MICROPROPAGATION

Axillary multiplication of *Swertia chirayita* (Roxb. Ex Fleming) H. Karst., a critically endangered medicinal herb of temperate Himalayas

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Abstract The present study describes the micropropagation of *Swertia chirayita*, an endangered medicinal herb of the temperate Himalayas, through axillary multiplication from 4-wk-old seedling-derived nodal explants. We obtained 4.5-fold multiplication every 4 wk on Murashige and Skoog (MS) medium supplemented with 4 μM benzyl amino purine (BA) and 1.5 μM 6-(γ,γ-dimethylallylamino) purine (2iP). Rooting was optimized on modified MS medium supplemented with 1 μM naphthalene acetic acid (NAA) and 500 mg l^{-1} of activated charcoal (AC). A success rate of 94 % was obtained by *in vitro* hardening in the growth-room and by *ex vitro* hardening in greenhouse conditions. The present study can serve as a tool for the mass multiplication of elite genotypes of this critically endangered species and can also be utilized for multiplying large numbers of quality planting material for the replantation in their natural habitat. This approach can also help meet the requirements of the growing pharmaceutical industry by ensuring the regular supply of authentic planting material.

Keywords Conservation · Gentianaceae · Micropropagation

Introduction

Swertia chirayita (Roxb. ex Fleming) H. Karst. (syn. *S. chirata*, *Ophelia chirata*; Fam. Gentianaceae), is an annual/

biennial medicinal herb, native of the temperate Himalayas (Wealth of India 1976; Kirtikar and Basu 1984; Edwards 1993). It inhabits the pastures and slopes in the Himalayas from 1,500 to 3,000 m above sea level. Traditionally, the herb's root serves as a drug and is used for fever, cough, and the common cold. Herbal medicines such as Ayush-64, Diabecon (Himalayan herbal care), and Melicon V ointment (Cadila Parmaceuticals) contain *chiretta* (colloquial name) extract in different proportions for its antipyretic, hypoglycemic, antifungal, and antibacterial properties (Edwin and Chungath 1988; Mitra et al. 1996; Valecha et al. 2000). In spite of its increasing pharmaceutical demand, there is a conspicuous absence of any large-scale commercial plantations of the herb. The low viability and germination percentage of seeds and the necessarily delicate field handling of the seedlings are some of the factors that discourage agrotechnology development and, hence, commercial cultivation of *S. chirayita*. Therefore, the raw material for the herbal industry is being consistently collected from the natural stands of the herb. Destructive harvesting beyond sustainable limits and destruction of the plant's habitat have lead to a decline in numbers and, in some places, extinction of natural populations. It is because of these reasons that *S. chirayita* finds its name among the 32 highly prioritized medicinal plants of India as identified by National Medicinal Plant Board, Government of India (http://www.nmpb.nic.in).

Plant tissue culture provides a viable alternative for managing these valuable resources in a sustainable manner. It serves as an alternative means of secondary metabolite production through cell and organ cultures. Most importantly, micropropagation provides an efficient method for *ex situ* conservation of plant biodiversity and multiplication of the endangered species from a minimum of available plant material. In fact, the technique of micropropagation has

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been effectively employed for the multiplication and conservation of endangered and commercially exploited medicinal plants such as *Picrorhiza kurroa* (Lal et al. 1988), *Aconitum heterophyllum* (Giri et al. 1993), and *Saussurea obvallata* (Joshi and Dhar 2003). However, very few micropropagation studies dealing with large-scale propagation of *S. chirayita* or its allied species have been reported. This situation has remained practically unchanged since the 1970s when *in vitro* culture of *S. japonica*, a species of genus *Swertia* (Miura et al. 1978a,b), was reported for the first time. A few efforts have been made to propagate *chiretta* by *in vitro* techniques. Whereas Wawrosch et al. (1999) described adventitious shoot formation from root explants of *in vitro* germinated seedlings of *S. chirata* Buch. Ham. ex Wall., Ahuja et al. (2003) patented the axillary multiplication of *S. chirayita* from mature explants.

