respectively. In Table 1 the emission from India is 8.26×10^{11} g and in Table 2 it is 1.22×10^{12} g. Such a discrepancy arises because of the weightage of average for the whole rice-growing area as against estimation for each region separately. The above calculations have the following important features which make them overestimates rather than underestimates:

1. During the entire period of growth the maximum methane emission for a period of 10–15 days accounts for 90% of the total emission. This 10–15 days period is the period of maximum biomass. The present calculations have considered 30 days emissions with the maximum biomass obtained from grain yield values. This is for a crop of 90 days duration. Thus, these estimates are in fact overestimates.
2. The average yield of a country is not a weighted average based on productivity of the area. For example, eastern India has an average yield of 1171 kg ha^{-1} for a rice area of 18.4 million hectare. North India has an average yield of 2283 kg ha^{-1} yield from an area of 8.4 million hectare. The average of these yield averages is 1722 kg ha^{-1} for the two regions. The emission from 18.3 million hectare will increase by 50% but would be reduced by only 20% for an area of 8.4 million hectare. Thus, the estimates based on larger area are likely to be higher.

Theoretical consideration

Methane production in a reducing environment is accomplished by methanogenic bacteria using a source of carbon. When the rice plant is attributed as the source of methane, we are assuming that all the required carbon source (carbohydrate) would have been supplied by the plant to the rhizosphere. Assuming that this supply is as glucose, the following reactions would occur:

$$C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 \quad (1)$$

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2O_2 \rightarrow 2CH_4 + 4CO_2 + H_2O. \quad (3)$$

Thus, for two molecules of methane to be produced one molecule of glucose would be needed. This means that for 32 g methane to be produced, 180 g glucose would be required. When we consider the reports of 50 g m^2 methane release, it means that 281 g m^2 glucose would have been provided. This amount of glucose would be equal to 225 g m^2 dry matter or 2250 kg ha^{-1} (300 g m^2–400 g m^2) dry matter. In all those regions where biomass production is only 3000–4000 kg ha^{-1} such a conclusion would be theoretically impractical. Normally, in most studies only 10–15% of the total assimilate is assigned to root growth, which would include leaching from roots also. Thus, the estimates of 20 g m^2 methane release or above would be impractical to achieve theoretically.

Conclusions

This study brings out estimates of methane emission based on biomass as an important means of assessing global methane emissions. On this basis it is estimated that the annual global methane emission from rice paddies would be 7.08×10^{12} g as against 110×10^{12} g stated in IPCC report[8]. Therefore, there is a need to make more realistic measurements of methane in situations where rice grows. Extrapolations of results from California and Europe have serious limitations from agricultural viewpoint.

This study emphasizes that it is likely that we are missing an important source of methane for which more efforts are needed.

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Received 3 November 1992, revised accepted 6 October 1994

A low-cost strategy for *in vitro* propagation of banana

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A simple low-cost method for micropropagation of banana has been standardized. To reduce the cost of production of tissue-cultured bananas, multiplication of shoots was carried out on a medium prepared with tap water and commercial-grade sugar as the carbon source. Shoot tips grown on such a medium showed

multiple shoot formation (4–5 shoots/culture). Rooting and further growth of shoots into plantlets was achieved on Knop's salts solution supplemented with NAA. Shoot tips encapsulated in sodium alginate were also grown on similar media and plantlets were developed. The plantlets were successfully established in soil.

BANANA is propagated vegetatively by suckers. However, this method is seriously limited by its low rate of multiplication. Several attempts have been made to increase the number of suckers[1–3] but the rate of increase has been only marginal and hence commercial production of planting material in some of the elite varieties of banana has not succeeded. In the recent years tissue culture propagation of banana through shoot tip as well as floral apices has been demonstrated successfully[4–7]. The major limitation in employing tissue culture technique on a commercial scale is the high cost of production per plant compared to suckers and the appearance of off-type plants in the progeny. As labour and media constitute more than half the cost of a tissue culture operation, in the present study an attempt has been made to reduce the cost of media by using tap water, commercial-grade sugar and minimizing the salt components for plantlet production.

