit, NAA 0.25–1.0 mg/lit , GA_3 0.5-1.0 mg/lit were tested and hormone-free medium was used as a control. The pH of all media was adjusted between 5.5 and 5.8 before they were autoclaved for 15 min at 121°C. All cultures were placed in a culture room at 25°C and 16 hrs photoperiod under cool white fluorescent light.

2.4.2 Shoot multiplication and elongation:

The *in-vitro* initiated shoots were transferred onto a fresh medium for shoot proliferation. For shoot proliferation, MS, WPM, B5 and SH media were used, each supplemented with 1.0 mg/lit BAP + 0.5 mg/lit NAA + 0.25 mg/lit GA_3 over a period of four weeks. Each experiment had ten culture bottles containing four explants each and experiments were repeated thrice. The different treatments were quantified as the mean number of multiple shoots per subculture. The data were statistically analyzed by using Analysis Tool Pack (ANOVA). Observation was noted after 30 days of sub-culturing.

2.4.3 Rooting:

Well developed and proliferated shoots obtained after shoot multiplication were transferred for root induction in rooting medium containing full strength and half strength MS fortified with 0.5-1.0 mg/lit IBA .

RESULTS AND DISCUSSION

3.1 Selection of suitable explants for tissue culture:

Young nodal segments and shoot tips were used as explants. Nodal portion and shoot tips were excised carefully from the plant material with the help of a sterilized blade. The size of the explants ranged from 0.5 to 1 cm, were washed under running tap water for 30 mins.

3.2 Surface sterilization of explants excised from mother plant:

Explants were sterilized by 1% bavistin, followed by mercuric chloride in different concentration and for different time exposure to minimize the contamination in *in-vitro* cultures.

Table 1: Standardization of Surface Sterilization

S. No.	Treatment	Time Duration
1.	0.1% $HgCl_2$	2 min
2.	0.1% $HgCl_2$	5 min
3.	0.5% $HgCl_2$	2 min
4.	0.5%$HgCl_2$	3 min

4.3 Effect of growth regulators on *in-vitro* regeneration of nodal explants

Culture of nodal explants inoculated on MS medium supplemented with plant growth regulators showed positive result in culture initiation as per the data collected after 15 days of inoculation. (Table-2). The shoots obtained did not elongate on the same medium up to 4 weeks time. Later on, these explants were transferred to fresh MS basal medium of the same composition but still they did not showed any further growth up to a period of 4 weeks. Finally they turned yellowish in color and afterwards necrosis. The initiated cultures were when sub-cultured after 2 weeks showed increase in multiple shoot production as compared to hormone free medium in all the four types of medium (MS, WPM, B5, SH) within 3 weeks of inoculation (Table-3). As sufficient shoot length was not obtained, the shoots were transferred to a shoot elongation media. For shoot elongation, the media (MS, WPM, B5, SH) were supplemented with 1.0mg/lit BAP, 0.5mg/lit, GA_3 0.25mg/lit NAA. The results were obtained after a time period of 2-3 weeks. The combinations in which the highest number of shoots and shoot length observed were described in table-3. In shoot initiation, MS was found to be superior to B5, WPM, SH media. Similarly Bhatnagar *et al.*, (2001) also found MS media to be superior to B5 (Gamborg) media, while investigating the regeneration capabilities of *M.indica* and *M.multicaulis.* In shoot elongation, MS was found to be superior to the other three media. WPM was found to be satisfactory after MS media. When nodal explants inoculated on MS medium containing BAP (3.0mg/lit), a maximum regeneration efficiency of 80% and 73% was observed at 2.0mg/l BAP, respectively. Mean shoot number was 1.11±0.22 and mean shoot length was 2.12 ± 1.01. (Table- 3).

4.4 Statistical data analysis:

The observations were monitored at the interval of every fifteen days for above objectives like shoot initiation and shoot multiplication. The data of shoot number and shoot length were represented as Mean ± Standard deviation, using Analysis Tool Pack (ANOVA

Table 2: Effect of different concentration of growth regulators in shoot initiation from nodal explants of *M. indica* (after 15 days of inoculation).

