Acc.77–170. The QTL identified in this study did not overlap with this major salt-tolerant gene[10], indicating elite or novel loci for salt tolerance. In anticipation of its significant contribution and pleiotropic nature, the QTL on chromosome 6 may contain a new major gene for salt stress tolerance at seedling stage in rice. This needs to be confirmed by conducting field trials in saline soils for two or three seasons to test if the QTL are stable across seasons and growth phases of the crop. Chromosome substitution at this loci can be done after fine-mapping and back-crossing to desired lines.

Rice breeders are resorting to molecular marker technology for developing salt-tolerant varieties, as traditional breeding practices, many a times, turned out to be difficult exercise in tackling complex traits. QTL mapping is the first step in applying marker technology 'o the molecular breeding programme. QTL identified by this technique, after fine-mapping, could be used for indirect selection of salt-tolerant traits to be used in MAS.

1. Yeo, A. R. and Flowers, T. J., in *Salinity Tolerance in Plants–Strategies for Crop Improvement* (eds Staples, R. C. and Toenniessen, G. H.), Wiley-Interscience Publication, Canada, 1984, pp. 151–170.
2. Guiderdoni, E., Gallinato, E., Luistro, J. and Vergara, G., *Euphytica*, 1992, **62**, 219–224.
3. Huang, N., McCouch, S. R., Mew, T., Parco, A. and Guiderdoni, E., *Rice Genet. Newsl.*, 1994, **11**, 134–137.
4. Zapata, F. J., Alejar, M. S., Torrizo, L. B., Novero, A. U., Singh, V. P. and Senadhira, O., *Theor. Appl. Genet.*, 1991, **83**, 6–11.
5. Prasad, S. R., M Sc (Agri.) thesis, UAS, GKVK, Bangalore, 1998.
6. Lander, E. S. and Botstein, D., *Genetics*, 1989, **121**, 185–199.
7. Lincoln, S., Daly, M. and Lander, E., *Mapping Genes Controlling Quantitative Traits with MAPMAKER/QTL 1.1.* (Version 1.1b), Whitehead Institute Technical Report, 1992, 2nd edn.
8. McCouch, S. R., Cho, Y. G., Yano, M., Paul, E., Blinstrub, M., Morishima, H. and Kinoshita, T., *Rice Genet. Newsl.*, 1997, **14**, 11–13.
9. Bagali, P. G., M Sc (Agri.) thesis, UAS, GKVK, Bangalore, 1997.
10. Zhang, G. Y., Guo, Y., Lin, C. S. and Chen, S. Y., *Plant Sci. Limerick*, 1995, **2**, 227–234.

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Somatic embryogenesis and plantlet regeneration in Amrapali and Chausa cultivars of mango (*Mangifera indica* L.)

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Somatic embryogenesis has been obtained from nucellus of two monoembryonic Indian mango cultivars 'Amrapali' and 'Chausa'. Among the four auxins (IAA, IBA, NAA & 2,4-D) tested, only 2,4-D stimulated callus initiation and induction of proembryogenic callus in cultured bisected ovules containing nucellus minus zygotic embryos. The proembryogenic calli produced up to 130 somatic embryos when transferred to 2,4-D-free medium. The presence of 2,4-D in the medium inhibited progression of development of somatic embryos. The best medium for the production, development and maturation of somatic embryos was the modified M4E medium which contained full-strength B5 macrosalts, MS microsalts, MS iron-EDTA and MS organics along with 400 mg/l L-glutamine, 6% (w/v) sucrose and 0.8% (w/v) agar. The mature somatic embryos gave rise to plantlets in liquid medium containing half-strength B5 macrosalts and 1.0 mg/l GA_3. The *in vitro* raised plantlets of Amrapali cultivar have been successfully transplanted in earthen pots containing garden soil, but those of Chausa failed to survive in the garden soil but have been established in pots containing sand and soil (3:1) mixture.

