5.460 g, respectively. Two types of seeds, viz. black and white are observed in mature fruits. The average dry weight of black and white seeds is 4.250 g. Various morphological parameters of seeds studied are given in Table 1.

A seed produces more than one seedling due to its polyembryonic nature, viz. one to four seedlings from a single seed (Figures 2 and 3). The germination percentage was 36.25 in black seeds under nursery conditions during July 1999. Out of the total germinated seeds, nearly 58.62% seeds produced one seedling per seed, 27.58% produced two seedlings per seed, 10.34% produced three seedlings per seed and 3.45% seeds produced four seedlings per seed.

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High frequency *in vitro* shoot multiplication of *Plumbago indica*, a rare medicinal plant

Plumbago indica L. (Plumbaginaceae) is a rare medicinal plant species found in Assam and other parts of north-east India[1]. It is important due to the presence of the active principle 'Plumbagin', an orange–yellow pigment. Plumbagin, in small doses has stimulant action on the central nervous system, on muscle pain and on the secretion of sweat, urine and bile[2]. Roots are reported to be vesicant, sialogogue and abortifacient, and are used in leucoderma, syphilis and leprosy. Roots are also recommended as a substitute for cantharides. Tincture of roots is used in dyspeptic and other digestive disorders and in piles[2,3]. In traditional medicinal practices of Assam, the aerial parts fried with fresh fish are eaten to cure piles. Tender twigs fried with eggs of domestic duck are given in asthma and epilepsy. Root paste is applied on wounds to get relief from pain[4].

P. indica L. is found in natural habitats of Jorhat, Golaghat and Dibrugarh districts of Assam[1,4]. The species is becoming rare due to massive collection by medicinal plant traders and also destruction of the natural habitat. For conservation as well as mass multiplication of these medicinally-important species, tissue culture may prove to be an important tool. In an attempt to standardize a protocol for micropropagation of *P. indica*, multiplication of shoots was considered an important step. Culture of shoot meristem, especially through enhanced axillary branching, permits rapid clonal propagation of certain plants and a high degree of genetic uniformity of the progeny. The paper presents the results of *in vitro* shoot multiplication of *P. indica* from nodal explants.

Small tender twigs collected from field grown mature plants of *P. indica* were thoroughly washed with tap water several times. Twigs were cut into 1–1.5 cm nodal segments and soaked in Tween 20 (20%) for 15 min. The shoot segments were then washed several times with sterile distilled water (SDW) in a laminar flow and then surface sterilized with a fungicide, carbendazim (1%), followed by mercuric chloride 0.1% (W/V) for 10 minutes. The explants were further rinsed in 70% ethanol for 30 s and washed thrice with SDW. These surface sterilized nodal segments were used as explants.

Explants were inoculated in MS medium[5] containing 0.8% agar and 3% sucrose supplemented with 6-benzyl adenine (BA, 0.25–1.0 mg/l), indoleacetic acid (IAA, 0.05–0.2 mg/l) and adenine sulphate (AS, 10–40 mg/l) in various combinations in 25 × 150 mm culture tubes. The pH of the medium was adjusted at 5.8 prior to autoclaving at 1.06 kg/cm^2 for 15 min. The cultures were maintained at 25 ± 2°C under 2000 lux light intensity provided by a white fluorescent lamp for 16 h photoperiod. In all experiments 20 replicates were used and each experiment was repeated thrice. Results presented are based on 60 days of observation.

The explants were cultured in two groups of hormone combinations with different concentrations. The first group consists of BA and IAA, while the second group consists of BA and AS. The nodal explants inoculated in the MS medium with BA (3 mg/l) and IAA (0.1 mg/l) showed shoot initiation after