The present communication describes axillary multiplication of *S. chirayita* from nodal explants derived from 4-wk-old aseptically raised seedlings and successful establishment of the plantlets in the field. In this study, shoot clusters obtained from seedling-derived nodal explants gave 4.5-fold multiplication every 4 wk and each cluster resulted in five rootable shoots at the end of the multiplication cycle. The patent report (Ahuja et al. 2003) cited a shoot multiplication rate ranging from 11.4 to 26.2 using single nodal explants. As compared to 50–80% rooting success at the end of the 8-wk inoculation period reported by Ahuja et al. (2003), in the present study, 100% rooting was obtained within 4 wk of shoot inoculation on rooting medium.

Eventually, the rooted shoots from the above experiments were hardened and more than 90% acclimatization success was achieved, both during *in vitro* and *ex vitro* hardening. The plants were finally transplanted to the field station at Mandal (30° 4′N, 79° 1′E) in Uttaranchal, India, and 65.3% transplantation success was achieved in the present study.

Material and Methods

Axillary multiplication from seedling derived nodal explants. Seeds of *S. chirayita* were obtained from the High Altitude Plant Physiology Research Center (HAPPRC), Uttranchal, India. The seeds were soaked overnight in 400 ppm (1.146 M) gibberellic acid (Sigma, St Louis, MO) solution. Surface sterilization was done using 1% sodium hypochlorite for 5 min. The seeds were rinsed with sterile water and then inoculated on semisolid MS (Murashige and Skoog 1962) 1/2 medium (MS major inorganic salts were reduced to half the original MS concentration). Nodal explants derived from 4-wk-old seedlings were inoculated on MS medium supplemented

with 6-benzyl amino purine (BA) or kinetin (Kn) or 6-(γ,γ-dimethylallylamino)purine (2iP) at an equimolar concentration of 3 μM to optimize medium composition for culture establishment. The response from nodal explants (Fig. 1a) was stabilized after the fifth subculture and, by that time, the nodal cultures started forming shoot clusters (Fig. 1b).

Therefore, during the establishment phase, the data was recorded as the number of shoot buds per nodal explant. The shoot clusters derived from nodal explants during the establishment phase were further used to study the synergistic effect of different cytokinins, auxins, and additives in combination with 4 μM BA during the multiplication cycles. Hence, the data was recorded as the number of shoot cluster per mother clusters during the multiplication cycles. A two-factor experiment was designed to study the effect of auxins and activated charcoal on root induction in *S. chirayita*.

The pH of all the nutrient medium was adjusted to 5.8 using 1 N NaOH and 1 N HCl before autoclaving for 15 min at 121°C at 95-atm pressure. The cultures were kept at 25±1°C for 16 h photoperiod with light intensity of 40 μmol m^{-2} s^{-1}, provided by cool, white, fluorescent tubes of 36 W (Phillips, Mumbai, India). The observations were recorded 4 wk after inoculations, both for multiplication and rooting experiments.

Statistical Analysis. The experiments were repeated thrice and each set had 24 experimental units. The data was analyzed by one-way and two-way ANOVA using Costat. Significant differences between the means were assessed by Duncan's multiple range test (DMRT) at $P=0.05$.

Results and Discussion

Axillary multiplication from seedling derived nodal explants. A protocol for the axillary multiplication of *S. chirayita* was established. In the absence of mature authentic plants, 4-wk-old aseptically raised seedlings were used for procuring initial nodal explants for culture initiation.

Seed germination. There are not many reports on studies relating to germination and viability of *S. chirayita* seeds. In one of the reports by Raina et al. (1994), chilling of the seeds at 3°C for 15 d was found to result in 91% germination as compared to 3.4% germination in control seeds. Difficulty in seed germination was also observed in our study. GA$_3$ was found to have a pronounced effect and 80% of the pretreated seeds showed germination. The seeds, however, failed to germinate when not pretreated with GA$_3$.

Figure 1. *S. chirayita* plantlets from *in vitro* multiplication to final transplantation in the field. (*a*) Nodal explant of *S. chirayita* derived from 4-wk-old *in vitro* germinated seedlings. (*b*) Shoot clusters derived from nodal explants after the initial establishment stages on MS medium supplemented with 3 μM BA and 3% sucrose for a culture period of 4 wk. (*c*) An *in vitro* rooted plantlet after a culture passage of 4 wk on rooting medium (1/2 MS+2% sucrose+ 1 μM NAA). (*d*) Shoots with basal callusing showing growth of small, thick, and tuberous roots after inoculation on rooting media without activated charcoal. (*e*) Few plantlets after hardening in greenhouse. (*f*) A bare root micropropagated *chiretta* plant ready for transplantation. (*g*) Micropropagated plants after 6 mo. of growth in the field. An average height of 15 cm, growth area of 0.15 m², and biomass of 106 g was recorded for the plants 6 mo. after transplantation to the field.

