J. Phytol. Res. **8** (2): 141-144, 1995

REGENERATION OF PLANTLETS FROM EXCISED ROOTS OF *BAUHINIA MALABARICA* ROXB.

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One cm long root explants from the seedlings of *B. malabarica* produced shoots when cultured on MS basal medium containing 2,4-D (2 mg/1) + BAP (1 mg/l) and NAA (2 mg/l) + BAP (1 mg/1). The shoots originated in 25 to 30 days, either directly or after slight callusing. Production of plantlets from excised seedling roots can be developed as a method of clonal propagation of elite plants.

Keyw ·ds :BAP; Callusing, 2,4-D;Explants; Excised roots; elite plants; NAA, Plantlets.

Introduction

The production of shoot buds from intact roots is a natural phenomenon and constitutes an integral part of the development of a wide variety of plant species[1]. Extensive investigations have been carried out by us to understand the factors that regulate shoot bud differentiation from roots. Several reports are available on the origin of shoot buds from root explants *in vitro*[2,3]. Evidence for differentiation of plantlets from cultured roots of legumes is lacking inspite of numerous studies made on the growth of callus derived from roots[4].

The present report describes the formation of entire plantlets from excised seedling roots of *B. malabarica*, an important wood and tannin yielding legume tree[5].

Materials and Methods

Mature seeds were collected from Dehradun, Uttar Pradesh. They were washed thoroughly in 1% Teepol and surface sterilized with 0.1% Mercuric chloride for 10 mins and implanted on Agar base (Difco) medium under aseptic conditions. The seeds germinated within 5-6 days and developed into seedlings having a slender, 2 to 3 cm long, creamish white, branched or unbranched tap-root system after another 5-8 days. Explants meauring approsimately 1.0 cm, without the apical meristem were obtatined from tap-roots and subcultured on MS basal medium (HIMEDIA) containing 2,4 - Dichlorophenoxy acetic acid (2,4-D), α - napthaleneacetic acid (NAA) and 6-Benzylamino purine (BAP) at different concentrations. Ten replicates were used for each treatment and the cultures were maintained at 25 ± 1°C under 2000lux of light for 16/8 hours of light/dark period. The experiments were repeated several times.

Results and Discussion

The explants started callusing within 6-8 days of culture, irrespective of the nature and concentrations of the hormones used. The epidermis split open exposing a creamish-green, semi friable callus in 10 to 12 days. Explants raised on MS + 2,4-D + BAP produced a larger amount of callus than those raised on MS + NAA +BAP. The nature of the callus was compact, soft, nodular and off white in colour. However, the callus induced on 2,4-D (1 mg/1), NAA (1 mg/1) and BAP (0.5 mg/1) did not show any organ differentiation. Contrastingly, when raised

on 2,4-D (2 mg/1), NAA (2 mg/1) and BAP (1 mg/1), the cultures showed vigorous callusing followed by regeneration of the plantlets (Table 1). Interestingly, the cultures raised on 2,4-D (2 mg/1) + BAP (1 mg/1) and NAA (2 mg/1) + BAP (1 mg/1) develoepd tiny patches of pale green callus bearing several green hump like structures (arrowed) within 15-20 days of culture. Subsequently these differentiated into shoot buds after 22-25days (Fig.1). These buds were initially somewhat irregular in appearance but became gradually established into normal, slender shoots measuring 2.5 - 3.5 cm. The developing shoots had normal, simple bilobed-leaf (Fig.2). The shoots derived from different cultures, when dissected and transferred to a medium of same concentrations of hormones that were used for shoot bud formation, invariably produced one or two roots from their basel cut ends in 4-5 days. These roots grew through the medium without callusing and produced laterals after another 10-15 days. The leaves also expanded and assumed their characteristic shape (Fig.2). Thus on an average 2 or 3 plantlets were formed per root explant within a period of about 30-40 days. Attempts are being made to transfer these plantlets to the soil.

The literature review indicates that auxin applied at relatively high concentrations suppress the inception of the shoot buds and promote the growth of the lateral roots[6]. In certain instances auxin can be stimulatory to bud initiation at lower concentrations[7,8]. In the present study, BAP alone was ineffective in causing shoot bud initiation and required the presence of 2,4-D and NAA. 2,4-D was more effective than NAA. Similar reports were also noted in *B. acuminata* and *B.variegata* (S. Dasgupta,unpublished), as well as in root tip cultures of *Dalbergia sisso*, a timber yielding legume tree[9].

A survey of the steadily growing literature on *in vitro* plantlet differentiation in legumes[10-12] shows that it generally takes longer than 6 to 8 weeks for a plantlet to be established in cultures. However, in *B.malabarica*, a plantlet is obtained in 4-5 weeks, especially on MS + 2,4-D (2 mg/1) + BAP (2 mg/1) medium, using seedling root as the explant.

Thus from the results presented by us and from a survey of literature it may be stated that production of plantlets from excised seedling roots can be developed as a method

Table 1. Calllus induction and differentiation of plantlets from excised seedling roots of *B.malabarica* Roxb.

(Data represent an average of ten replicates/treatment)

Medium + Homone (mg/1)	Callusing pattern	% of cultures showing callusing response	Frequency of Plantlet formation.(%)
Control (only MS)	Poor callusing	2 - 5	-
MS + 2,4-D (1) + BAP (0.5)	Moderate callusing	10 - 42	No organogenesis
MS + 2,4-D (2) + BAP (1)	Profuse callusing	75 - 80	55 - 60
MS + NAA (1) + BAP (0.5)	Moderate callusing	30 - 35	No organogenesis
MS + NAA (2) + BAP (1)	Profuse callusing	45 - 50	40 - 45

Fig. 1. Differentiation of plantlets from excised seedling roots of *B. malabarica* showing formation of shoot buds (arrowed).

Fig. 2. A well developed plantlets bearing roots and shoots with normal leaves.

of clonal propagation of elite plants. Yet, another advantage of using seedling material lies in the fact that, it is essentially free of phenolic compounds, the accumulation of which inhibits organogenesis in relatively more mature tissues. Actually, the present communication deals with the successful regeneration of *B.malabarica* plantlets for the first time through *in vitro* cultures.

Acknowledgement

Kalyani University Research Scholarship grant is thankfully acknowledged by S. Dasgupta for the present work.

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