MANGO is a prized summer fruit crop of India with over one thousand recognized varieties consumed as fresh fruit or variously processed. Mango pickles constitute an important ingredient of the poor man's daily meal. The wild trees are a source of timber. India is a major producer of monoembryonic mango cultivars, many of which are esteemed for the high quality fruit and considered far superior to the polyembryonic counterparts[1]. Cultured tissues of mango are recalcitrant to plantlet regeneration. Any biotechnological approach, aimed at improvement of plant quality and yield, requires an efficient plant regeneration system[1]. Somatic embryogenesis has been reported in several mango cultivars (most of which are polyembryonic); yet reports on the development of plantlets from somatic embryos are limited to a few cultivars[1,2]. As the responses are cultivar-dependent, the methods may not be applicable to other varieties[2]. Serious efforts have not been made to utilize the advantages of tissue culture for enhancing the availability of planting material of Indian monoembryonic

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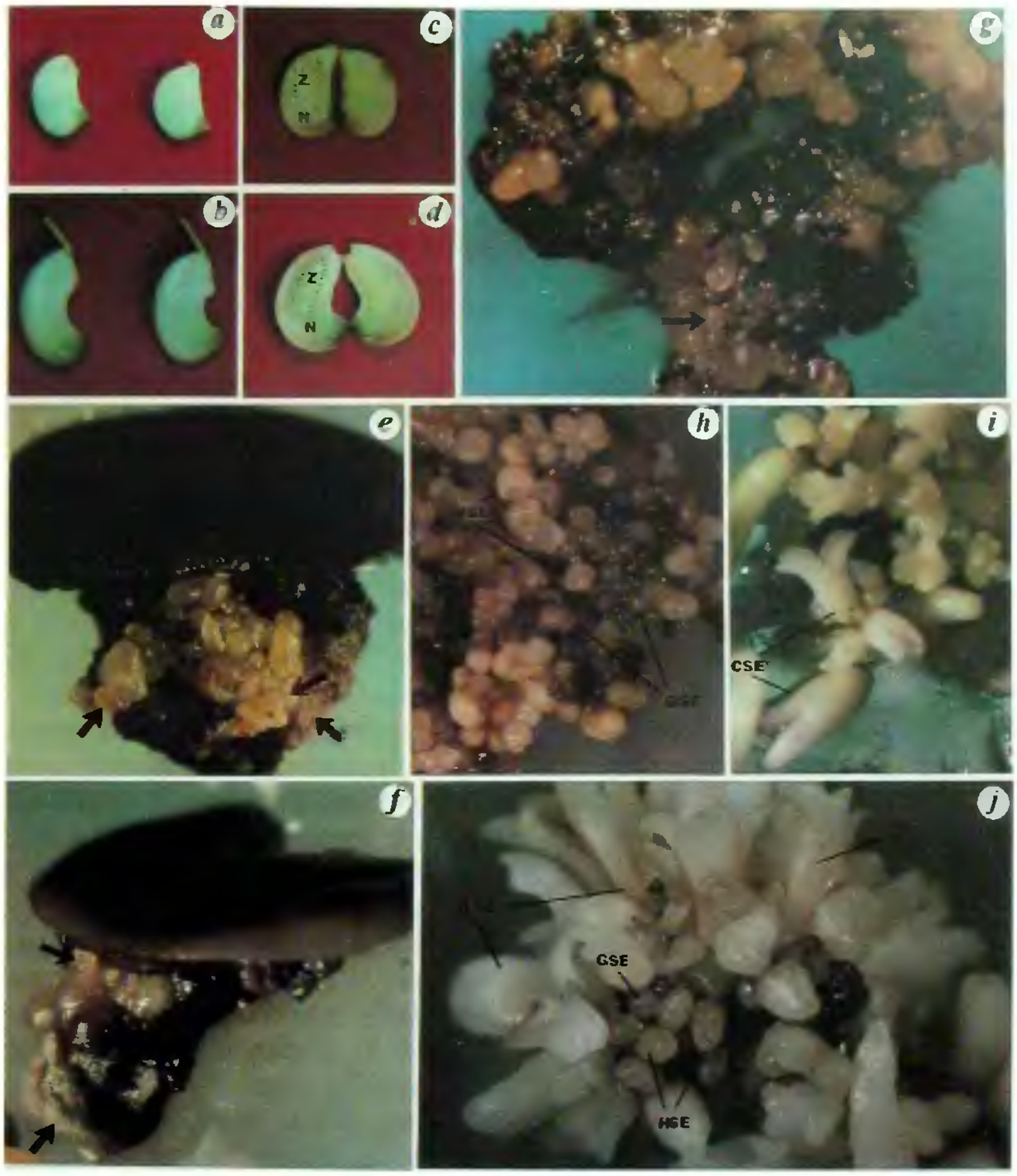

