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# Effect of nutrients on in-vitro culture of Morus alba L. (White mulberry)

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## Abstract

Morus alba L. is an economically important plant used extensively in sericulture, also possessesing medicinal properties. In vitro culture provides an effective means to overcome the common problems encountered in conventional propagation by stem cuttings and seeds. Effect of phytohormones, growth adjuvants, sugars and essential minerals on the in vitro response of M. alba explants has been described in the study. Phytohormones and growth adjuvants resulted in explicit response in terms of organogenesis. Sucrose and glucose containing culture media elicited best response among sugars in nodal explants. Typical symptoms were exhibited by the growing shootlets when the medium was deprived of essential minerals such as nitrogen and sulphur.


## Introduction

The mulberry plant (*Morus alba* L.) is chiefly exploited by the sericulture industry for its foliage, used as feed for silk worm (*Bombyx mori* L.), and for feeding ruminants[3]. Depending on the location where it is grown, the plant is also valued for its fruit (consumed fresh, in juice or as a preserve) and aerial parts (stem and leaves are used as vegetable)[25]. *M. alba* also contains a considerable amount of dietary supplements such as proteins, carbohydrates, fats, fibers, essential minerals[22], ascorbic acid and ?-carotene[10].

The plant is known to possess anti-hyperglycemic and anti-hyperpigmentation activity[12]. The root bark of the plant possesses astringent and anthelmintic properties, hypotensive activity, anti-tumour activity and anti-microbial activity and is used in treatment of cough and cold[25]. Traditional reports of the application of *M. alba* in treatment of atherosclerosis and diabetes were experimentally validated by Enkhmaa[821].

Stem cuttings and seeds have been used conventionally for propagation of mulberry plants which have assumed tremendous economic importance over the years. Genotype, environmental factors and physiological state of the cuttings play a significant role in determination of success of rooting[14]. Propagation by seeds is undesirable owing to a long juvenile period[27] coupled with cross pollination which in turn leads to a high level of heterozygosity[2]. *In vitro* culture or micropropagation is a sought-after alternative, providing an effective means of rapid propagation of uniform genotypes of the plant. Application of modern techniques for development of new high-yielding varieties and cultivars depends on the availability of an efficient *in vitro* regeneration system[1]. This communication provides an account of studies on the *in vitro* response of stem-bit and nodal explants of *M. alba* to select phytohormones, growth adjuvants, minerals and sugars. This study was designed with the aim of identifying specific growth supplements which would have a positive influence on growth of *in vitro* plantlets. Another objective of the study was to evaluate the occurrence of the known *in vivo* physiological effects of some of the selected supplements on the plantlets *in vitro*.

## Materials and Methods

Local varieties of *M. alba* were procured from local farms in and around Puttaparthi and grown in the experimental garden of the Sri Sathya Sai University, Prasanthi Nilayam campus. These constituted the mother plants for the explants used during the course of the in vitro study. The stem-bit and nodal explants were washed under running tap water for 30 minutes, surface sterilized using 0.1% (w/v) aqueous solution of Mercuric Chloride and rinsed five to six times with sterile double distilled water.

The surface-sterilized explants were inoculated on Murashige and Skoog medium[15] under a laminar flow hood (KLENZAIDS, India). The cultures were maintained at 24 ± 2°C under 16/8 h photoperiod at a light intensity of around 3000 lux.

MS medium fortified with auxins (2,4-D, NAA and IBA), cytokinins (Kn, BAP) and growth adjuvants (coconut milk and casein hydrolysate) as individual treatments was used in the study. MS medium without its constituent nitrogen and sulphate mineral stock solutions was used to study the effects on explant growth and development. Each of the phytohormones was used at a concentration of 2mg $L^{-1}$ of MS medium and the growth adjuvants at 15% w/v or v/v. Aliquots of MS medium containing different sugars such as glucose, sucrose, fructose, mannose, maltose, lactose and galactose at a concentration of 3% (w/v) was used to study the effect of different sugars. Basal MS medium without addition of any supplements represented the control.

## Results and Discussion

Studies on the *in vitro* response of explants of *M. alba* have been undertaken to understand the effects of different nutrients with respect to organogenesis. This study focuses on the effect of phytohormones, growth adjuvants, sugars and minerals as observed in mulberry explants *in vitro*.

Nodal explants showed a better response as compared to stem- bits in the study of the effect of phytohormones on *in vitro* culture of *M. alba*. Callus initiation and proliferation occurred in MS medium supplemented with 2,4-D. The callus was semi-transparent and off-white in the beginning gradually turning brownish in colour. Both NAA and IBA were effective in initiating a response in terms of shoot formation and growth; the former resulting in development of unbranched roots and the latter, profusely branched roots. The cytokinins Kn and BAP promoted shoot growth. Kn resulted in shoots with considerably longer internodal distance compared to any other treatment and BAP treatment led to formation of leaf clusters. Treatment with casein hydrolysate in MS medium inoculated with nodal explants gave good results in shoot formation while coconut milk was more effective in callogenesis. Coconut milk showed similar response using stem-bit explants.