Field-grown plants of banana cv. Basrai (AAA) – a leading commercial dwarf variety in India – were used as the experimental material. Shoot tip cultures were established for the induction of multiple shoots. The procedure for establishing the cultures and encapsulation of shoot tips has been given in an earlier report[8]. Multiple shoot cultures were established on Murashige and Skoog's[9] (MS) medium supplemented with 2 mg/l benzyl aminopurine (BA). The individual shoots isolated from such cultures were used for experiments Multiplication of shoots was carried out on a medium supplemented with BA (2 mg/l) and either sucrose or commercial-grade sugar (3%) as a carbon source. The media were prepared either in distilled water or in tap water. The composition of tap water[10] was: total dissolved solids 70 mg/l, $CaCO_3$ 42 mg/l, Mg 4 mg/l, Ca 10 mg/l, K 0.5 mg/l, Na 4 mg/l, Si 16 mg/l, Fe 0.1 mg/l, Cl_2 5 mg/l and SO_4 2 mg/l. For obtaining complete plantlets, the following media – (i) MS salts solution; (ii) Knop's salts solution (250 mg/l KNO_3, 1000 mg/l $Ca(NO_3)_2$, 250 mg/l, $MgSO_4 \cdot 7H_2O$, 250 mg/l KH_2PO_4) with MS minor, iron and vitamins; or (iii) Knop's salts solution alone – were used along with sucrose or commercial-grade sugar (3%), 1 mg/l naphthalene acetic acid (NAA) and 0.1% activated charcoal. The media were prepared either in distilled water or in tap water. For each treatment 24 shoots were used and the experiments were conducted under controlled conditions of light (1000 lux), temperature (25 ± 2°C) and relative humidity (50–60%).

