On Biodiversity, Economics and Protected Areas (Technical Session VII), H. C. Pokhriyal (H. N. B. Garhwal University, Srinagar) highlighted the need to include income generation activity in biodiversity-related studies and proposed a development model for Uttaranchal. Sanjeeva Pandey (Forest Department, HP Government) talked about Women Saving and Credit Groups initiative for conservation and management of biodiversity in protected areas. Sameer Sinha (Forest Department, Uttar Pradesh Government) related biodiversity conservation with ecodevelopment in Uttar Pradesh and focused on the role of women in this activity. S. S. Samant (GBPIHED, Almora) spoke on the role of Lead/Coordinating Institutions in conservation and management of biodiversity of the biosphere reserves.

On Peoples' Participation (Technical Session VIII), Ashish Kothari (Kalpvriksh, Pune) dwelt, at length, on some success stories from Himalaya where conservation and natural resource management have yielded rich dividends. Sejal Worah (WWF-Asia/Pacific, Mussoorie) focused on strengthening linkages between conservation and livelihood and opined that attempts be made to enhance benefits from biodiversity. Shyamala Krishna (CEE, Delhi) while speaking on conservation through education, came up strongly with the need to promote involvement of school and college students in conservation programmes. Ashwini Chhatre (Navrachna, Palampur) dealt with community-led initiatives in the protection and management of forests in HP. Training and awareness modules for biodiversity conservation were presented by Abdesh Gangwar (CEE, North East Regional Cell, Guwahati) and finally Indira Khurana (CSE, New Delhi) spoke on the role of campaign strategies to bring out appropriate changes in biodiversity conservation programmes. She opined that political, financial, technical, social, cultural and knowledge, capitals are prerequisites in mass movement programmes, including biodiversity conservation.

Convention and IPRs (Technical Session IX) was dealt with in detail by A. K. Ahuja (Government of Rajasthan) who highlighted major objectives of the Convention and stressed that dissemination of information was important and could be achieved through collation and compilation of existing work in standard formats. Finally, Ashish Kothari (Kalpvriksh, Pune) spoke on National Biodiversity Strategy and Action Plan (NBSAP) process. He opined that this process would identify issues and problems related to Himalayan biodiversity as well. He also felt that the country needed effective documentation to prevent patents, protection of documents through copyrights and registration of innovative patent systems.

The group discussions, which followed technical sessions, identified key issues under various themes to be taken up on priority. These were further discussed in a final round of group discussions to facilitate the identification of Action Points. Action Points thus finalized will become the basis for the preparation of an Approach Paper on Himalayan Biodiversity.

Among others, K. C. Malhotra, Lalit Pande, S. P. Kulrestha, S. Bhattacharya, E. Sharma, Bansuri Taneja, David Hopkins, James Khargonkar, Shalini Shrivastava, Smita Chaudhary and Lalit Tewari actively participated in Technical Sessions and Group Discussions in various capacities.

ACKNOWLEDGEMENTS. We thank the Director, G. B. Pant Institute of Himalayan Environment and Development, Kosi Katarmal, Almora for encouragement and support. Partial financial assistance from DBT and DST, Government of India, New Delhi, is gratefully acknowledged.

Uppeandra Dhar*, R. S. Rawal, S. S. Samant, Uma Shankar and H. K. Badola, G. B. Pant Institute of Himalayan Environment and Development, Kosi-Katarmal, Almora 263 643, India
*For correspondence.
e-mail: joshidc@vsnl.com

SCIENTIFIC CORRESPONDENCE

High regenerative nature of *Paspalum scrobiculatum* L., an important millet crop

Coarse cereals (millets) have the potential to provide food and nutrition as well as ensure sustainability of poor farmers in fragile ecosystems. *Paspalum scrobiculatum* L. (Kodo millet) is an important millet crop cultivated almost throughout India. It forms an important component of dry land, tribal and hilly agriculture. It is recommended for diabetic persons as a substitute for rice and has medicinal and insecticidal properties, which are uncommon and relatively unknown to modern societies[1]. Therefore genetic improvement of this crop will have a great effect on the socio-economic status of the people in the rural areas, where it is widely cultivated. The application of the biotechnological approaches to the Gramineae relies on the availability of reproducible and reliable regeneration procedure. Mostly plants have been regenerated through somatic embryogenesis from several explants of many graminious species[2]. Morphogenetic responses gradually decline with increase in the age of the culture and number of subcultures[3]. Establishment, selection and preferential culture methodology of embryogenic callus have been recognized as critical factors in long-term morphogenetic potential in cultures. Most of the transformation procedures published recently, have relied on inducing long-term morphogenetic potential after repeated subculture, and it is critical for successful transformation. The higher transformation rates are likely due to the induction of long-term morphogenetic callus[4]. There are a few reports of somatic embryogenesis of the genus *Paspalum*, that include

