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Shoot regeneration via direct organogenesis from in vitro derived leaves of mulberry using thidiazuron and 6-benzylaminopurine

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Abstract

A brief culture of mulberry leaves for 8–10 days on MS medium with 18.17 μM TDZ followed by transfer to 8.88 μM BAP supplemented medium triggered high frequency shoot organogenesis (77.6–89.2%) and favoured shoot elongation in *Morus* spp. Shoot proliferation was highest in the presence of 2.22 μM BAP with induction of 9.4–10.6 shoots per culture. High frequency of root induction (76.0–86.6%) was observed on medium supplemented with 0.49 μM IBA whereas increase in the level of IBA (4.92 μM) resulted in induction of roots along with development of callus from the base of the shoots. The regenerated plants established in soil at higher frequency in rainy season compared to winter and summer.

Keywords: Direct shoot organogenesis; Leaf explants; *Morus indica* L.; *Morus alba* L.; Thidiazuron; 6-benzylaminopurine

1. Introduction

Mulberry is of great economic importance in the sericulture industry because its foliage is used as food for silkworms. The dioecious nature of mulberry is a major barrier for genetic improvement by conventional hybridization. Moreover, the perennial and highly

Abbreviations: BAP, 6-benzylaminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog (1962) medium; NAA, α-naphthalene acetic acid; TDZ, thidiazuron (1-phenyl-3- [1,2,3-thiadiazol-5-yl] urea); Kn, kinetin

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heterozygous nature of the plant coupled with a prolonged juvenile period limits the speed of improvement using traditional methods (Ravindranan and Lakshmi Sita, 1994). Further, in vegetatively propagated plants like mulberry it takes many years to evolve a desirable clone from an economic and commercial point of view by routine propagation methods. Therefore, application of biotechnological tools for genetic improvement of mulberry attains greater significance, which in turn depends upon the availability of an efficient regeneration system.

The in vitro regeneration of plantlets from the adventitious buds formed on the leaf explants derived from aseptically grown shoots (Oka and Ohyama, 1981; Mhatre et al., 1985), cultured embryos (Kim et al., 1985) or axillary buds (Yamanouchi et al., 1999; Vijayan et al., 2000) has been reported in different species of *Morus*. Most of the regeneration work from leaf explants dealt with temperate varieties, which are non-adaptive to tropical environments, and also there are limited reports available on regeneration from leaf explants of tropical varieties (Vijayan et al., 2000). Kapur et al. (2001) obtained high percentage of regeneration from leaf explants of mulberry on MS medium supplemented with 11.41 μM IAA, 4.99 μM TDZ and 11.77 μM $AgNO_3$. In the present study, an efficient protocol for inducing direct shoot organogenesis from leaf explants and subsequent recovery of plants of economically important cultivars of mulberry is presented.

2. Materials and methods

Shoot cultures were established by culturing nodal explants obtained from 3-year-old plants of *Morus indica* L., cultivars M-5, S-36 and S-13, and *Morus alba* L., cultivar China White. The excised nodal explants with axillary buds were surface sterilized in 70% alcohol for 1 min followed by 0.1% mercuric chloride ($HgCl_2$) for 15 min. They were rinsed four to five times in sterile distilled water with 5 min duration each. The sterilized explants of M-5, S-36, S-13 and China White cultivars were cultured on MS (Murashige and Skoog, 1962) medium with 3% sucrose and 0.8% agar. The culture media were supplemented with 1.36 μM 2,4-D for inducing axillary bud sprouting in M-5, S-36 and S-13 cultivars whereas those of China White cultivar were cultured on the same basal medium with a supplement of 9.29 μM Kn (Chitra and Padmaja, 2002). Shoot multiplication was achieved by culture of shoot tips on MS medium with 2.22 μM BAP. Leaves without petiole derived from in vitro multiplied shoots were used for induction of shoot organogenesis. Two leaf explants were cultured in sterile culture tubes (25 mm × 150 mm) containing 15 ml of MS basal medium with 3% sucrose, 0.8% agar (Agar Powder, Extra pure, Hi-Media, Mumbai, India). The effect of BAP and TDZ on induction of direct organogenesis from leaf explants was tested by supplementation into MS medium in varied concentrations. In another experiment, the leaf explants were precultured on MS medium containing TDZ (9.08, 13.62, 18.17 and 22.7 μM) for different durations and subsequently transferred to the medium supplemented with 8.88 μM BAP for induction of direct organogenesis. Leaves of different sizes were cultured either with the abaxial or adaxial side in contact with the medium. Each treatment was repeated thrice at different times having at least 20 explants per treatment. The appearance of shoot buds