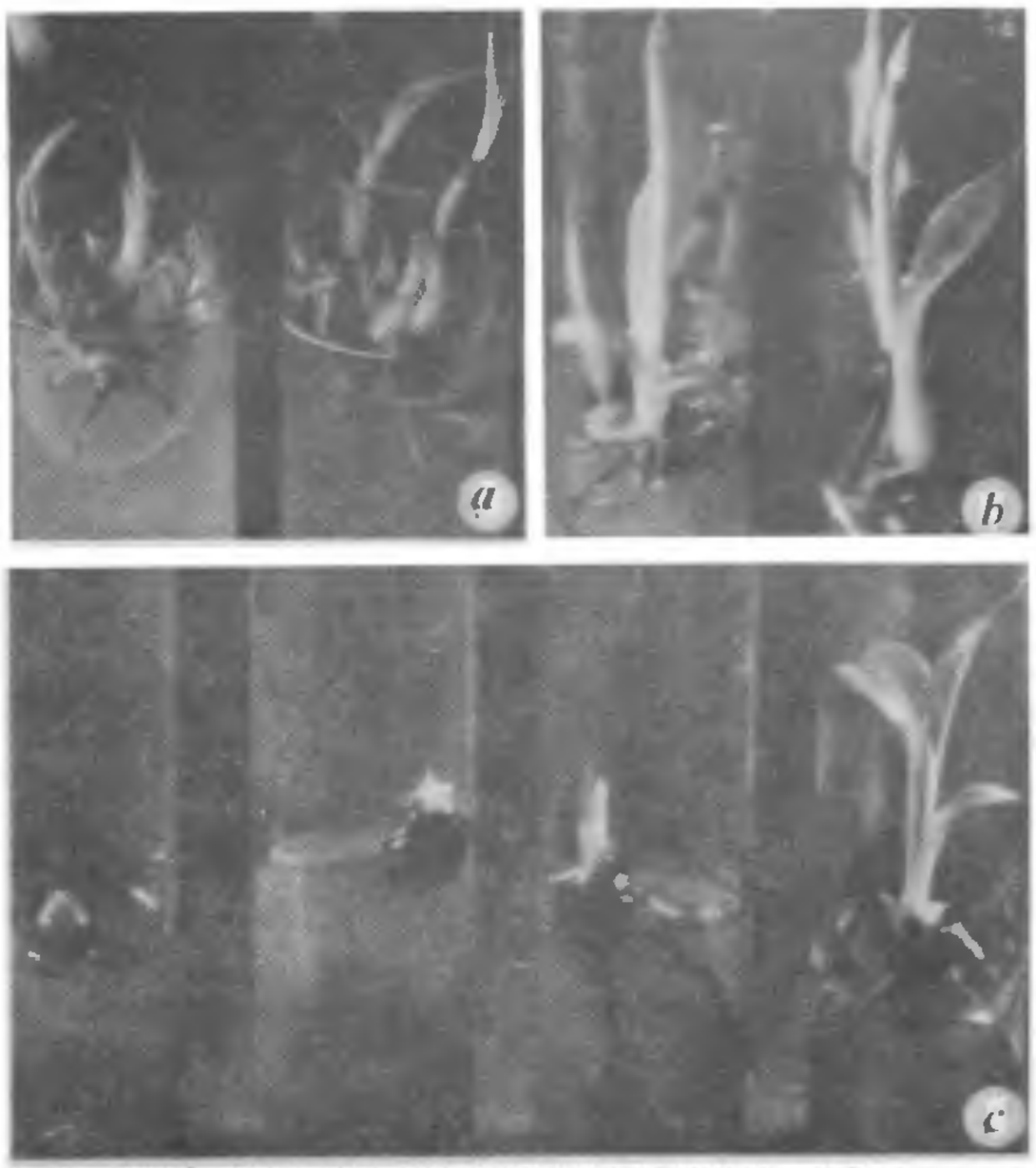

Figure 1. *a*, Multiple shoot formation on MS + 2 mg/l BA prepared with commercial-grade sugar. *b*, Plantlet development on Knop's salts solution + 1 mg/l NAA with commercial-grade sugar. *c*, Encapsulated shoot tips developing into plantlets on Knop's salts solution + 1 mg/l NAA with sucrose *d*, Hardened banana plants in polybags in the greenhouse.

Individual shoot tips were excised from the multiple shoot cultures and were subcultured on MS medium (prepared either in distilled water or in tap water) supplemented with BA (2 mg/l) and 3% sucrose or commercial-grade sugar. The shoot tips grown on the above media prepared in distilled water containing sucrose or commercial-grade sugar showed the formation of multiple shoots, 4–6 shoots per culture (Figure 1 *a*) in four weeks in 100% of the cultures. There was no significant change in the frequency of multiple shoot formation on both types of media. On a similar medium prepared in tap water too the frequency of response was 100%. The

Table 1. Effect of different media on plantlet formation from shoot tips of banana

Medium + carbon source (3%) + NAA (1 mg/l)	Cultures forming plantlets (%)	No. of roots/ plantlet	Plantlet height after 4 weeks (cm)
A MS basal salts +			
(i) sucrose/commercial-grade sugar in distilled water	100	2–4	7–9
(ii) sucrose/commercial-grade sugar in tap water	95	2–3	7–8
B *Knop's salts + MS minor,* iron and vitamins +			
(iii) sucrose/commercial-grade sugar in distilled water	85	2–3	6–8
(iv) sucrose/commercial-grade sugar in tap water	85	2–3	6–8
C. Knop's salts +			
(v) sucrose/commercial-grade sugar in distilled water	75	2–3	6–7
(vi) sucrose/commercial-grade sugar in tap water	75	2–3	6–7

Data obtained after 4 weeks; 24 explants per treatment.

growth of the multiple shoots on tap-water-prepared medium was, however, comparatively slow and the sub-culture passage was extended to 6 weeks; this may be attributed to the composition of tap water.

To develop complete plants, elongated shoots (5–6 cm in length) were isolated and cultured on either one of the following media – (1) MS medium; (2) Knop's salts solution along with MS minor, iron and vitamins; or (3) Knop's salts solution alone – along with NAA (1 mg/l) + 0.1% activated charcoal and 2% sucrose or commercial-grade sugar. The media were prepared either in distilled water or in tap water.

The shoots cultured on MS medium prepared in distilled water with either sucrose or commercial-grade sugar showed considerable elongation and 2–4 roots with laterals were noticed after three weeks. The frequency of plantlet formation on both the media was 100%. However, on a similar medium prepared in tap water the shoots showed slow growth and the frequency of plantlet formation was 95%.

Plantlets were also developed (85%) on medium containing Knop's salts along with MS minor, iron and vitamins, with sucrose or commercial-grade sugar, and prepared either in distilled water or in tap water (Table 1). Even on Knop's salts solution alone with sucrose or commercial-grade sugar prepared with distilled water or tap water, 75% of the shoots developed into plantlets (Figure 1 *b*).