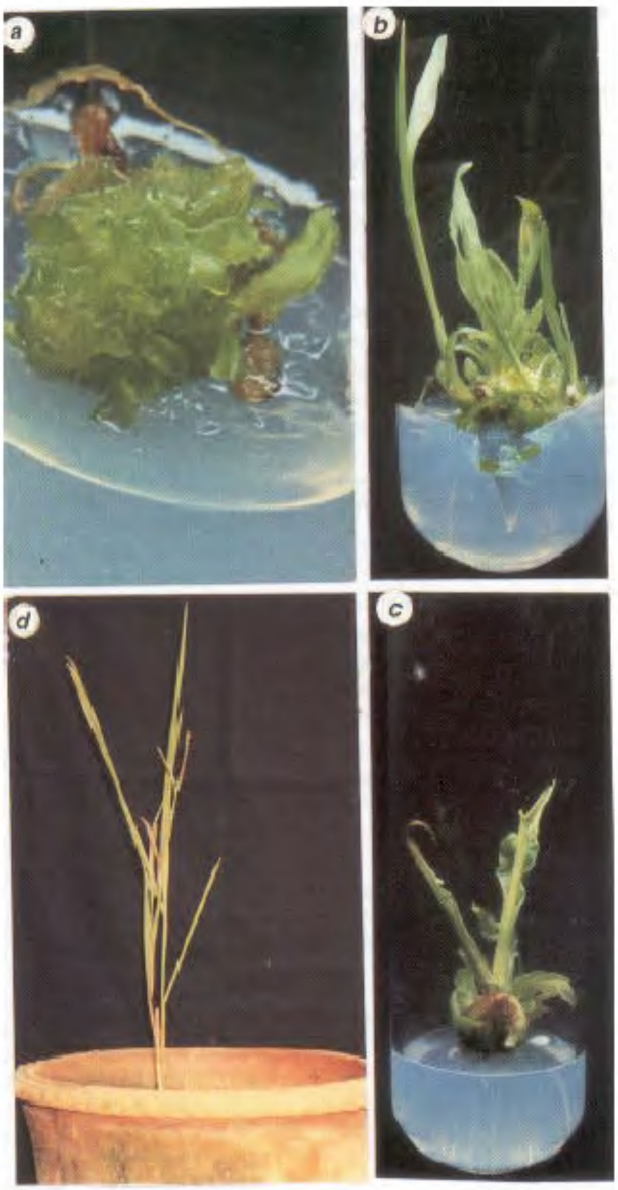

Figure 1. ***a***, Shoot apex-derived callus showing differentiation of shoots (0.5 cm); ***b***, Elongation of regenerated shoots; ***c***, Regenerated shoots showing root induction; and ***d***, Regenerated fertile plants in the pot.

P. notatum[5,6], *P. dilatatum*[7] employing embryonic explants or through protoplasts of immature inflorescence in *P. scrobiculatum*[8]. However plant regeneration from callus cultures has not been reported so far. The present work deals with plant regeneration from short-term and long-term callus cultures derived from shoot apex explants.

Seeds of *P. scrobiculatum* L. (cv. Co 4) were obtained from Tamil Nadu Agricultural University, Coimbatore. They were washed with 0.1% (v/v) detergent solution of Tween-20 for 10 min and thoroughly washed with running tap water. Then the seeds were surface sterilized with 70% ethanol for 3 min, 0.1% $HgCl_2$ (w/v) for 6 min and rinsed with sterile double-distilled water 7–8 times. MS basal medium[9] containing 3% sucrose and 0.75% agar (Himedia, Mumbai) was used for seed germination. Seeds cultured on this medium were incubated at 27 ± 2°C with the photoperiod of 16 h light and 8 h dark for five days. Shoot apices were dissected and cultured on MS medium containing 3% sucrose, 0.75% agar and 2,4-D (4.5, 9.0, 13.5 and 18.1 µM) for callus induction. For each treatment 10 explants were cultured and the experiment was repeated at least three times. The callus was subcultured on callus induction medium once every month and was maintained over a period of three years. The regeneration ability of the long-term callus cultures was also tested at different time intervals.