Effect of type and concentration of cytokinins on shoot bud induction during culture establishment. Preliminary experiments were conducted to study the effect of equimolar concentrations of BA, Kn, and 2iP with MS basal as control medium on shoot bud induction from nodal explants. The number of shoots induced per nodal explant and the cluster

height were regularly recorded at the end of the 4-wk culture period.

The nodal explants inoculated on MS basal and on MS medium supplemented with 3 μM each of BA, Kn, and 2iP separately showed a significant variation in terms of number of shoot bud induced per explant. A maximum number of 5.9 and 5.8 shoot buds were induced per nodal explant on 2iP and BA-based MS medium, respectively. On the other hand, only 4.9 shoot buds per explant were induced on medium supplemented with an equimolar concentration of Kn. In comparison to the response of nodal explants on media supplemented with cytokinins, only 1.65 shoot buds per explant were formed on basal media. The role of cytokinins in promoting shoot bud induction has also been illustrated in many other studies on medicinal plants. For instance, BA was found most effective for shoot bud induction in *Chlorophytum borivilianum* (Purohit et al. 1994). Similarly, in *Vitex negundo*, Sahoo and Chand (1998) reported BA as the most effective for shoot bud induction and multiple shoot formation. In our study, both 2iP and BA were found to be equally conducive for initial culture establishment and shoot bud induction.

The effect of cytokinins on shoot length was, however, not much pronounced. In fact, during the initial stages of culture establishment, stunted shoot growth was observed and no significant variation in shoot length was recorded on different medium combinations. Nevertheless, by the end of 5–6 culture passages, the clusters attained an average shoot length of 2 to 2.5 cm on cytokinin-supplemented medium.

Effect of different cytokinins on cluster multiplication and elongation. The regulatory action of cytokinin on apical dominance in *in vitro* shoot induction and multiplication has been well-documented (Sachs and Thiman 1964) and was also evident in our results. Shoot clusters obtained from nodal explants during culture initiation were employed to study the effect of different concentrations (0.5 to 10 μM) of BA, Kn, and 2iP on cluster multiplication and elongation. It was observed that cluster proliferation and elongation varied considerably for different concentrations of cytokinins, revealing that the type and concentration of plant growth regulator employed had a significant effect on multiplication rate ($P=$0.0001). Graphical representation of the effect of cytokinins on culture growth and elongation is presented in Fig. 2.

Among the 18 combinations tested, the highest multiplication rate of 3.6 was obtained on medium supplemented with 4 μM BA, whereas, the lowest multiplication rate of 2 was obtained on medium supplemented with 0.5 μM of 2iP. Thus, the superiority of BA over other cytokinins for culture multiplication was established in this experiment. Benzyladenine has often been found to be more effective at stimulating axillary shoot development in many medicinal plants such as *Chlorophytum borivilianum* (Dave et al.

2003), *Rotula aquatica* (Martin 2003), and *Orthosiphon stamineus* (WaiLeng and LaiKeng 2004).

It was of significance to note that optimal multiplication rates were obtained at considerably low concentrations for all the cytokinins tested. The low concentration of cytokinin level reduces the occurrence of adventitious bud formation and the consequent risk of somaclonal variations. This assures propagation of clonally uniform plants as was achieved in the present study (Joshi and Dhawan 2007). Any increase in concentration of cytokinins beyond these concentrations resulted in the decrease in the multiplication rate. For instance, among the six concentrations of BA tested, the highest multiplication rate was achieved at 4 μM concentration and a decline in multiplication rate was noticed with further increase in BA concentration (Fig. 2). A similar trend was also noticed for medium supplemented with Kn and 2iP at varying concentrations. The reduction in the number of shoots generated from each explant at cytokinin concentrations higher than the optimal level has also been reported in *Kaempferia galanga* (Vincent et al. 1992), *Vitex negundo* (Sahoo and Chand 1998), and *S. chirata* (Wawrosch et al. 1999), and this is usually attributed to callusing, adventitious bud formation, and hyperhydricity.

