*Indian Journal of Plant Sciences ISSN: 2319-3824 (Online)*
*An Online International Journal Available at http://www.cibtech.org/jps.htm*
*2013 Vol. 2 (2) April-June, pp.107-110/Aparna et al.*
***Research Article***

# MULTIPLE SHOOT REGENERATION THROUGH SHOOT TIP AND NODAL SEGMENT CULTURE OF *MORUS ALBA* L.

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**ABSTRACT**
An efficient and reproducible protocol has been established for micropropagation from nodal and shoot tip explants in *Morus alba* L. Multiple shoot induction (5-7 per plant) was obtained through shoot tip culture by supplementing MS basal medium with BAP (3 mg/l), NAA (0.5 mg/l), asparagine (25 mg/l) and glutamine (1 mg/l). Indirect shoot regeneration was obtained on medium supplemented with 2, 4-D (2 mg/l), BAP (0.5 mg/l) and both aminoacids i.e. asparagine and glutamine. Omission of aminoacids resulted in lateral bud proliferation along with slight callusing from the cut end. The plantlets were transferred to the rooting media containing 1 mg/l NAA. Rooted plantlets were successfully transferred to the field.

***Key Words:*** *Regeneration, Micropropagation, Proliferation, Shoot buds, Reproducible protocol.*

## INTRODUCTION

Mulberry (Morus alba L.), a woody perennial tree plays a very significant role in sericulture as its foliage constitutes the main diet for the silkworm (Bombyx mori L.). Conventionally, mulberry is propagated by cuttings as well as through seeds. Often cuttings prove difficult to root, thus posing problems for mulberry breeders. Propagation through seed is undesirable because of cross pollination and enormous heterozygosity in this plants. Tissue culture techniques provide a fast and dependable method for micropropagation in order to produce large number of elite plantlets in a short time. The in vitro production of plantelts from axillary buds has been reported by various workers in different species of *Morus. M. alba* (Oka and Ohyama 1974; 1981; Ohyama and Oka 1987; Kim et al. 1985; Jain et al. 1990; Sharma and Thorpe 1990, Pattnaik and Chand 1997; Chitra and Padmaja 1999) and through both adventitious and axillary buds in Morus indica (patel et al. 1983; Mhatre et al. 1985). The present study was undertaken to determine the culture conditions for rapid induction, proliferation and maintenance of calli through shoot tip and nodal explants whihc in turn is to be exploited for regeneration of mulberry plants.

## MATERIALS AND METHODS

Nodal explants and shoot tips were collected from actively growing shoot of Mulberry, growing in the Botanical garden of the University of Rajasthan. The excised nodal explant (2-3) cm) were washed thoroughly under running tap water for 30 minutes and then with 5% teepol for 8-10 minutes and rinsed several times in autoclaved single distilled water. Thereafter, the explants were surface sterilized in 0.1% $HgCl_2$ solution for 5-7 minutes followed by thorough washing with sterile water.

The sterilized single nodal explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 2, 4-D, IAA, NAA, BAP, KN, and two aminoacids (Glutamine and Asparagine) in varied concentrations individually as well as in combination of each other for inducing sprouting and shoot differentiation. The pH of the media was adjusted between 5.6-5.8 before autoclaving at 15 Ibs/$cm^2$ at $121^0$C for 15 minutes. Cultures after inoculation were incubated at $25^0$C +- $2^0$C and 65-70% relative humidity with photoperiod of 16/8h at 3000 lux intensity by fluroscent tubes.

## RESULTS

### *Induction of shoots from axillary buds of nodal explants*

Nodal explants which were slightly tender medium in thickness and having greenish axillary buds responded efficiently for shoot bud differentiation. Nodal explants cultured on MS medium supplemented with BAP (2 mg/l) showed callusing at lower cut ed while at upper end, proliferation of lateral bud took place after 15 days of inoculation. the callus formed was transferred to MS medium fortified with BAP (4 mg/l), NAA (1 mg/l), asparagine (25 mg/l) and glutamine (1 mg/l) which showed multiple shoot formation and inflorescence development after 40 days of transfer. The inflorescence so obtained was further transferred to MS medium containing IAA (2 mg/l) + BAP (0.5 mg/l) + asparagine (25 mg/l) + glutamine (1 mg/l) which resulted in direct shoot regeneration after 10 days of inoculation. Ten replicates of each treatment were taken during these experiments.

### *Induction of Multiple Shoots*

Multiple shoots were induced with varying frequencies by culturing shoot tip of 2-3 cm on MS medium supplemented with BAP (3 mg/l), NAA (0.5 mg/l), asparagine (25 mg/l) and glutamine (1 mg/l) after 30 days of inoculation. When the concentration of BAP was increased beyond (3 mg/l) there was no enhancement of multiple shoot induction.

