that the sedimentary record of the Gangetic Plain mimics the monsoon fluctuation, besides a tectonic component.

To conclude, the chronological and sedimentological data in the present study show that: (1) Fluvial activity in the interfluve areas of the Central Ganga Plain existed during 8–7 ka which coincides largely with a phase of enhanced monsoonal precipitation. With this perspective it seems logical to suggest that initiation and/or reactivation of the fluvial channels on interfluve areas should have occurred at around 13 ka, coinciding with the re-establishment of the SW monsoon activity at ~13 ka (ref. 24). (2) Abandonment of many of these channels took place during 7–5 ka, and aeolian activity at 5–4 ka resulted in ridge-like Bhur deposits. (3) A neotectonic activity was reported to be one of the causes for cessation of the channel process at Gangaganj[9]. This can be bracketed between 7 and 5 ka.

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Regeneration of plants from *in vitro* root segments of *Holostemma annulare* (Roxb.) K. Schum., A rare medicinal plant

C. G. Sudha*,†, P. N. Krishnan*, S. Seeni* and P. Pushpangadan**

*Plant Biotechnology Division, Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, India
**National Botanic Garden and Research Institute, Lucknow 226 001, India

Plant regeneration was achieved from chlorophyllous root segments derived from *in vitro* rooted plants of *Holostemma annulare* (Roxb.) K. Schum (*H. adakodian* R. BR. ex. Schult., Asclepiadaceae), a rare medicinal plant grown for 8–9 weeks in half-strength Murashige and Skoog (MS) medium with 0.3 mg/l indole-3-butyric acid (IBA). Up to 82% solitary shoot bud initiation with small laterals was obtained from the excised (3–4 cm) root segments implanted horizontally on MS agar medium supplemented with 0.2 mg/l benzyladenine (BA) within 2 weeks. Rapid elongation of both shoot and lateral root initials was observed upon transfer of the responded root segments to a half-strength MS medium lacking growth regulators (basal medium). The shoots attained a length of 13–14 cm with 8–9 nodes in a period of 3–4 weeks of culture in the basal medium. After 5-6 weeks incubation, the regenerated shoots having elongated roots with parental root segments were transferred to the field, and showed 80% survival after a hardening period of 4 weeks in the mist chamber. For further shoot multiplication, the rhizogenic shoot provided 8–9-fold multiplication rates in the MS medium supplemented with 0.5 mg/l BA and 0.05 mg/l α-naphthaleneacetic acid (NAA).

HOLOSTEMMA annulare (Asclepiadaceae) is a laticiferous, twining perennial shrub with tuberous roots native

†For correspondence.

to India, Burma (Myanmar) and Sri Lanka[1]. The tuberous roots are medicinally important and are major ingredient of the drug *Jivanthi* in the indigenous system of medicine[2]. Approximately 150 metric tons of root tubers are needed per annum for Ayurvedic drug preparations in major south Indian pharmacies[3]. A recent survey conducted on the commercial exploitation of medicinal plants in the drug industry revealed that the annual requirement of the root of this plant in four districts of southern Kerala was 6.16 tons per annum[4]. The destructive and ruthless collection of root tubers in recent times has led to acute scarcity of the plant and consequently it is listed out as vulnerable[5] and rare[6]. Conventional propagation of the plant is rather difficult and insufficient to meet the demand owing to seasonal dormancy, yearly flowering, scarce fruit set and impractical use of root cuttings as propagules. A rapid clonal propagation system is therefore of immense use and was recently achieved through multiple shoot formation in nodal explant cultures of the plant[7].

Roots of certain angiosperms are also endowed with a unique capacity to form shoot buds in nature. Shoot bud differentiation on the root is a species-specific attribute particularly among perennials, and is one of the major reasons for the success of the many perennial herbs[8]. The shoots developing on root segments of the same plant are genetically uniform[9]. Chaturvedi and Sharma[10] suggested that root culture is an alternative method for clonal propagation and germplasm preservation. Furthermore, regenerative root culture is an ideal tool for basic and applied studies like effect of plant hormone and apical dominance[11] and biotransformation[12]. To our knowledge, no reports are available on shoot regeneration in root tissues of *H. annulare*, which could be useful for both basic and applied studies. We report here direct plantlet formation in root segment culture of *H. annulare*, useful in conservation of this rare plant.