The effect of three cytokinins on cluster elongation was contradictory to that on shoot multiplication. Whereas 4 μM BA produced the highest multiplication rate, the maximum shoot elongation was attained on medium supplemented with 4 μM 2iP. Shoot clusters inoculated on 2iP supplemented medium showed a consistent gain in shoot length as the concentration was raised from 0.5 to 4 μM. However, further increase in 2iP concentration did not enhance shoot growth (Fig. 2). The elongation obtained for six concentrations of 2iP ranged from 2.13 to 2.73 cm. In case of BA supplemented media, these values ranged between 0.9 and 2.6 cm. In case of explants inoculated on Kn supplemented medium, shoot elongation ranged from 1.3 to 1.8 cm for all the six concentrations that were tested. Thus, among the three cytokinins, Kn had the least stimulating effect on shoot elongation.

Synergistic effect of 4 μM BA along with 2iP and Kn on cluster multiplication and elongation. As 4 μM BA produced the most desirable results, both in terms of multiplication fold (3.6) and cluster elongation (2.6 cm), the synergistic effect of 4 μM BA with 2iP and Kn at varying concentrations was studied for cluster multiplication and elongation. The average multiplication rate varied significantly with the type and concentration of cytokinin that was added to the MS+4 μM BA medium ($P=0.0071$). For instance, the maximum multiplication of 4.5-fold was obtained on MS+4 μM BA+1.5 μM 2iP, which was followed by 3.9-fold multiplication on medium

The effect of different cytokinins on cluster multiplication and elongation
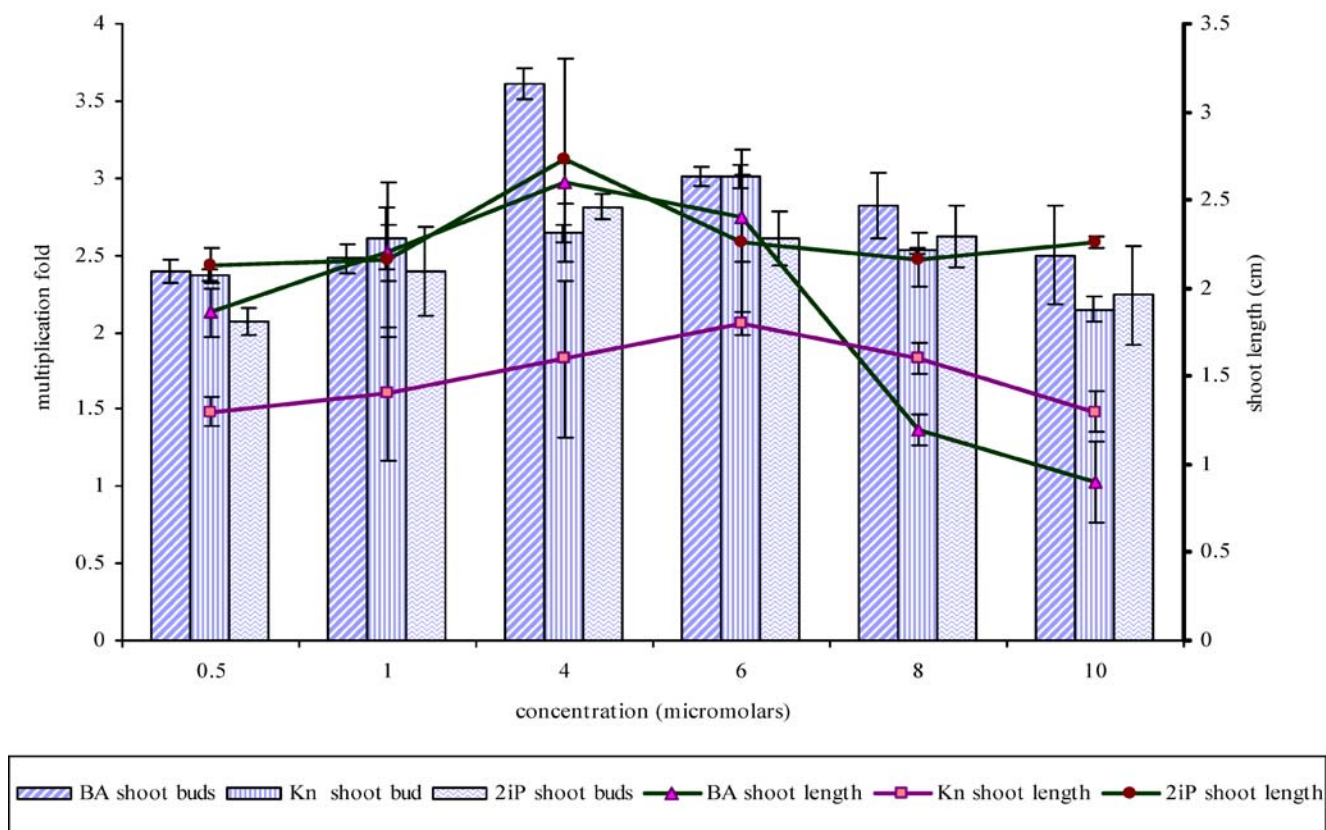