Figure 1. Somatic embryogenesis from nucellus of Amrapali and Chausa cultivars of mango; *a*, *b*, Isolated whole ovules of Amrapali and Chausa (× 1.5) respectively; *c*, *d*, Ovules cut open lengthwise and the halves placed side by side (c Amrapali; d Chausa) showing nucellus and zygotic embryo (both × 1.5); *e*, *f*, Side view of ovular halves (*e* Amrapali; *f* Chausa) showing light-yellow coloured PEC (arrows) arising from dark-brown nucellar calli (both × 10); *g*, Initiation of globular somatic embryos (arrows) from PEC of Chausa (× 10); *h*, Globular and early heart-shaped somatic embryos produced from PEC of Amrapali (× 10); *i*, *j*, Later stage somatic embryos of Chausa and Amrapali (both × 10). [N, nucellus; Z, zygotic embryo; GSE, globular somatic embryo(s); HSE, heart-stage somatic embryo(s); CSE, cotyledonary-stage somatic embryo(s)].

mangoes. In this paper we present the results of our efforts to develop a protocol for plantlet regeneration through somatic embryogenesis from nucellus of highly prized monoembryonic mango cultivars 'Amrapali' (a hybrid variety evolved at IARI, New Delhi, in 1978 as a result of a cross between Dashehari and Neelum)[3] and 'Chausa' (a superior chance seedling variety)[3] in which production of uniform rootstocks and other planting materials are needed for large-scale clonal multiplication.

Young fruits of Amrapali (2.0–3.5 cm long) and Chausa (1.5–2.5 cm long) were collected from the Horticultural Garden, Institute of Agricultural Sciences, BHU, Varanasi. They were surface-sterilized with 0.1% (v/v) sodium hypochlorite, 2 or 3 drops of Tween-20 and 3% (v/v) cetrimide (ICI, India Ltd.) solution in tap water for 20 min. Thereafter, the fruits were opened and ovules were isolated under aseptic conditions and rinsed with 0.05% (w/v) $HgCl_2$ solution for 3 min with gentle shaking. After washing with sterilized double distilled water (3 or 4 times) the ovules were bisected longitudinally (Figure 1 *a–d*). The zygotic embryos were discarded from each ovule and the intact ovular halves containing nucellar tissue were placed in a nutrient medium such that the nucellus was in direct contact with the medium. Initially the explants were inoculated in

150 × 25 mm test tubes containing agar-gelled M4 medium with various concentrations (0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0 and 10.0 mg/l) of auxins (2,4-D, NAA, IAA or IBA) for initiation of callus and induction of proembryogenic callus (PEC). The M4 medium used in the present work contains half-strength MS macrosalts and iron-EDTA with full-strength MS microsalts and organic supplements, 400 mg/l L-glutamine, 100 mg/l ascorbic acid, 6% (w/v) sucrose, and 0.8% (w/v) agar. This was further modified (details are given in Table 2). pH of the medium was adjusted to 5.80 ± 0.05 prior to autoclaving at 108 kPa and 121°C for 15 min. At least 12 explants were used for each treatment and the experiment was done three times.

All the cultures were maintained in complete darkness at 25 ± 2°C. To avoid necrosis of the explants with phenolic exudates, the explants of both the cultivars were transferred to fresh medium of the same composition at one or two-day intervals in the first week of inoculation and later at seven to ten day intervals. The number of explants showing callus initiation and PEC induction were recorded 5 and 8 weeks after inoculation respectively for each treatment and their per cent frequency was calculated.