Sl.No	Treatments	Response of Shoot Initiation%	Shoot no. mean ± S.D
1	MS	20%	0.40±0.02
2	MS+0.5 mg/l BAP	30%	0.50±0.04
3	MS+1.0mg/l BAP	65%	0.82±0.03
4	MS+2.0mg/l BAP	70%	0.94±0.02
5	**MS+3.0mg/l BAP**	**80%**	**1.11±0.22**

Table 3: Effect of different concentration of growth regulators in shoot multiplication and elongation of *M.indica* (after 30 days of subculture)

Sl.No.	Media	Mean Shoot No.± S.D	Mean shoot length ±S.D
1.	MS	0.40±0.02	0.40±0.01
	MS+1.0mg/l BAP	1.5±0.35	0.82±0.35
	MS+1.0mg/l BAP+0.5 mg/l GA_3	2.6±0.87	2.0 ± 0.32
	MS+1.0mg/l BAP+ 0.25mg/l NAA	1.05±0.33	0.45 ± 0.50
	MS+1.0mg/l BAP+ 0.5mg/l GA_3+0.25mg/l NAA	**3.78±0.56**	**2.12 ± 1.01**
2.	WPM	0.35±0.27	0.28±0.12
	WPM+1.0mg/l BAP	2.0±1.15	0.75±0.28
	WPM+1.0mg/l BAP+ 0.5mg/l GA_3	2.5±0.57	1.0±0.70
	WPM+1.0mg/l BAP + 0.25mg/l NAA	1.25±0.50	0.55±0.10

	WPM+ 1.0mg/lBAP+1.0mg/l GA_3+0.5mg/l NAA	**3.16±1.16**	**2.0±1.37**
3.	B5	0.38±0.04	0.23±0.09
	B5+ 1.0mg/l BAP	0.83±0.40	0.58±0.37
	B5+1.0mg/l BAP+0.5mg/l GA_3	2.50±0.97	1.12±0.69
	B5+ 1.0mg/l BAP+ 0.25mg/l NAA	0.52±0.22	0.43±0.11
	B5+1.5 mg/l BAP+ 0.5mg/l GA_3+ 0.25mg/l NAA	**2.83±1.51**	**1.56±1.04**
4.	SH	0.20±0.03	0.20±0.10
	SH+ 1.0mg/l BAP	2.33±0.57	1.33±0.28
	SH+ 1.0mg/l BAP + 0.5mg/l GA_3	**2.50±0.70**	**2.50±0.35**
	SH +1.0mg/l BAP + 0.25mg/l NAA	0.50±0.23	0.36±0.20
	SH+ 1.0mg/l BAP+ 0.5mg/l GA_3 + 0.5mg/l NAA	1.50±0.70	1.50±0.70

****** *BAP – 6-benzylaminopurine, NAA – indole-3-butyric acid, MS – Murashige and Skoog medium, WPM – woody plant medium, B5 – Gamborg medium, SH – Schenk, Hildebrandt medium; SE – standard error, mean ±1 SE – each experiment consisted of ten replicates and repeated three times, means separated using ANOVA.*

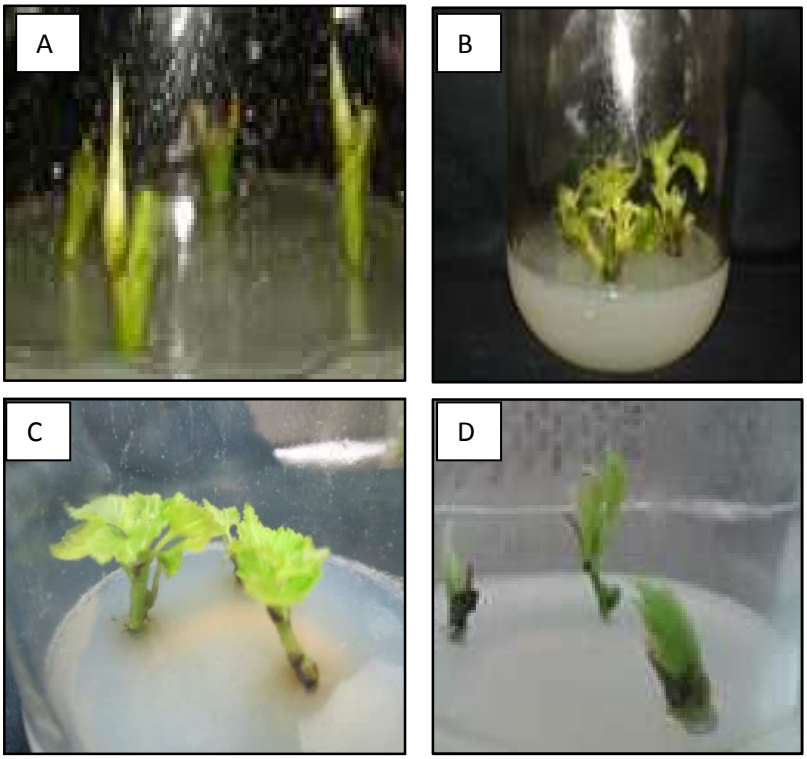

Fig A.- Nodal explant inoculated in MS medium containing 3.0mg/l BAP
Fig.B.- Shoot initiation in MS + 3.0mg/l BAP media
Fig.C & Fig.D.- Multiple shoot formation and shoot elongation in MS medium supplemented with 1.0mg/l BAP, 0.5mg/l GA_3 and 0.25mg/l NAA

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