Figure 2. Pictorial representation of the variations in cluster multiplication and cluster height in *S. chirayita* as obtained on the varying concentrations of BA, Kn, and 2iP after 4 wk of culture period. Among the 18 different combinations tested, 4 μM BA produced the most desirable results, both in terms of multiplication fold (3.6) and cluster elongation (2.6 cm).

supplemented with MS+4 μM BA+0.5 μM 2iP (Table 1). However, an increase in concentration of 2iP to 3 and 4 μM did not result in any further increase in the multiplication rate for the tested concentrations. When MS+4 μM BA medium was supplemented with kinetin at different concentrations, the multiplication remained statistically similar to that obtained on MS+4 μM BA medium. At 0.5 and 1.5 μM concentrations of Kn, multiplications of 3.6- and 3.5-fold were obtained, which decreased to 3- and 2.9-fold when the concentrations of kinetin were increased to 3 and 4 μM, respectively. The synergistic effect of different cytokinins in combination with BA on the promotion of shoot multiplication is well-documented for medicinal plants, such as *Kaempferia galanga* (Vincent et al. 1992) and *Piper longum* (Philip et al. 2000). In the present study, we observed that BA along with 1.5 μM 2iP produced the best multiplication of 4.5-fold in *S. chirayita* explants followed by 3.9-fold multiplication rate on MS+ 4 μM BA+0.5 μM 2iP.

Shoot elongation achieved in the above-discussed medium combinations were found to be statistically nonsignificant with *P* value of 0.524. Hence, it can be said that in the present study, the effect of different cytokinin combinations was not significant for shoot elongation in *S. chirayita*. Highest elongation of 2.8 cm was obtained on medium supplemented with 1.5 μM 2iP along with 4 μM BAP, followed by 2.76 cm of cluster elongation obtained on medium combination of 1.5 μM Kn+4 μM BA and 4 μM BA+3 μM 2iP.

Rooting

A two-factor experiment was designed to study the effect of auxins and activated charcoal on root induction in *S. chirayita*. Although a consistent 100% rooting was noted on all the medium combinations supplemented with auxins, almost negligible rooting was induced in shoots inoculated on MS basal medium and the root primordia that were formed gained a maximum length of 0.3 cm even after a culture period of 4 wk. Among the three auxins tested (namely, indole acetic acid (IAA), indole butyric acid

Table 1. Synergistic effect of BA (benzyl amino purine) with varying concentrations of 2iP (6-[γ,γ-dimethylallylamino] purine) and Kn (kinetin) on multiplication rate and elongation in *S. chirayita* shoot clusters after 4 wk of culture period

Values followed by the same letter within the columns are not significantly different at the 5% level of significance according to Duncan's multiple range test.