The encapsulated shoot tips were grown on different basal media containing either sucrose or commercial-grade sugar, prepared in distilled water or tap water, 1 mg/l NAA and 0.1% activated charcoal (Figure 2). MS medium prepared in distilled water and supplemented with 3% sucrose and 1 mg/l NAA showed 100% plant conversion. Plantlets of transplantable stage were ob-

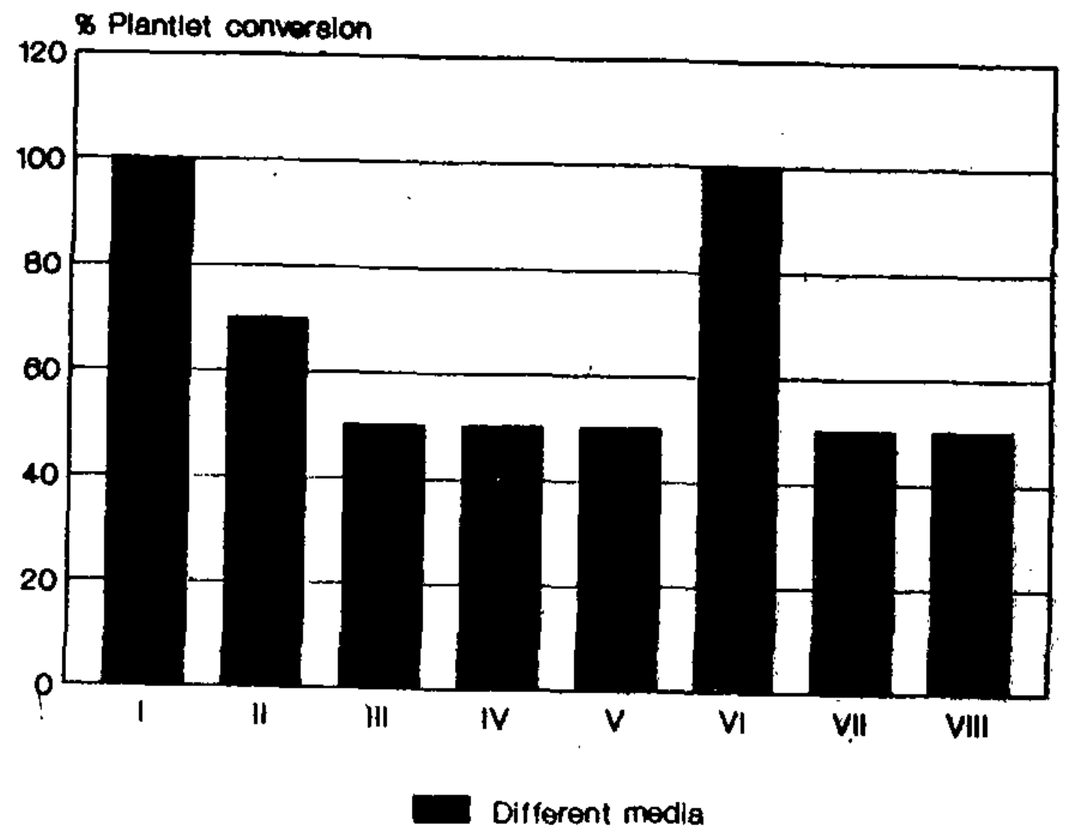

Figure 2. Effect of different media on plantlet conversion from *encapsulated shoot tips* of banana Sucrose/commercial-grade sugar (3%) + 1 mg/l NAA. 24 shoot tips per treatment were taken.

I – MS basal salts + sucrose in distilled water/tap water.
II – MS basal salts + commercial-grade sugar in distilled water.
III – MS basal salts + commercial-grade sugar in tap water.
IV – Knop's salts solution + MS minor, iron, vitamins + sucrose in distilled water/tap water.
V – Knop's salts solution + MS minor, iron, vitamins + commercial-grade sugar in distilled water/tap water
VI – Knop's salts solution + sucrose in distilled water.
VII – Knop's salts solution + commercial-grade sugar in distilled water.
VIII – Knop's salts solution + sucrose/commercial-grade sugar in tap water.