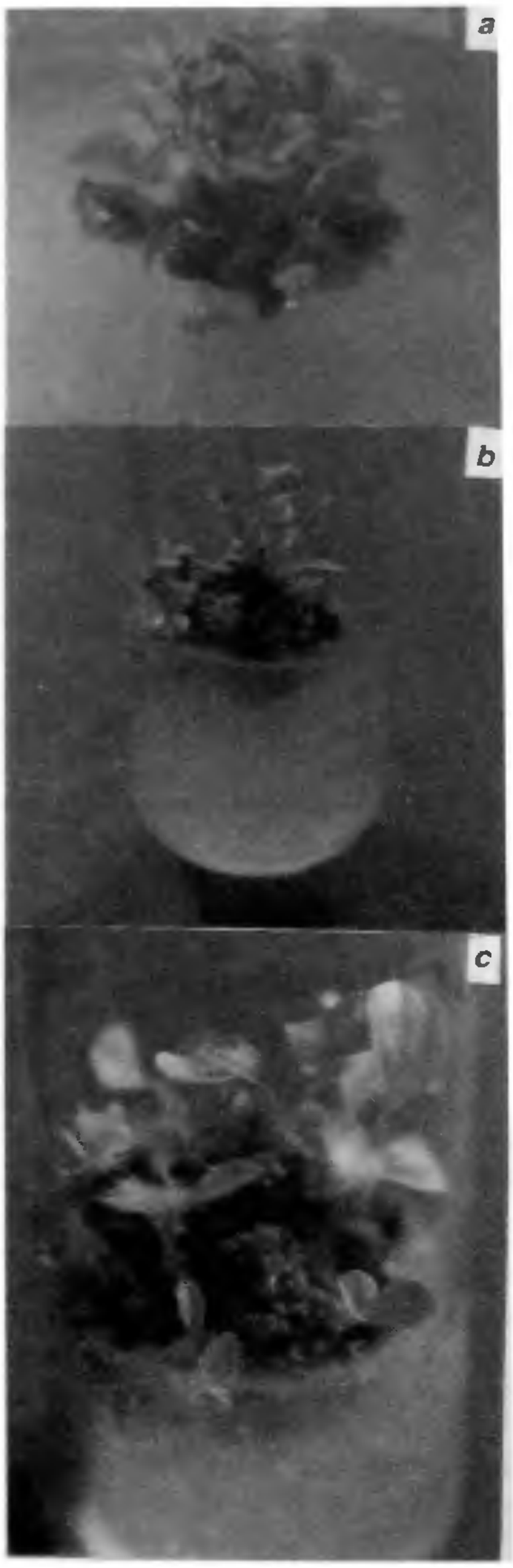

Figure 1. *In vitro* shoot multiplication of *P. indica* L.; *a*, Shoot multiplication in MS medium containing BA and IAA; *b*, *In vitro* development of buds in MS medium containing BA and AS; *c*, Differentiation of buds into shoots.

10 days of culture. These shoots were allowed to grow for another 10 days and then subcultured to the same medium for further growth and multiplication. Shoot multiplication occurred after 15 days of subculture. A maximum of 17 shoots developed from a single nodal explant (Figure 1 *a*). However, the growth of shoots was very slow reaching a height of 3 cm after maintaining for another 25 days.

The explants inoculated in the MS medium containing BA (3 mg/l) and AS (25 mg/l) developed small buds within 25–30 days of culture (Figure 1 *b*). But no further growth occurred when maintained in the same medium. Hence they were transferred to MS medium with low concentration of BA (0.1–1.5 mg/l). The buds differentiated into shoots after 18–20 days of transfer (Figure 1 *c*). A maximum of 35 shoots was obtained from a single nodal explant. Shoot multiplication through the formation of buds resulted into higher number of shoots in comparison to the cultures maintained in the MS medium with BA and IAA giving direct shoot initiation and multiplication.

Earlier reports on *P. indica*[6] and *Ixora singaporensis*[7] described that bud differentiation occurs only in the presence of higher concentration of adenine (10 mg/l). Further increase in AS caused decrease in shooting efficiency. In the present work it was observed that a higher concentration of adenine led to the formation of buds with a reduced bud differentiation. But these buds differentiated into shoots on transfer to a medium containing low concentration of adenine (BA, 0.1–1.5 mg/l) in contrast to the earlier reports.

The present result demonstrated the two-stage treatment of adenine for bud formation and subsequent differentiation of buds into shoots. Bud formation took place in higher adenine concentration [BA (3 mg/l) + AS (25 mg/l)] but the buds failed to grow further. When these buds were transferred to lower concentration of BA (1–1.5 mg/l) differentiation occurred. However, earlier reports[6,7] showed that bud differentiation and development required higher concentration of adenine (10 mg/l).

In this study, it was observed that adenine (BA, 3 mg/l) induced multiple shoots, but a still higher concentration of adenine, i.e. BA (3 mg/l) and AS (25 mg/l) together led to the development of adventitious buds in a high frequency (average number 30).

During the study about 90% explant contamination was observed initially even when explants were treated with carbendazim (1%) for 10 min. Later when the explants were dipped in fungicide overnight, the contamination was reduced up to 90%. However, fungal contamination still posed a problem during the study. This may be due to the presence of endofungus in the explant.

The protocol standardized through this study demonstrated the high frequency *in vitro* shoot multiplication of *P. indica* which can be used for successful mass propagation of the species.

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