The previous study on *H. annulare*[7] induced shoots from nodal explants in Murashige and Skoog[13] (MS) medium with 1.0 mg/l 6-benzyladenine (BA) and 0.1 mg/l α-naphthaleneacetic acid (NAA) which were subcultured and maintained in the same medium with 0.5 mg/l BA and 0.05 mg/l NAA. These shoots were used for initiating roots. The chlorophyllous long roots

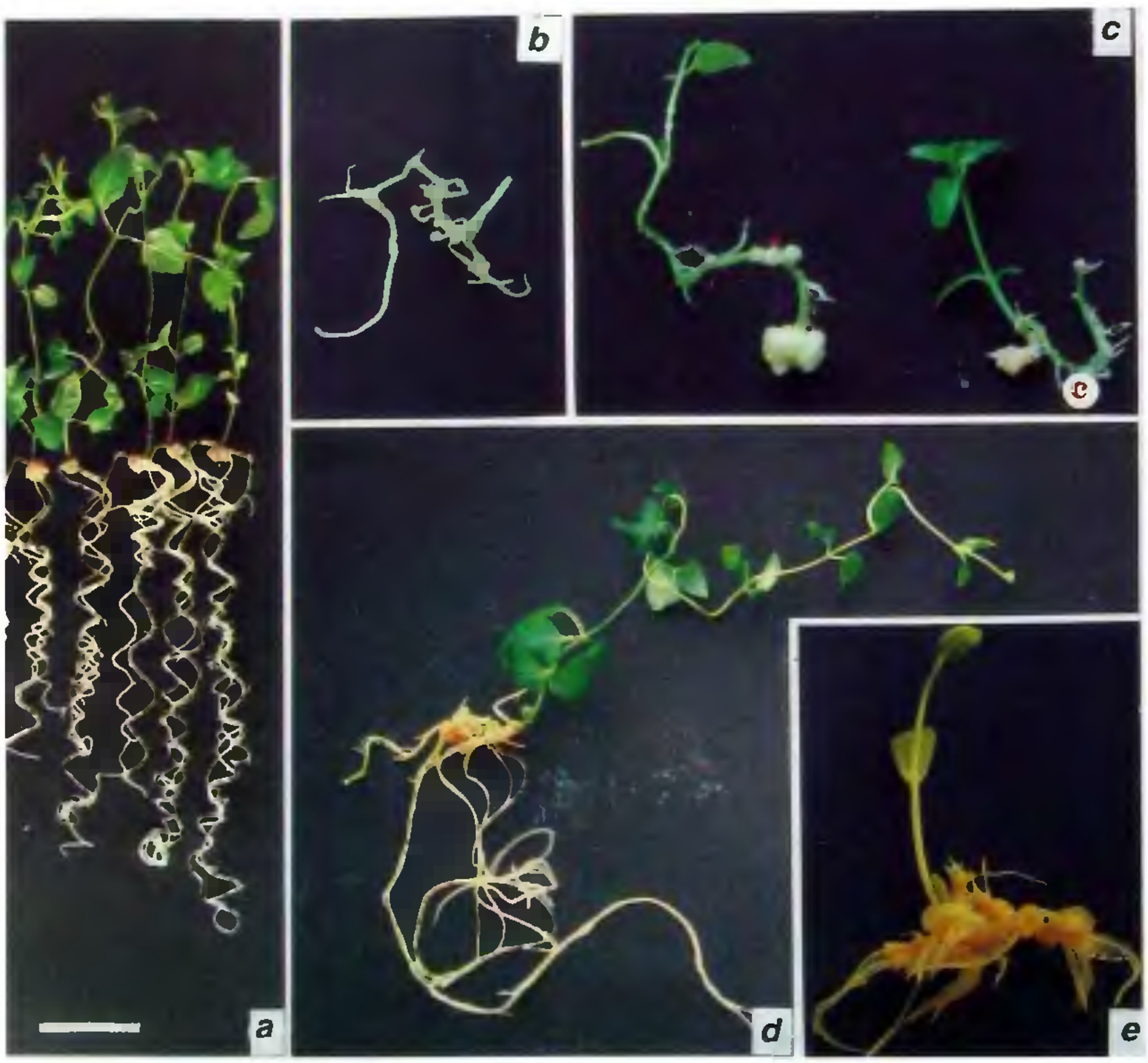

Figure 1. Plant regeneration from *in vitro* root segments of *Holostemma annulare* (Roxb.) K. Schum. ***a***, *in vitro* rooted plants (8–9 weeks old) grown in half-strength MS medium with 0.3 mg/l IBA (root length – bar = 2.0 cm); ***b***, achlorophyllous distal root segment cultured in medium with 0.2 mg/l BA alone; ***c***, single shoot initiation on proximal (left) and middle (right) chlorophyllous root segment after 3 weeks of culture in medium with 0.2 mg/l BA; ***d***, rapidly elongated shoot and lateral roots upon transfer of the responded segments from BA containing medium to basal medium; ***e***, flaccid shoot formation on root segments cultured on medium containing 0.1 mg/l IBA.