The explants showing PEC induction after 8 weeks of starting the culture were transferred to medium of the same composition and kept in darkness at 25 ± 2°C to promote further proliferation of PEC. With the lapse of three weeks the PEC (approximately 10 mg/test tube) was isolated and subcultured on media of different composition (see Table 2) with or without 1.0 mg/l 2,4-D to study the differentiation and development of somatic embryos (SEs). The number of somatic embryos of each stage (globular, heart and cotyledonary) were recorded after four weeks in each treatment. Thereafter the somatic embryos (late heart stage/early cotyledonary stage) were allowed to mature on a medium containing full-strength B5 macrosalts with full-strength MS microsalts, MS iron-EDTA, MS organic supplements, 400 mg/l L-glutamine, 6% (w/v) sucrose and 0.8% (w/v) agar. The 9-week-old somatic embryos (cotyledonary stage) having opaque, white and thick cotyledons, measuring 8 mm or more in length were transferred to germination medium containing half-strength B5 macrosalts with full-strength each of MS microsalts, MS iron-EDTA and MS organic supplements, 400 mg/l L-glutamine, 3% (w/v) sucrose, 1.0 mg/l GA_3. Both liquid and semi-solid (with 0.6% w/v agar) conditions were provided in the culture medium. Each treatment had at least 30 somatic embryos. The cultures were exposed to 16 h photoperiod (60 μE m^{-2} s^{-1} light intensity provided by cool white fluorescent tubes, Philips) at 25 ± 2°C. The number of germinated somatic embryos (which showed emergence of tap root) were counted after four weeks and the latter transferred to a liquid medium of the same composition but without GA_3 to stimulate growth of root and shoot since the medium containing GA_3 inhibited growth of these. During the next 4 weeks, the number of plantlets developed in each treatment was counted. The percentage of germinated and converted (those that bore shoots and roots) SEs was estimated.

The well developed plantlets were washed and transferred to plastic pots containing a mixture of sand and soil (collected from Botanical Garden, BHU) in the ratio of 3:1 under artificial light (60 μE m^{-2} s^{-1} light provided by cool white fluorescent tubes) at 25–30°C. Initially the plantlets were irrigated with tap water and covered with individual polyethylene bags to maintain high humidity. After 20 days, the pots were transferred to sunlight for a short duration (30 min) each day for about one week. Gradually the daily exposure time was increased. The polyethylene bags were removed after 30 days, the plants were transferred to the earthen pots containing only garden soil and kept under sunlight.

One-way or two-way analysis of variance (ANOVA) was used to analyse the significance of factors at 1% or 5% probability level and comparison among means of specific pairs of treatments was made by the least significant difference (LSD) test wherever ANOVA was found to be significant.

Table 1. Effect of concentration (conc.) of 2,4-D on frequency of callus initiation and PEC induction from nucellus explants of Amrapali (A) and Chausa (C). (Data represent mean ± SE of three independent experiments.)

	Callus initiation (%)		PEC induction (%)	
2,4-D conc. (mg/l)	A	C	A	C
0.0	0.00	0.00	0.00	0.00
0.1	9.72 ± 6.37	2.78 ± 2.41	0.00	0.00
0.5	15.28 ± 4.81	5.56 ± 2.40	2.78 ± 2.41	0.00
1.0	54.16 ± 11.79	27.78 ± 6.37	37.12 ± 12.27	15.28 ± 6.37
1.5	12.50 ± 4.17	5.56 ± 2.40	2.78 ± 2.41	1.39 ± 2.41
2.0	6.95 ± 4.81	4.17 ± 0.00	0.00	0.00
2.5	2.78 ± 2.41	2.78 ± 2.41	0.00	0.00
5.0	0.00	0.00	0.00	0.00
10.0	0.00	0.00	0.00	0.00
$LSD_{1\%}$	13.97	6.25	19.27	8.51

Table 2. Effects of the type of medium and 2,4-D on production and development of SEs from PEC of Amrapali (A) and Chausa (C). (Data collected 4 weeks after subculturing of PEC and pooled from three independent experiments.)

