Rapid micropropagation of five cultivars of mulberry

B.S. BHAU* and A.K. WAKHLU

Plant Tissue Culture Laboratory, Department of Botany, University of Jammu, Jammu 180 006, India

Abstract

Multiple shoots were initiated from nodal and shoot tip explants collected from mature trees of *Morus alba* L. cultivars Chinese White, Kokuso-27 and Ichinose, and *M. multicaulis* Perr. cultivars Goshmore and Rokokuyaso after 2 weeks of culture. Nodal explants were more responsive than shoot tip explants. Murashige and Skoog basal medium was found to be most suitable medium and 6-benzylaminopurine was the most effective cytokinin for shoot induction. Explants collected between April and September evoked better response than the explants collected between October and March. Shoots were multiplied by transferring nodal explants excised from *in vitro* raised shoots onto a medium containing cytokinins. Sucrose was the most suitable carbon source examined for shoot multiplication. An increase in shoot multiplication rate was noticed up to 4-5 subcultures. Nodal explants rooted on an auxin-supplemented medium. The acclimatized plants were successfully transplanted in the field.


Introduction

The genus *Morus* belonging to the family Moraceae comprises nearly 35 species. Many members of this genus are cultivated on a commercial scale. *Morus alba* L. cvs. Chinese White, Kokuso-27, Ichinose and *Morus multicaulis* Perr. cvs. Goshmore and Rokokuyaso are promising cultivars for sericulture industry in temperate regions of the world. Their foliage is used as a source of feed for rearing silkworms (*Bombyx mori* L.). Low rooting potential of cuttings from these cultivars is a serious bottleneck to their large-scale propagation. Mulberry plants are out breeder and as a result their progeny show genetic variability, which makes them unsuitable for the commercial purpose. Methods of conventional vegetative propagation like production of plants through grafting is not economically viable because it involves lot of skilled manpower, expensive nursery facilities and a long wait of 4-5 years to obtain plants ready for harvest (Bhau 1999, Bhau and Wakhlu 2001). Propagation of plants through cuttings is also not viable for these cultivars due to their extremely low rooting ability. An attempt made in the past to induce rooting in stem cuttings of these cultivars by the use of auxins has not yielded encouraging results (Fotadar et al. 1990).

Micropropagation provides an alternative method for mass clonal propagation. The successful regeneration of plants *in vitro* has been achieved in several mulberry species by axillary shoot proliferation (for review see Wakhlu and Bhau 2000). These studies have revealed that *in vitro* micropropagation in mulberry is dependent on the growth regulator combinations, explant type and the genotype. New mulberry cultivars require development of successful *in vitro* regeneration system. The objectives of this study were to establish an efficient protocol for micropropagation of *M. alba* cvs. Chinese White, Kokuso-27, Ichinose and *M. multicaulis* cvs. Rokokuyaso and Goshmore and optimize conditions for acclimatization and transplantation of plants to field.

Received 24 July 2001, accepted 4 March 2002.

Abbreviations: BAP - 6-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; B4 - Gamborg's medium; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; Kn - kinetin; MS - Murashige and Skoog; NAA - α-naphthaleneacetic acid; WPM - woody plant medium.

Acknowledgements: Authors are grateful to Professors N. S. Rangaswamy and S. S. Bhojwani, Department of Botany, University of Delhi, Delhi, for helpful discussions and Drs. B. B. Bindroo and R. K. Fotadar (CSRTI, Jammu) and Dr. A. Koul (RHRS, Jammu) for providing the plant material. AKW is thankful to the Central Silk Board and Department of Biotechnology (Govt. of India) for providing the financial support and SSB is grateful to UGC for the award of NET fellowship.

*Corresponding author; present address: Plant Molecular Biology Laboratory, Tata Energy Research Institute, India Habitat Center, Lodhi Road, New Delhi 110 003, India; fax: (+91) 11 4682144, e-mail: bhau-bs@hotmail.com
Materials and methods

The explants were collected from 10-year-old mature trees maintained in the Mulberry Germplasm, Regional Horticulture Research Station, Sher-e-Kashmir University of Agricultural Sciences and Technology, Udhaywala, Jammu, India. For each cultivar, the explants were collected from a single tree between February and October, 1993 - 1997 unless otherwise stated. Nodal segments (1 - 2 cm long) and shoot tips (1 cm long) were excised from current branches. The explants were washed with detergent under running tap water for 1 h, then immersed in 0.5 % Bavistin solution (BASF India Ltd., Mumbai, India) for 30 min, rinsed 3 - 4 times with sterile distilled water. These explants were surface-sterilized with 70 % ethanol for 1 min, followed by a dip in 4.6 % sodium hypochlorite solution containing 2 - 3 drops of Tween-80 (Loba Chemie, Mumbai, India) for 20 min. The explants were finally rinsed 4 - 5 times with sterilized distilled water.