(12–15 cm) obtained from shoots grown for 8–9 weeks in half-strength MS medium supplemented with 0.3 mg/l indole-3-butyric acid (IBA) in which the growing tip (distal end) was achlorophylious, were used for explants (*Figure 1 a*). The aseptically removed long roots were repeatedly washed in sterile distilled water to free them from nutrient medium and then dissected into 3–4 cm long proximal, middle and distal segments with apical meristem. The segments were inoculated horizontally on agar-gelled (0.5% (w/v): CDH, India Ltd.) MS medium supplemented with BA alone (0.05–0.2 mg/l) for initial trial experiments. Subsequently, the roots devoid of distal segments were dissected without considering the position and inoculated on a medium supplemented with different auxins, NAA, IBA and indole-3-acetic acid (IAA) individually at 0.1 mg/l and in combination with 0.2 mg/l BA, BA alone (0.05–1.0 mg/l) and the medium lacking growth regulators (basal medium). The medium was dispensed in 90 ml aliquots into 250 ml Erlenmeyer flasks after adjusting the pH to 5.8 prior to autoclaving (121°C and 108 kPa for 18 min). The cultures were incubated at 24 ± 2°C under 12 h photoperiod at a photon flux density of 50 µE mol m^{-2} s^{-1} provided by daylight fluorescent tubes (Philips India Ltd., Mumbai). Each treatment consisted of twenty explants and was repeated twice.

Three weeks after culture, the root segments with initiated shoot buds were transferred to fresh half-strength MS basal medium for normal and rapid growth of the shoot buds. After 5–6 weeks of incubation in this medium, the regenerated shoots were used for further multiplication or direct field establishment. An average of 8–9 nodes was excised from each regenerated shoot and subcultured in the standardized medium (MS + 0.5 mg/l BA + 0.05 mg/l NAA) as per the previous work[7]. The regenerated shoot along with parental root segments were transferred in 5 × 5 cm clay pots containing potting mixture of sand:soil:cow dung (1:1:1) and covered with perforated polythene bags and kept inside the mist chamber. The polythene bags were removed for 1 h during the first week and the exposure time increased slowly during the subsequent weeks. The plants were well irrigated and hardened by the end of 4 weeks and subsequently established in the field.

Among the different root segments tried during the initial trials using BA alone, achlorophyllous distal root segments remained nonresponsive to shoot buds (Figure 1 *b*), while the proximal and midddle segments exhibited single shoot regeneration and apparently no difference in the frequency and mode of shoot bud differentiation. A subsequent experiment using varying concentrations of BA did not help to enhance the number of shoot buds. The bud initiation usually started within 2 weeks of culture and was observed just distal to the cut ends of root segments with small laterals. A low frequency (40%) of shoot bud initiation was noticed in the basal medium (Table 1) and the initiated buds were seldom elongated during the later period of culture. Incorporation of 0.2 mg/l BA was found to be optimal for highest frequency (82%) of bud initiation (Table 1). The shoot buds grew normally to an average length of 3 ± 0.3 cm with scanty callus formation from cut ends or along the root axis after a period of three weeks (Figure 1 *c*). However, prolonged incubation of these caulogenic root segments in the same medium resulted in intensive callus formation and inhibited shoot elongation. Three weeks after culture in the optimal medium, when root segments with initiated shoot buds were transferred to half-strength MS basal medium, rapid elongation of both shoot and lateral root initials was observed in addition to the new laterals. These shoots attained a length of 13–14 cm with 8–9 nodes in a period of 3–4 weeks culture in the basal medium and were absolutely healthy and normal (Figure 1 *d*). High concentration of BA (0.5–2.0 mg/l) initiated stunted shoot buds with more callus which was not desirable.