Medium*	Total number of SEs/10 mg PEC		Cotyledonary stage SEs (%)	
	A	C	A	C
M4A	67.82 ± 3.37	62.14 ± 6.20	8.18 ± 3.19	5.78 ± 3.17
M4A + 2,4-D (1.0 mg/l)	49.58 ± 1.97	48.16 ± 2.82	2.25 ± 1.50	1.36 ± 0.59
M4E	128.33 ± 3.82	129.56 ± 3.14	27.28 ± 4.16	15.28 ± 2.26
M4E + 2,4-D (1.0 mg/l)	87.44 ± 4.40	84.55 ± 4.17	6.22 ± 3.06	5.18 ± 1.50
Modified M4E	130.44 ± 3.21	129.55 ± 5.28	63.68 ± 2.71	41.18 ± 3.60
Modified M4E + 2,4-D (1.0 mg/l)	90.58 ± 3.36	83.52 ± 5.20	16.42 ± 1.39	15.44 ± 3.36
ANOVA results				
d.f.	5, 12	5, 12	5, 12	5, 12
F	639.79^S	562.22^S	234.46^S	98.23^S
Probability level	1%	1%	1%	1%
LSD	4.46	4.20	1.98	0.96

S, Significant; *Composition of the medium; M4A (i.e. M4 medium without ascorbic acid) = MS macrosalts and MS iron-EDTA (both half-strength) + MS microsalts + MS organics (both full-strength) + 400 mg/l L-glutamine + 6% (w/v) sucrose; M4E = B5 macrosalts, MS microsalts and MS organics (full-strength each) + MS iron-EDTA (half-strength) + 400 mg/lL-glutamine + 6% (w/v) sucrose; Modified M4E = B5 macrosalts + MS microsalts + MS iron-EDTA and MS organics (full-strength each) + 400 mg/l L-glutamine + 6% (w/v) sucrose.

Among 2,4-D, IAA, NAA and IBA only 2,4-D stimulated callus initiation and induction of PEC in the nucellus of ovular halves of both Amrapali and Chausa cultivars. The explants inoculated on medium supplemented with IAA, NAA and IBA became black and did not show any further response. Callusing of the nucellus occurred in 3–5 weeks only on the medium supplemented with 0.1 to 2.5 mg/l 2,4-D (Table 1). Higher concentrations (5.0 and 10.0 mg/l) of 2,4-D caused total inhibition (Table 1). The initial callus was dark-brown or black and moist. From these calli pale-yellow or cream-coloured, shiny and translucent proembryogenic calli (PEC) (Figure 1 *e*, *f*) initiated in the next 2–3 weeks on fresh medium of the same composition. Nevertheless, PEC was not induced in all explants which showed initiation of dark-brown or black callus. Thus, the per cent frequency of callus initiation and PEC induction varied among treatments (Table 1). PEC induction from nucellar callus of Amrapali was noted on medium supplemented with 0.5 to 1.5 mg/l 2,4-D, whereas from the nucellar callus of Chausa PEC induction occurred only in response to 0.5 and 1.0 mg/l 2,4-D (Table 1). The percentage of PEC induction was highest on a medium supplemented with 1.0 mg/l 2,4-D in both the cultivars (Table 1). The variation in per cent frequency of callusing [($F_{Amrapali} = 19.91$; d.f. = 5, 12); ($F_{Chausa} = 26.68$; d.f. = 5, 12)] and PEC induction [($F_{Amrapali} = 15.24$; d.f. = 2, 6); ($F_{Chausa} = 13.87$; d.f. = 2, 6)] in response to 2,4-D concentrations was significant ($P < 0.01$) in both the cultivars.

Simultaneously with proliferation, the 3–5 weeks old PECs of both the cultivars showed formation of shiny, transparent to translucent, cream-coloured, fleshy and compact embryogenic calli which produced globular somatic embryos (Figure 1 *g*, *h*) from one week onwards. Development of globular somatic embryos into heart- and cotyledonary-stage was asynchronous. Thus somatic embryos of all developmental stages (i.e. globular, heart and cotyledonary-stage) were observed in the cultures (Figure 1 *i*, *j*). Depending upon the composition of the medium and the presence or absence of 2,4-D, the production (i.e. total number of somatic embryos) and development of SEs varied from globular to cotyledonary stage. The PEC of both the cultivars was able to produce up to 50 SEs on M4A medium which contained MS macrosalts and 2,4-D (1.0 mg/l); nevertheless the SEs rarely reached the cotyledonary stage (Table 2). A slight increase in the number of SEs and the percentage of those developing into cotyledonary stage was observed on medium of the same composition in the absence of 2,4-D (Table 2). However, approximately twice the number of SEs were produced on M4E and modified M4E medium (which contained full-strength B5 macrosalts with half- and full-strength MS iron-EDTA respectively) along with 2,4-D (1.0 mg/l) as compared to M4A medium containing half-strength MS macrosalts and MS iron-EDTA with 2,4-D (1.0 mg/l) (Table 2). Highest production and development of SEs into cotyledonary stage was observed on M4E and modified M4E medium without 2,4-D (Table 2). Presence of MS iron-EDTA in half- or full-strength with full-strength B5 macrosalts in M4E and modified M4E media respectively had no influence on the production of SEs (Table 2). A significant effect on the develop-