For plant regeneration, the calli were transferred to regeneration medium containing MS with 3% sucrose and 0.75% agar supplemented with different concentrations of BAP (0.44–11.07 µM) singly and in combination with NAA (0.53 µM). The regenerated shoots were transferred to MS medium containing different concentrations of indolebutyric acid (IBA, 0.1–2.0 µM) for root induction. The pH of the medium was adjusted to 5.8 prior to autoclaving at 1.4 kg/cm^2 for 15 min. All the cultures were incubated at 27 ± 2°C and given a light and dark cycle of 16/8 h. The fully developed plantlets were transferred to paper cups containing sterile vermiculite, covered with polythene bags and kept in the culture room for three weeks. The plants were irrigated with Hoagland solution[10] for two weeks and subsequently with tap water. Polythene bags were removed from the cups at the end of the third week and plants were transferred to earthen pots containing garden soil after four weeks.

The first evidence of callus initiation was noted from the basal portion of shoot apex after ten days of culture on MS medium supplemented with 2,4-D (4.5, 9 and 13.5 µM) and in five weeks of culture a good amount organogenic callus was obtained. In evaluating the influence of various concentrations of 2,4-D on callus induction medium, it was observed that 11.25 µM of 2,4-D was found to be optimal for callus induction than the other concentrations (4.5, 9 and 13.5 µM). The callus was compact, nodular, white and embryogenic in nature. The frequency of callus formation was higher (60%) at 11.3 µM of 2,4-D. Induction of embryogenic callus from shoot apex explants was also reported in finger millet and fox-tail millet[11,12]. For maintenance of callus for three years, 9.0 µM 2,4-D was optimum to induce embryogenic callus from the shoot apex explants. The degree of cellular organization and shoot meristems in this millet can be controlled by manipulations of 2,4-D concentration, as reported in wheat[13].

Plant regeneration was achieved by transferring the plants to regeneration medium and it was characterized by the development of green pigment sectors distributed discretely throughout the callus.

Table 1. Morphogenetic response of shoot apex explants of *P. scrobiculatum* on MS medium supplemented with different concentrations of BAP, alone and in combination with NAA

BAP (μM)	NAA (μM)	Explant formed shoots (%)	Mean no. of plantlets/ explant callus
0.0	0	0.0	0.0
0.44	0	26.6	3.3 ± 1.05^{a}
2.21	0	33.3	5.0 ± 1.05^{acf}
4.43	0	63.3	9.4 ± 1.89^{b}
6.64	0	46.6	7.5 ± 2.07^{bcf}
8.86	0	43.3	3.2 ± 1.93^{a}
11.07	0.53	16.6	4.9 ± 1.66^{ag}
2.21	0.53	28.3	6.4 ± 1.5^{cfg}
4.43	0.53	76.6	15.4 ± 3.27^{d}
6.64	0.53	50.0	12.6 ± 2.98^{e}
8.86	0.53	40.0	5.6 ± 2.06^{fg}

Values followed by the same alphabet do not differ significantly at $P < 0.05$ according to One Way ANOVA test and means were separated by LSD; data were scored after four weeks.
Values are in mean ±SD.

Table 2. Effect of age of callus in regeneration frequency of *P. scrobiculatum* on MS medium supplemented with 4.43 μM BAP, alone and in combination with 0.53 μM NAA after culture for one month

Age of the callus in months	No. of culture produced shoots (%)	Mean no. of shoots/explant callus
2	88.3	13.8 ± 1.31^{a}
4	80	10.6 ± 1.3^{b}
6	80	10.4 ± 1.57^{b}
12	70	8.0 ± 1.49^{c}
18	70	7.3 ± 1.33^{c}
24	65	6.0 ± 1.15^{d}
30	55	6.1 ± 1.19^{d}
36	45	5.1 ± 1.37^{d}

Values followed by the same alphabet do not differ significantly at $P < 0.05$ according to One Way ANOVA Analysis and data were scored after four weeks. Means were separated by LSD.
Values are in mean ± SD.