Table 1. Effect of BA in MS medium on shoot bud differentiation on chlorophyllous root segments derived from *in vitro* rooted plants of *H. annulare*

BA (mg/l)	Frequency of response (%)	Mean length of the shoot (cm)	Callusing
0.0	40.6	0.8 ± 0.6	–
0.05	60.3	1.6 ± 0.5	–
0.1	71.5	2.3 ± 0.7	–
0.2	82.0	3.0 ± 0.3	–
0.3	63.8	2.0 ± 0.5	+
0.5	51.7	1.5 ± 0.5	++
1.0	30.3	1.2 ± 0.3	+++
2.0	33.2	0.5 ± 0.8	+++

Observations after 3 weeks; Number of + or – signs indicates the degree of callusing.

Root segments failed to initiate shoot buds in a medium supplemented with auxins alone or in combination with BA. An enhanced induction of lateral roots from cut ends or root axis was observed in a medium supplemented with 0.1 mg/l auxins (IAA, IBA, NAA) alone. However, pale and flaccid shoot formation was noticed occasionally in an IBA-containing medium and they were unhealthy (Figure 1 *e*). The inefficiency of auxins alone for shoot induction on root segments in the present system is similar to the report on *Aegle marmelos*[14]. The presence of surplus endogenous auxins in root segments of the present study as they were taken from auxin-containing rooting medium together with exogenous auxin though in low concentration, probably inhibits the shoot differentiation and promotes lateral root formation. This is also supported by the view that relatively high concentration of auxin suppresses the inception of shoot buds and promotes the growth of lateral roots[15]. The present observation of lateral root formation

on cut root segments may also be due to the loss of apical dominance as there is a hypothesis that root apex also exerts dominance, thereby inhibiting lateral root formation[16]. When the root segments were cultured in a medium with optimal concentration of BA (0.2 mg/l) with 0.1 mg/l auxins (NAA, IAA, IBA) a remarkable decrease in lateral root initiation with a discontinuous production of compact, globular pale green calli along the root axis was observed. Maintaining these explants in the same medium for over 4-weeks or upon transfer to the half-strength MS basal medium did not favour shoot initiation. The present observation is contradictory to the report that a combined effect of cytokinin and auxin was inevitable for shoot initiation on root segments of *Dalbergia sissoo*[17] or enhanced production of shoot buds on root segments of *Aegle marmelos*[14].

Restriction of shoot bud formation to the chlorophyllous portion of the root in *H. annulare* might be due to the fact that chlorophyll in the root provided necessary photosynthase for the energy requirements for shoot bud differentiation. This can be correlated to the study of Thorpe[18] that starch serves as a source of energy for the process of shoot bud differentiation. Bud initiation and its formation uniformly from the proximal or mid portion of the root indicate that some secondary growth also may be essential for shoot initiation as in *Comptonia peregrina*[19].

The inefficiency of the distal root segment with apical meristem of *H. annulare* for shoot bud induction is contradictory to the findings in *Limnophylla* where the shoot bud differentiation on root segments is obligatorily dependent on the presence of active apical meristem[20]. However, the precise role of root tip meristem of *H. annulare* has to be analysed further, because, root tip meristems provide some essential ingredients for the differentiation of shoot buds and have been established as a seat of cytokinin synthesis[21].

When the parental root segments with regenerated shoot and laterals were transferred to the field for acclimatization, 80% survival was obtained by adjusting the humidity conditions inside the mist chamber by removing the polythene covering for 1 h during the first week and increasing the exposure time in subsequent weeks. Further multiplication of shoots using *in vitro* nodes derived from the rhizogenic shoots in MS medium with 0.5 mg/l BAP and 0.05 mg/l NAA supported 8–9-fold multiplication rate. The *in vitro* rooted plants, from which the chlorophyllous roots were harvested for the study, had two or more similar roots which were found to be sufficient for their safe field establishment.

In conclusion, success in the development of a plant regeneration system on excised *in vitro* root segments of *H. annulare* is credited by the following aspects. (a) Since explants were derived aseptically they did not face the problem of contamination and were therefore ideal for germplasm exchange and cryopreservation. (b) The chlorophyllous root used for this study was harvested from a fully developed rooted plant, which had two other roots sufficient for its safe field establishment. (c) The protocol can be considered as an alternative means to enhance the *in vitro* multiplication rate for clonal propagation and is also technically advantageous as it eliminates the stage of rooting prior to deflasking. (d) The system will be useful for biochemical and physiological studies in relation to organ differentiation. (e) The shoots regenerated on root segments can also be used for enhancing the *in vitro* propagation rate.

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