Table 3. Effects of physical state of the medium on germination and conversion of SEs of Amrapali (A) and Chausa (C). (Data represent mean ± SE of three independent experiments.)

Physical state of germination medium	Germinated SEs (%)		Converted SEs (%)	
	A	C	A	C
Semi-solid	12.67 ± 1.42	6.82 ± 1.50	0.00	0.00
Liquid	71.56 ± 3.14	51.56 ± 4.12	41.67 ± 3.36	26.62 ± 1.27

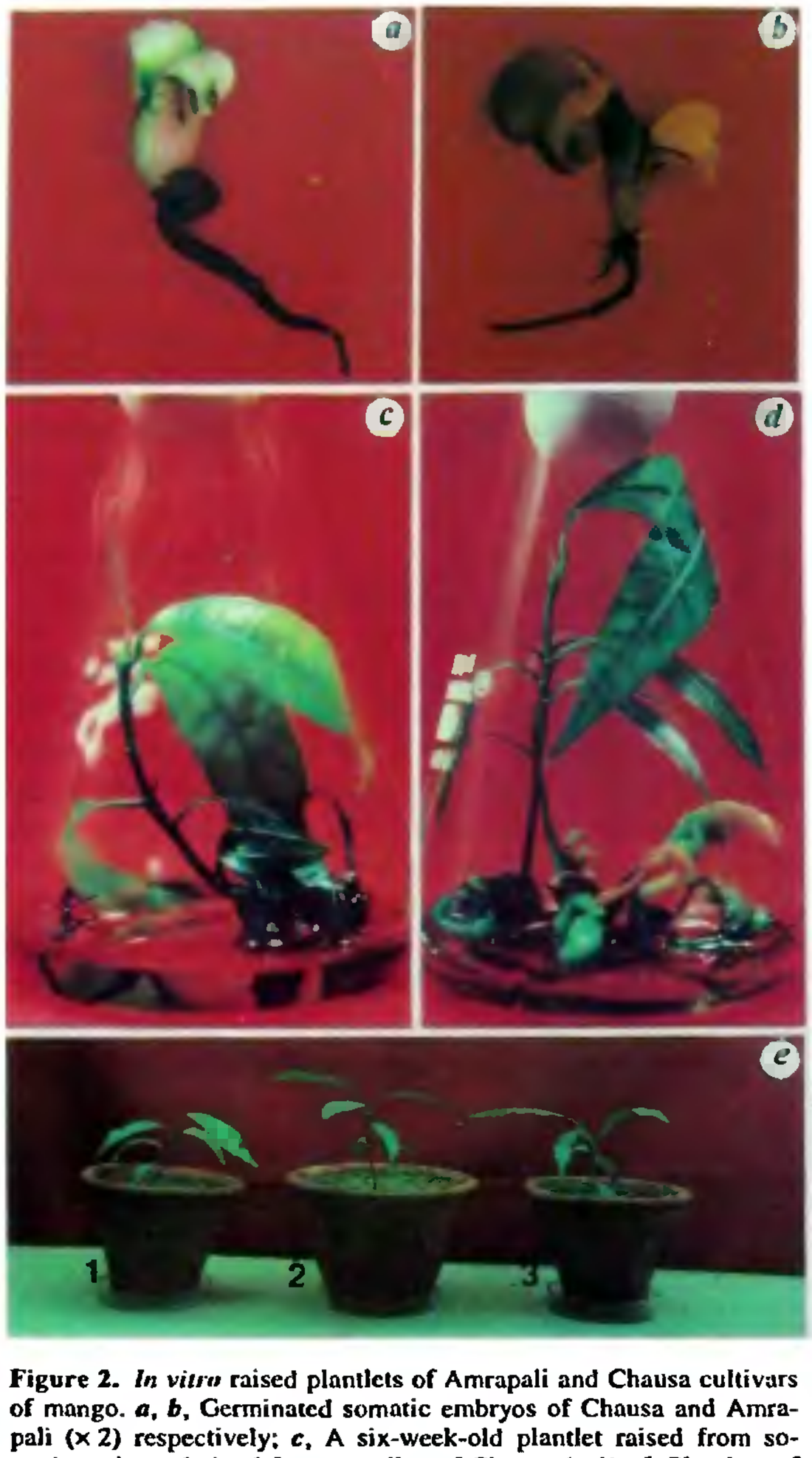

Figure 2. *In vitro* raised plantlets of Amrapali and Chausa cultivars of mango. *a*, *b*, Germinated somatic embryos of Chausa and Amrapali (× 2) respectively; *c*, A six-week-old plantlet raised from somatic embryo derived from nucellus of Chausa (× 1); *d*, Plantlets of Amrapali similar to that shown in *a* (× 1); *e*, *In vitro*-raised plantlets of Amrapali (1, eight-month-old; 2, 3, twelve-month-old) after establishment in pots (× 0.18).

ment of SEs into cotyledonary stage was observed as the percentage of cotyledonary stage was highest on modified M4E medium which contained full-strength MS iron-EDTA along with B5 macrosalts (Table 2).

The cotyledonary stage SEs showed variation in the number of cotyledons and the length of cotyledons and hypocotyl. On modified M4E medium the cotyledonary stage SEs of both the cultivars, irrespective of morphological variations showed increase in length of the cotyledons and/or hypocotyl. With increase in size, the translucent and soft cotyledons became opaque and firm on the same medium. In a period of 9 weeks the cotyledonary stage SEs reached a length of approximately 1.0 to 1.5 cm, and subsequently germinated (Figure 2 *a*, *b*) and became converted into plantlets (Figure 2 *c*, *d*). Germination and conversion of SEs into plantlets were significantly ($P < 0.01$) affected by the physical state of the medium. On the semi-solid medium germination was poor and no plantlet developed. However, there was a marked enhancement in germination and conversion into plantlet when liquid medium was employed in both the cultivars (Table 3).