Table 2. Cost-effectiveness of different media used for banana *in vitro* propagation

Medium used	No. of plantlets anticipated per year (in lakhs)[a]	Cost/plantlet (media-based) (Rs)
MS + sucrose + DW	3.75	0 45
MS + CG sugar + DW	3 75	0 42
MS + sucrose + TW	3 56	0 46
MS + CG sugar + TW	3 56	0 43
MS + Knop's[b] + sucrose + DW	3 18	0 53
MS + Knop's[b] + CG sugar + DW	3.18	0 50
MS + Knop's[b] + sucrose + TW	3.18	0 52
MS + Knop's[b] + CG sugar + TW	3 18	0 48
Knop's[c] + sucrose + DW	2.81	0 36
Knop's[c] + CG sugar + DW	2 81	0.33
Knop's[c] + sucrose + TW	2 81	0 35
Knop's[c] + CG sugar + TW	2 81	0.31

DW. distilled water, TW: tap water, CG commercial grade
[a]The initial explants used were 100 shoot tips
[b]Knop's salts + MS minor, iron and vitamins were employed at the rooting phase.
[c]Only Knop's salts were employed at the rooting phase
The subculture regime was 4–6 weeks

tained after 4 weeks. However, if sucrose was replaced with 3% commercial-grade sugar, the conversion frequency was brought down to 70%. On MS medium of the same constitution prepared in tap water and with 3% sucrose the frequency was 100%, while with 3% commercial-grade sugar it was 50%. Encapsulated shoot tips grown on Knop's salts solution with MS minor, iron and vitamins prepared in distilled water or tap water and supplemented with NAA (1 mg/l) and 3% sucrose or commercial-grade sugar showed 50% conversion frequency. Interestingly, Knop's salts solution alone prepared in distilled water and fortified with 3% sucrose and 1 mg/l NAA showed a 100% conversion (Figure 1 *c*), whereas on similar media with 3% commercial-grade sugar the conversion frequency was 50%.

The objective of this investigation was to develop an *in vitro* method for the micropropagation of banana by substituting sucrose and distilled water with commercial-grade sugar and tap water, respectively, and minimizing the use of chemical components of the nutrient media (Tables 1 and 2). In banana, though the tissue-cultured plantlets have advantages over suckers, cost-wise they are 5–10 times more expensive. To minimize the cost of tissue-cultured plantlets, various strategies have been used in many plant species[11–15].

In *Leucaena leucocephala* the optimum level of sucrose for shoot cultures was 4% and it was found that 'Analar' grade (AR) sucrose can be successfully replaced by a commercial form of table sugar (sugar cubes) of sufficient purity, which is ten times cheaper than the AR grade sucrose. The micronutrients and organic salts included in the MS medium were also not essential for growth and multiplication of *Leucaena* shoots. Further, it is stated that the omission of $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$ and KI did not affect the rate of multiplication, and among the organic nutrients only thiamine was found to be essential[16]. Similarly, in the present study on banana tissue cultures, sucrose was replaced with commercial-grade sugar, and for the preparation of media, instead of distilled water, clean tap water was used. Though all the components of MS medium were found to be necessary for initial shoot multiplication, rooting and development of plantlets from shoot tip as well as from encapsulated shoot tips was readily achieved on Knop's salts solution with NAA and commercial-grade sugar. An overall consideration of the cost-effectiveness of the various media shows the superiority of Knop's salts alone for plant conversion, followed by MS media prepared either with commercial-grade sugar or with tap water (Table 2). We believe that these modifications in the medium would be eventually useful in reducing the total cost involved in the tissue culture production of bananas.

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ACKNOWLEDGMENT We thank Mr R. M Mudaliar for photography

Received 19 January 1994, revised accepted 1 October 1994

Plants regenerated from protoplasts of sugarcane (*Saccharum officinarum*)

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Protoplasts were isolated from calli derived from leaf spindles of sugarcane cultivar CoJ-76, released for commercial plantation. The purified protoplasts cultured in Kao and Michayluk (KM) medium were provided gradually reduced osmoticum by addition of amino acids-containing KM medium at weekly intervals. Regeneration of plants required the developing microcalli to be initially cultured on callus proliferation medium, followed by Murashige and Skoog (MS) basal plant regeneration medium. The regenerated plants were transferred to modified MS liquid medium for shoot proliferation and root development. Plants with well-developed roots were successfully transferred to soil for further development.

