Plantlets from somatic callus tissue of the woody legume
Sesbania bispinosa (Jacq.) W.F. Wight

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ABSTRACT

In vitro regeneration of plantlets and multiplication of Sesbania bispinosa (Jacq.) U.F. Wight plants from cultured callus tissue were demonstrated. Callus was established from both cotyledons and mature leaflets on Murashige and Skoog (MS) basal medium supplemented with BAP (0.5 mg/l) and 2,4-D (2 mg/l). Callus mediated shoot bud differentiation was studied under defined nutritional, hormonal and cultural conditions. Various concentrations of BAP or kinetin (Kn) with coconut milk (CM) in MS media induced different levels of shoot bud differentiation as well as multiplication. Multiple shoot bud differentiation occurred in most of the primary calli. The best medium for shoot bud differentiation from cotyledon derived callus, contained BAP (2 mg/l) and 15% CM (V/V). More efficient shoot bud organogenesis was recorded with BAP than Kn. Supplementation with CM in MS media accelerated shoot bud organogenesis in differentiating callus tissue. Rooting of differentiated shoots was achieved by a three step culture procedure involving (a) MS solid medium containing IBA (2 mg/l), (b) growth regulator free half strength MS medium with 1% charcoal, and (c) half strength MS liquid medium free of vitamins, growth regulators and charcoal.

ABBREVIATION

IAA, indoleacetic acid; IBA, indole-3-butyric acid; NAA, naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine; Kn, kinetin; CM, coconut milk, MS, Murashige and Skoog’s medium; SBI, shoot bud inducing medium.

INTRODUCTION

Plant regeneration and propagation is based on proper manipulation of the cultural environment and appropriate selection of genotype and tissue. There has been a number of reports on plant regeneration through either organogenesis or somatic embryogenesis of various legumes (Skolman and Hapes, 1976; Mohan Ram et al., 1982; Venketeswarn and Romano, 1982; Gharyal and Haheshwari, 1983; Hroginski and Kartha, 1984; Xu et al., 1984; Bovo et al., 1986). Recently, application of tissue culture techniques for regeneration and propagation of forest trees is becoming a promising area of research, and a number of reports on plant regeneration have been published (Rao and Lee, 1986; Rai and Chandra, 1988; Sharma and Chandra, 1988; Sinha et al., 1988; Tomar and Gupta 1988; Mascarenhas and Huralidharan, 1989; Hittal et al., 1989). However, no major woody legumes species can be mass propagated in vitro. The majority of tree species are known to be recalcitrant and it is difficult to achieve organ formation from callus. This has made many woody legumes unsuitable for the development of a suitable callus mediated plant regeneration method for both propagation and conservation.

Sesbania bispinosa (Jacq.) W.F. Wight is an important fast-growing woody legume used for gum, fuel, fiber, pulp, green manure and fodder (Anonymous, 1984). The heavy nodulation capacity of this species (Allen and Allen, 1981) has recently demonstrated its significant role in green manuring and nitrogen nutrition with the yield of rice (Goswami and Sarkar, 1989; Hanna et al., 1989; Panda et al., 1989) in India.

The present paper describes the induction of successful plant regeneration and multiplication in vitro from both cotyledon and leaf-derived calli of S. bispinosa. Callus mediated successful plant regeneration and multiplication of S. bispinosa has not been reported previously.

MATERIALS AND METHODS

Seeds of Sesbania bispinosa (Jacq.) W.F. Wight supplied by the Indian Horticulture Society, Calcutta were used as explant source. Seeds were washed thoroughly with 5% Teepol (a commercial detergent) and
then in running tap water. Seeds were then surface sterilized by immersion in a 0.1 per cent mercuric chloride solution for 10 minutes and were thoroughly washed three times with autoclaved distilled water. The seeds were aseptically germinated in growth regulator and vitamin free HS basal medium. Two cotyledons of 14 day old in vitro grown seedling were then aseptically dissected out and used as primary explants. Prior to inoculation, cotyledonary explants were cut from cotyledonary node and subsequently cultured in solidified HS basal medium supplemented with growth regulators for callus initiation and growth. In one experiment fully expanded leaflets obtained from mature plants were employed as source of explants. The leaflets were disinfected using the same procedure as cotyledons and cultured in HS basal medium containing growth regulators for callus establishment.

All the nutrient media consisted of major and minor salts as well as vitamins according to Murashige and Skoog (1962), 3% sucrose and 0.7% bacteriological grade agar. Growth regulators used for callus induction and maintenance were 2,4-D (1-4 mg/l) and BAP (0.2-1 mg/l). For shoot bud differentiation and subsequent regeneration of plantlets from callus tissue, various concentrations and combinations of BAP (0.5-4.0 mg/l), Kn (0.5-4.0 mg/l) and CH 15% (V/V) were used in different shoot bud inducing (SBI) media. All media were adjusted to pH 5.7 before autoclaving and chemicals used for culture were of analytical grade (E. Herck, Sigma or Difco). Cultures were incubated at 22 ± 2°C and a relative humidity of 55-60% with 16h photoperiods at a light intensity of 3000 lux from daylight fluorescent lamps. Initially the callus was separated from the mother tissue and routinely maintained through successive subculture at three week intervals for over 16 months.

RESULTS AND DISCUSSION

Successful induction of callus tissue was observed in both cotyledon and mature leaf explants of S. bispinosa in media containing 2,4-D singly or in combination with BAP. Use of low levels of BAP (0.2-0.5 mg/l) with 2,4-D (2 mg/l) supported better dedifferentiation and callus growth. These calli were relatively compact and pale yellow in colour (Fig. 1). Callusing occurred in less than three weeks on the callus inducing medium. However, in subsequent subcultures, calli derived from both the explants were found highly competent for shoot bud initiation particularly in media containing BAP (0.5-4.0 mg/l) in combination with 15% CH (V/V). The earliest sign of shoot bud formation was noticeable within 15-21 days. The amount of shoot bud organogenesis observed per unit callus (600 mg fresh weight) ranged from 44 to 100% during 42 days of incubation in several SBI media supplemented with different concentrations and combinations of BAP, Kn and CH (Table I). The optimum level of shoot bud formation was recorded in SBI medium containing BAP (2 mg/l) and 15% CH (V/V) (Fig. 2).

Experiments aimed at the induction of shoot bud organogenesis and subsequent plant regeneration involved transferring callus to shoot bud inducing medium (SBI) containing different BAP or Kn concentrations in combination with CH. Callus tissue (approx. 600 mg fresh weight) transferred to different SBI media produced more compact green and moderately hard callus with or without shoot bud formation. However, calli derived from both the explants were found highly competent for shoot bud initiation particularly in media containing BAP (0.5-4.0 mg/l) and 15% CH (V/V). The earliest sign of shoot bud formation was noticeable within 15-21 days. The amount of shoot bud organogenesis observed per unit callus (600 mg fresh weight) ranged from 44 to 100% during 42 days of incubation in several SBI media supplemented with different concentrations and combinations of BAP, Kn and CH (Table I). The optimum level of shoot bud formation was recorded in SBI medium containing BAP (2 mg/l) and 15% CH (V/V) (Fig. 2).

In all the SBI media containing either BAP alone or in combination with CH, regeneration of shoot buds as well as regeneration of multiple shoots were readily