Cytokinins (μM)			Number of propagules	Length (cm)
BA	2iP	Kn		
4	0.5	–	3.9[ab]	2.2[d]
4	1.5	–	4.5[a]	2.8[a]
4	3	–	3.7[bc]	2.7[abc]
4	4	–	3.7[bc]	2.5[abcd]
4	–	0.5	3.0[cd]	2.2[cd]
4	–	1.5	3.6[bcd]	2.7[abc]
4	–	3	3.5[bcd]	2.4[abcd]
4	–	4	2.9[d]	2.4[abcd]
4	–	–	3.4[bcd]	2.3[bcd]
LSD			0.704	0.40

(IBA), and NAA), MS medium supplemented with 1 μM NAA was most optimal for root induction and, on average, 6.5 roots per shoot were induced in 4 wk, each with an average length of 0.69 cm (Table 2, Fig. 1c). In case of medium supplemented with auxins such as IBA and IAA at 1 μM concentration each, the root length remained more or less similar (0.49 and 0.50, respectively), however, the number of roots induced on IBA supplemented medium (11.5) far exceeded that obtained both on IAA and NAA media (7.8 and 6.5, respectively). Analysis of variance (ANOVA) revealed that the influence of auxins was highly significant both for the number of roots induced ($P<0.001$) and root length ($P= 0.0004$) for each medium combination tested.

This experiment also brought out the influence of activated charcoal on the quality of roots and shoots. The shoots inoculated on medium without activated charcoal developed calluses at the base of the shoot and the roots remained thick, tuberous, and small (Fig. 1d). The addition of activated charcoal improved the quality of roots. The roots formed were longer and thinner (Fig. 1c). The callus observed at the base of the shoots was conspicuously missing in medium combinations supplemented with activated charcoal.

Table 2. Effect of various auxins at 1 μM concentration on shoot length, root length, and root number in *S. chirayita*

Growth regulators (1 μM)	Shoot length (cm)	Root length (cm)	Root number
Basal	2.33[ab]	0.30[c]	2.83[c]
IBA	2.53[a]	0.49[b]	11.5[a]
IAA	2.56[a]	0.50[b]	7.8[b]
NAA	2.26[b]	0.69[a]	6.5[b]
LSD 0.05	0.22	0.14	1.73

Values followed by the same *letter* within the *columns* are not significantly different at the 5% level of significance according to Duncan's multiple range test.

Analysis of variance for the data recorded showed that activated charcoal had a highly significant effect on shoot length ($P=0.0015$) and root length ($P=0.001$). The average root length on medium containing activated charcoal was 0.61 cm compared to 0.37 cm long roots formed on medium without activated charcoal (Table 3). Similarly, shoots inoculated on medium with activated charcoal gained an average length of 2.6 cm compared to 2.2 cm shoots inoculated on medium without activated charcoal. However, the number of roots inoculated on medium with and without activated charcoal (7.7 and 6.6, respectively) remained statistically nonsignificant as was deduced from ANOVA values ($P=0.060$). The interaction of growth regulators and the activated charcoal was found to be nonsignificant for all three variables. The rooting medium thus optimized, consisted of 1/2 MS (major salts and iron reduced to 1/2 of the original MS strength) supplemented with 1 μM NAA and 500 mg l^{-1} activated charcoal. In an earlier study on *S. chirata*, Wawrosch et al. (1999) used considerably higher concentration of auxins (10 μM) for rooting, and this resulted in the induction of short, tuberous roots with callus. They subsequently altered the rooting procedure and rooting was obtained on basal medium after a short pulse treatment of 2 s in 15 ppm (0.08 μM) NAA. Ahuja et al (2003), on the other hand, reported 60–65% rooting in a time duration of 8 wk on MS media supplemented with IAA ranging between 1and 5 mg l^{-1} (5.71–28.57 μM).

In the present study, 1 μM auxin (either IAA, NAA or IBA) was sufficient to induce rooting. However, a thick swollen tuberous growth of roots was evident. This was circumvented by the addition of activated charcoal to the rooting medium.

The addition of activated charcoal results in the adsorption of various organic compounds, such as excess hormones, vitamins, abscisic acid, phenolic metabolites, and ethylene from the growing cultures (Van Winkle et al. 2003), thus leading to their improved growth. The carry-

Table 3. Effect of activated charcoal on root induction in *S. chirayita*

Treatment	Shoot length (cm)	Root length (cm)	Root number
Without activated charcoal	2.58[a]	0.37[b]	7.75[a]
With activated charcoal	2.26[b]	0.61[a]	6.5[a]
LSD 0.05	0.158	0.10	1.22