THE regeneration of complete plants from protoplasts is one of the most reliable ways to introduce foreign gene(s) for the production of transgenic plants in monocots. Regeneration of plants from protoplasts has been reported in several cereal crops like rice[1–6], wheat[7,8], barley[9] and oat[10].

In sugarcane, however, this system is limited due to poor release of good-quality, round-shaped protoplasts[11], the release of polyphenolic compound(s) from cultures[12] and difficulties in regeneration of plants from protoplasts of sugarcane[13–15] In this study, we have overcome the problems of release of (i) low numbers of good-quality protoplasts by identification of the callus type and (ii) release of polyphenolic compound(s) by use of amino acids in Kao and Michayluk (KM) medium[16] during osmoticum reduction of cultured protoplasts.

The materials for this study comprised sugarcane cv. CoJ-76, released for commercial plantation in the state of Punjab. The calli were obtained from the innermost young unfurled leaf spindle, cultured on MS medium[17] supplemented with 3.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.2 mg/l 6-benzylaminopurine (BAP). These cultures were maintained in the dark at 26 ± 1°C. The calli were separated according to morphological appearance and maintained by regular subculture on MS medium supplemented with 2.0 mg/l, 2,4-D and 0.2 mg/l BAP at every 15 days intervals.

Four–five-month old calli were used for isolation of protoplasts. Two grams of eight-day-old subcultured calli were incubated in a 90 mm Petri dish containing 15 ml of an enzyme mixture of 3% Cellulase 'Onozuka' *RS (Yakult Honsha Co. Ltd., Japan)*, 1% Macerozyme R-10 (Yakult Honsha Co. Ltd., Japan), 0.5% Pectolyase Y-23 (Seihin Pharmaceutical Ltd., Japan), 5 mM MES buffer and CPW salts[18] with 13% mannitol. The mixture was kept on a gyratory shaker and maintained at a speed of 50 rpm for 10 h, followed by 2 h stationary incubation at 26 ± 1°C in the dark. After incubation, the mixture was passed through a set of 64, 45 and 30 µm pore size sterile nylon mesh. The relatively uniform isodiametric protoplasts were obtained by sucrose density gradient technique[3]. The purified protoplasts were washed thrice in CPW salts with 13% mannitol and collected by pelleting at 1000 rpm. The viability of purified protoplasts was 76% when it tested by trypan blue. The protoplasts were cultured on agarose bed at a density of 1×10^6 protoplasts/ml in KM medium[16] supplemented with 0.8 M glucose and 0.15% agarose (Seaplaque, LGT, FMC, Rock Land, USA). The culture dishes were sealed with parafilm and incubated in the dark at 26 ± 1°C.

The agarose bed was prepared in KM medium supplemented with 0.4 M glucose and 0.8% agarose. The osmoticum of protoplast cultures was reduced at 8 days by feeding 0.5 ml of KMA medium (prepared by addition of amino acids of AA medium[19] and 0.5 M glucose in KM medium[16] instead of NH_4NO_3 and KNO_3). This was followed by KMA medium with 0.2 M glucose at weekly intervals. After 40 days of culture, the protoplast-derived calli were transferred on to callus proliferation medium (MS medium supplemented with 3.0 mg/l 2,4-D, 0.2 mg/l BAP, 30 g/l sucrose and 0.8% agarose) for 15 days, followed by transfer on to modified MS medium (MS salts with various combinations and concentrations of auxins with kinetin (e.g. 0.5 mg/l NAA and 3.0 mg/l kinetin, 1.0 mg/l NAA and 3.0 mg/l kinetin, 0.5 mg/l IAA and 3.0 mg/l kinetin, 1.0 mg/l IAA and 3.0 mg/l kinetin, 30 g/l sucrose and 0.8% agarose) for differentiation of calli. The cultures initially maintained in the dark for a week were transferred to a 16/8 h light/dark regime using an assortment of fluorescent light (2500 lux) at 26 ± 1°C. 8–10 cm plantlets dif-