Three basal media, MS (Murashige and Skoog 1962), B5 (Gamborg et al. 1968) and WPM (Lloyd and McCown 1980) media were used in this study. Each medium was fortified with 3 % sucrose and 0.8 % agar unless otherwise stated. The pH of the media was adjusted to 5.8 prior to autoclaving. Cultures were maintained at 28 ± 2 °C and 16-h photoperiod with a irradiance of 30 μmol m⁻² s⁻¹ provided by cool-white fluorescent tubes.

The effects of different growth regulators (0.5 - 10 mg dm⁻³ BAP or Kn and 0.1 - 0.5 mg dm⁻³ IBA, IAA, or NAA), basal media (MS, B5, WPM), explant type (nodal segments, shoot tips) and explanting period were tested for establishment of cultures. Shoots formed in vitro were cut into single nodes and used for shoot proliferation. The effects of above mentioned growth regulators and carbon source (sucrose, fructose, sorbitol, and mannitol in 3 % concentration) were tested for proliferation of shoots. Multiplication rate was evaluated after a regular gap of 4 weeks for 6 sequential subcultures on a medium supplemented with 1.5 mg dm⁻³ BAP. Data were taken in terms of the number of shoots formed per explant and the shoot length.

Nodal explants (1 cm) excised from in vitro raised shoots were used for rooting. The effect of growth regulators (0.1 - 2 mg dm⁻³ NAA, IBA, or IAA, and 0.1 - 1.0 mg dm⁻³ BAP or Kn) was tested for root formation. Data were taken in terms of the percentage of rooted explants, the average number of roots formed per explant and the root length. The regenerated plantlets were transferred to plastic cups (8-cm diameter) containing sand and vermiculite (1:1). The plantlets were covered with a polythene bag in order to maintain high humidity and were placed in a greenhouse. Plantlets were watered every 2 d with Knop's solution for 4 weeks. Hardened plantlets were then transplanted in polythene bags (16 × 13 cm) containing garden soil, sand and farmyard manure (1:1:1). These plants were transplanted in the experimental plots of Department of Botany, University of Jammu and Regional Sericulture Research Station, Miranshaib, Jammu, India.

Ten replicates with 50 explants were used for each treatment and repeated 3 times. The results were recorded at a regular interval of 4 weeks of culture and analyzed by analysis of variance using randomized block design method. Data taken in percentage was subjected to arcsin transformation for proportions before analysis and converted back to percentages for presentation in tables. Means were compared using Duncan's new multiple range test (Duncan 1955).

Results

Multiple shoot induction: At the beginning of the experiment ‘Kokusu-27’, ‘Ichinose’ and ‘Rokokuyaso’ cultivars grew well, while Chinese White and Goshooerami required an adaptation period to the in vitro conditions. Multiple shoots were initiated from nodal and shoot tip explants after 2 weeks of culture on a growth regulator supplemented medium (Fig. 1). The greatest numbers of shoots were regenerated on a medium with BAP. The most effective concentration of BAP was from 1 - 2.5 mg dm⁻³ for ‘Kokusu-27’, ‘Ichinose’ and ‘Rokokuyaso’ and 4 mg dm⁻³ in Chinese White and Goshooerami. Nodal segments were more responsive than shoot tip explants. Maximum number of shoots formed per nodal explant was higher than the number of shoots formed per shoot tip explant. Auxin type and concentration had no significant effect on multiple shoot induction and favored callus formation at the cut ends of the explants (data not presented).

MS basal medium was more suitable than B5 and WPM medium for shoot induction from nodal explants (Table 1). The type of basal medium did not influence multiple shoot induction from shoot tip explants. The season of explant collection had a significant influence on multiple shoot initiation. Maximum number of shoots per explant was obtained from nodal explants collected between April and September than from explants collected between October and March (Fig. 2).