Micropropagation studies on mulberry, most commonly, have used MS medium culture of nodal cuttings and axillary and apical buds[24]. Islam[11] observed that explants in medium containing 2,4-D showed best callogenesis and callus proliferation. Pattnaik and Chand[185] obtained multiple shoots in BAP fortified medium which enhanced bud-break frequency with the addition of $GA_3$. The combination of NAA and BAP induced shoot proliferation in mulberry and NAA, in particular, was found to be essential for root induction by Ponchia and Gardiman[20]. The increased inter-nodal distance in shootlets obtained in Kn containing medium, in this study, has not been reported hitherto in the available literature.

Nodal explants cultured in MS medium containing glucose and sucrose responded by producing best results in shoot length, leaf growth and the development of a highly branched, extensive root system. This was followed by medium containing fructose. Quite notably, medium containing galactose did not show any response. The results of the response of nodal explants to different sugars using MS medium are summarized in Table 1.

Table 1.

Comparative effect of different sugars on morphogenic response of *M.alba* explants

| Sugars used | Average shoot length (cm) | Average leaf width (cm) | Root system |
|---|---|---|---|
| Monosaccharides | | | |
| Glucose | 5.7 ± 0.3 | 3.0 ± 0.1 | Thick, long extensively branched |
| Fructose | 4.6 ± 0.3 | 2.7 ± 0.2 | Thin, short, few rootlests |
| Mannose | 1.8 ± 0.1 | 1.8 ± 0.1 | Thin, short, no rootlets |
| Disaccharides | | | |
| Sucrose | 6.0 ± 04 | 3.9 ± 0.3 | Thick, long extensively branched |
| Maltose | 2.9 ± 0.1 | 1.8 ± 0.2 | No response |
| Lactose | 3.6 ± 0.1 | 2.6 ± 0.3 | Thin, short, few rootlets |

Sucrose is the principal product of carbon fixation in plants[4]. Other sugars have to be converted to glucose, the starting point of carbohydrate metabolism, or its derivatives to enter the glycolysis cycle[7]. The varied response to the different sugars reflects on the ability of the plant in converting these sugars into suitable glycolysis intermediates. Enomoto[9] reported that sucrose containing media elicit the best response in *in vitro* mulberry culture. However Ohyama[161726] report fructose as being more favourable. The present study revealed that sucrose is the best for morphogenic response in shoot length, leaf growth and size. These characteristics are highly advantageous for the sericulture industry.

Alteration in the mineral constitution of MS medium produced pronounced physiological effects that reflected in the morphology of the developing explants.

Table 2 outlines the different morphological changes that occurred in the response of stem-bit explants in the absence of nitrates and sulphates in MS medium.

Table 2.

| Effect of minerals on explants of *M.alba* | |
|---|---|
| Alteration in MS medium composition | Effect on growing explants |
| MS without Nitrogen ($NH_4NO_3$ and $KNO_3$) | Shoots got dried up; leaves were shriveled and gradually turned yellow; rooting was negligible. |
| MS without Sulphates ($MgSO_4$, $MnSO_4$, $FeSO_4$ and $CuSO_4$) | Shoot were very short; leaves were small, drooping, light green to yellow in colour with black tips, roots were profusely branched. |

Absence of nitrogen compounds in MS medium resulted in very poor morphogenic response of explants. Nitrogen supplied as ammonium ($NH_4^+$) and nitrate ($NO_3^-$) ions promotes cation anion balance within the plant[6]. Under severe nitrogen deficiency, leaves turn yellow and eventually fall off[23]. Sulphur is a constituent of several co-enzymes and vitamins essential for plant metabolism. Its deficiency leads to downward hooking of leaves along with stunted growth of shoots[23]. Deficiency of sulphates also decreases chlorophyll content and rate of photosynthesis[13].

## Conclusion

The mulberry plant is reported to be the one of best plants for silkworm rearing[24]. The qualitative and quantitative improvements in this plant are very essential from the aspect of enhancement of the sericulture industry. Micropropagation is an established alternative to the conventional propagation methods for obtaining improved mulberry cultivars. The hormone treatments discussed herein provide means to obtain mulberry plants with desirable traits. Biochemical tests could be conducted on *in vitro* generated plants to ascertain changes in phytochemical constituents which may have a bearing on the feeding habits of silkworms reared on mulberry. Similar studies could be designed for obtaining stress resistant varieties of mulberry which would be of benefit to the silk industry.

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## APPENDIX 1

**Abbreviations**

MS: Murashige and Skoog

2,4-D: 2, 4- Dichlorophenoxy acetic acid

NAA: ? - Naphthoxy Acetic Acid

Indole -3- Butyric Acid

Kn: Kinetin

BAP: 6- Benzyl Amino Purine.

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