This led to differentiation of shoots (Figure 1 *a*). Heyser and Nabors[14] reported that green pigment production in the callus is required for the growth and the formation of shoots in oat. BAP in the MS medium induced shoot regeneration with a concentration range of 0.44–11.07 μM, alone and in combination with NAA (0.53 μM). The number of shoots per explant varied with respect to different concentrations of BAP, either alone or in combination with NAA (Table 1). However, the combination of 4.43 μM BAP and 0.53 μM NAA was found to be optimal among the concentrations tested, in which maximum number of shoots per explant was produced (Table 1). The findings are in agreement with those of Lambe *et al.*[15]. Loss of regeneration capacity, increase of albinism and lack of fertility are usually associated with prolonged period of cell/callus cultures[16,17]. In contrast to these findings, the calli retained morphogenic potential and were competent for plant regeneration as long as three years (Table 2). In this study, occurrence of albinism and fertility problems were not identified. However the regeneration frequency was found to decrease with increase in the age of the callus (Table 2). For the development of a good root system, the elongated shoots (Figure 1 *b*) were transferred to MS basal medium with or without IBA (0.49–9.81 μM) and at a concentration of 0.49 μM complete plantlets with well-developed roots were obtained (Figure 1 *c*). Out of 142 plants, 112 (78.8%) survived, flowered and set seeds (Figure 1 *d*). This reproducible regeneration protocol will be useful for the genetic transformation of this crop.

1. Roy, B. and Pal, D. C., Abstracts, Fourth International Congress of Ethnobiology, Lucknow, 1994, vol. 29, pp. 17–21.
2. Vasil, I. K., *Bio/Technology*, 1988, **6**, 397–402.
3. Lürhs, R. and Lörz, H., *Planta*, 1988, **175**, 77–81.
4. Zhang, L., Rybczynski, J. J., Langenberg, W. G., Mitra, A. and French, R., *Plant Cell Rep.*, 2000, **19**, 241–250.
5. Bovo, O. A. and Mroginski, L. A., *Plant Sci.*, 1989, **65**, 217–223.
6. Marousky, F. J. and West, S. H., *Plant Cell Tissue Org. Cult.*, 1990, **20**, 125–129.
7. Akashi, R. and Adachi, T., *Plant Sci.*, 1992, **82**, 213–218.
8. Nayak, P. and Sen, S. K., *Plant Cell Rep.*, 1989, **8**, 296–299.
9. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
10. Hogland, D. R. and Arnon, D. I., *Calif. Agric. Exp. Stn., Bull.*, 1950, 347.
11. Pius, J., George, L., Eapen, S. and Rao, P. S., *Proc. Indian Natl. Sci. Acad.*, 1950, **B60**, 53–56.
12. Osuna-Avila, P., Nava-Cedillo, A., Jofre-Garfias, J. E. and Cabrera-Ponce, J. L., *Plant Cell Tissue Org. Cult.*, 1995, **40**, 33–35.
13. Sears, R. G. and Deckard, E. L., *Crop Sci.*, 1982, **22**, 546–550.
14. Heyser, W. J. and Nabors, M. W., *Z. Pflanzenphysiol.*, 1982, **107**, 153–160.
15. Lambe, P., Mutabel, H. S. N., Deltour, R. and Dinant, M., *Plant Cell Tissue Org. Cult.*, 1999, **55**, 23–29.
16. Fellers, J. P., Guenzi, A. C. and Taliaferro, C. M., *Plant Cell Rep.*, 1995, **15**, 232–237.
17. Altpeter, F., Vasil, V., Srivastava, V., Stoger, E. and Vasil, I. K., *Plant Cell Rep.*, 1996, **16**, 12–16.

Received 25 October 2000; accepted 15 December 2000

S. AROCKIASAMY
S. PRAKASH
S. IGNACIMUTHU*

Entomology Research Institute,
Loyola College,
Chennai 600 034, India
**For correspondence.*
(e-mail: eri_lc@hotmail.com)