Values followed by the same alphabet within the *column* are not statistically different at the 5% level of significance according to Duncan's multiple range test.

over effect of plant growth regulators used during the multiplication cycles can lead to difficulty in root induction and can affect the quality of roots as well. Also in the present study, the addition of activated charcoal in the rooting medium, improved the quality of roots and thin, long roots developed directly from the shoot base without an intervening callus. This is most likely attributed to activated charcoal, which is known to eliminate the residual effects of cytokinins applied during the multiplication stage (Maene and Debergh 1985). The effect of activated charcoal on root induction has been previously reported in different plant systems such as *Rollina mucosa* (Figueiredo et al. 2001) and *Curcuma zedory* (Loc et al. 2005).

Hardening. *In vitro* hardening of ex-agar, rooted plantlets was tried in a growth-room under controlled conditions of temperature ($22\pm2°C$), light intensity ($200 \ \mu mol \ m^{-2} \ s^{-1}$) and humidity ranging between 65% and 70% during the months of May and August. The rooted plantlets (Fig. 1*c*) were transferred to culture vessels (6×13 cm in size) containing coir peat and irrigated with quarter strength liquid MS. The jars were tightly covered with lids that helped in maintaining the humidity to 100%, a condition to which the plants were accustomed to in the culture-room. After 2–3 d, the lids were gradually loosened, thus dropping the humidity levels to those in the growth-room (65–70%). This procedure subsequently resulted in *in vitro* hardening of the plants. Within 2 wk, the plants were completely acclimatized and survived in open containers. A good survival rate of 95.5% and 92.7% was observed for the 2 mo.

Hardening in the growth room is, however, not a commercially feasible proposition. The rooted plants were, therefore, taken out during December–April to see the possibility of successful hardening in the mentioned months in greenhouse conditions of controlled temperature ($27\pm2°C$) and humidity gradient of 65% to 80%. A success rate similar to that obtained in the growth-room was also obtained in greenhouse conditions. A total of 3,596 plants were taken out for hardening and a survival percentage of 94.54% was achieved. The study, thus, provides a successful micro-propagation protocol with viable options of *in vitro* and *ex vitro* hardening of the plants.

Ex vitro Performance. There are many reports available in literature that describes the micropropagation of medicinal plants. However, there are very few studies on the *ex vitro* performance of tissue cultured plants up to the field transplantation stage. If laboratory-to-field transfers of tissue culture are to be successful, such studies become imperative. Figure 1*e* and *f* illustrates the hardened plants ready for transplantation and a bare root hardened plant immediately before transplantation in the field, respectively. The micropropagated plants after 6 mo. of growth in the field are shown in Fig. 1*g*. The plants acclimatized initially in May and August were taken to Mandal (Uttranchal, India) for transplantation. Mandal is located at an altitude of 1,568 m above sea level in the Himalayas, and thus, it is climatically suitable for *S. chirayita*, a plant of temperate Himalayas.

Three raised beds (4.5×1.5 m) were made within the polyhouse and hardened plants were transplanted at a plant-to-plant distance of 45×45 cm. An average survival percentage of 65.3% was obtained as recorded after 6 mo. of growth. The reported figure is justifiable considering the drastic mortality observed in the third bed (16% survival, data not shown). It is very likely that the orientation of the plots vis-à-vis the sun-facing side had a definite role in determining the initial survival of the small and tender tissue culture-raised plantlets. The third bed was toward the lower side of the hill, which remained exposed to the sun throughout the day, and this possibly resulted in the high mortality in the initial stages of establishment. The results emphasize the significance of the initial care required while handling the tissue-cultured plants. In the other two plots, a survival of 86% and 80% was observed, respectively. The plants attained an average height of 12–18 cm and the roots grew up to a length of 11–15 cm in the three plots, suggesting healthy growth.

Conclusion

The results of this study connote the successful production of *S. chirayita* plantlets in high numbers, highlighting the role of tissue culture in the multiplication of economically important but endangered medicinal plants. Conservation and cultivation of medicinal plants is important under the

present scenario of the ever-mounting pressures of urbanization on the habitat of the plant, its destructive harvesting, lack of cultivation and agrotechniques. Micropropagation studies become crucial as they provide an alternative to wild harvesting of plants and also become a means of *ex situ* conservation of these medicinal herbs.

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