Shoot apex explants when placed on MS medium supplemneted with 2, 4-D (2 mg/l) induced friable callus after about a month of culture. After first sub culture (about 35 days) on the same medium fortified with asparagine and glutamine, it started inducing green shoot buds. The growth and multiplication of the shoot buds along with the callus culture were observed after transferring them to the medium which contained MS micro and macro-salts + BAP (1 mg/l) which was found most suitable.

### *Rhizogenesis*

Elongated shootlets formed on the above combination were clipped off from the proliferating callus cultures and were transferred to the rooting medium to regenerate whole plant. The rooting medium fortified with NAA (1 mg/l) induced roots within 20-25 days of inoculation. Further it was observed with the use of Activated charcoal (0.05 - 1%) to the shoot proliferating medium enhanced the production of adventitious roots by at least 2-3 weeks and inhibits the formation of callus at the basal end of the shoots.

The rooted shoots or the plants with well developed root and shoot system were transferred to the pots containing soil, soilrite (1:1) mixture and then the acclimatized plants were finally transferred to the soil under filed conditions. it was reported that about 40% such plants could survive in the field for another three weeks.

## DISCUSSION AND CONCLUSION

The present findings on *Morus alba* demonstrates the possibility for mass propagation of mulberry through the culture of nodal segments and shoot apex. Nodal explants that were slightly tender and having greenish axillary buds responded efficiently for bud sprouting compared to hard nodal explants with brownish buds which showed no sign of growth.

Rapid Multiplication of any plant can be achieved by inducing multiple shoots through *in-vitro* culture. Multiple shoots were induced in moderate frequency from nodal explants cultured on MS with BAP (2 mg/l). Chitra and Padmaja (1999) reported high frequency of shoot differentiation from nodal explant of *Morus alba* L. on MS with 2, 4-D (0.3 mg/l). Although 2, 4-D is considered to suppress organogenesis and is generally used in experiments involving callus induction but in Mulberry its low level triggers shoot differentiation. Anuradha and Pullaiah (1992) reported that low concentration of 2, 4-D (0.5 mg/l) stimulated sprouting whereas higher concentration (2 mg/l) resulted in rapid callus proliferation from axillary bud cultures of *M. alba*. Combination of BAP (1 mg/l) and NAA (1 mg/l) induced axillary bud sprouting at a higher frequency compared to BAP alone. The inhibitory effect of BAP on shoot proliferation at concentrations higher than (1 mg/l) was noticed earlier in *Morus* species (Ohyama and Oka, 1987).

In the present study various growth hormones like 2, 4-D, BAP, NAA and aminoacids (asparagine and glutamine) were used either singly or in combination in MS medium to see their effects on bud sprouting and shoot differentation. Sprouted axillary buds developed into shoots as well as inflorescence. Induction of inflorescence from cultured axillary buds would be of significance in studies related to another culture as it does not demand sterilization which has to be done when the inflorescence are obtained from field grown plants. Kumar et al. (1999) reported inflorescence development from axillary buds on MS medium with very low concentration of BAP while our results showed its development in a combined treatment of BAP (3 mg/l), NAA (0.5 mg/l), asparagine (25 mg/l), and glutamine (1 mg/l). Polyamines have been intricately associated with various in *vitro* plant developmental processes including organogenesis. Omission of polyamines in the present work resulted in the inhibition of growth of lateral buds and reduction in callus formation. It is apparent that both the polyamines have a definite role in inducing shoot development and multiple shoot induction from both apical shoot buds and nodal explants in *M. alba*.

Jagadishchandra and Gowda (2003) observed abundant and vigorous rooting from axillary bud cultures of *Morus indica* L. on MS medium with IBA (0.1 mg/l) which was sparse on NAA and IAA. In contrast, Anuradha and Pullaiah (1992) reported that NAA was a more effective rooting agent than IAA for *Morus alba*. Bhau and Wakhlu (2001) also reported best rooting in regenerated shoots on Murashige and Skoog medium containing NAA or IAA (0.5 mg/l). This supports our results also. Chitra and Padmaja (1999) did not get any response with NAA as rooting agent and reported 2, 4-D to be more effective rooting agent than IAA and IBA. Sharma and Thorpe (1990) reported the addition of activated charcoal to the shoot proliferating medium produces adventitious roots and inhibits the callus formation at the basal ends of the shoots. Similar effect of the Activated charcoal was observed in the present study too.

The method of plant regeneration from calli and shoot apices ensure a constant supply of plant materials for further morphogenetic and biochemical studies. And our results may provide an efficient in vitro methods for the rapid propogation of elite genotypes of mulberry.

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