The plantlets of both the cultivars survived when transferred to plastic pots containing sand and soil (3:1 ratio) mixture. The plantlets of Amrapali cv. showed better survival (14 out of 30) than those of the Chausa cv. in which blackening of the shoot tip caused death of 16 out of 20 plantlets. The plantlets of Chausa that survived, neither showed increase in their shoot length nor bore any new leaf when transferred to garden soil, whereas those of Amrapali showed further growth (Figure 2 *e*). The growth of the plantlets of Amrapali cv. was slow, new leaves developed at intervals of approximately 6 weeks with the internode elongating up to 1–3 cm. The leaves were morphologically similar to those of adult trees of the same variety growing in the orchard. Size of leaves of survived plantlets of Amrapali cv. could reach only up to 5.0 × 1.5 cm in pots containing a sand soil (3:1) mixture. When these plantlets were transferred to earthen pots containing garden soil and exposed to sunlight, new leaves developed which could grow to a bigger (12.0 × 2.5 cm) size.

It is evident from the results that it is possible to induce somatic embryogenesis and to recover plantlets in mango cultivars 'Amrapali' and 'Chausa'. Until now the monoembryonic mango cultivars in which somatic embryogenesis has been reported include 'Irwin', 'Ruby' and 'Tommy Atkins' of Florida, USA[4] and 'Alphonso', 'Mundan', 'Baneshan' and 'Arka anmol' of India[5,6].

In the present study somatic embryogenesis occurred through the intervention of a callus phase. Litz *et al.*[7] have described that the developmental pathway of somatic embryogenesis varies according to the seed type (monoembryonic/polyembryonic) of mango. According to them, from explanted nucellus of monoembryonic mango a transient embryogenic callus is formed that very quickly differentiates to form proembryogenic masses, whereas, the explanted nucellus of polyembryonic mangoes forms somatic embryos directly from embryogenically competent cells already present in the explant. However, in *Citrus* spp. (both polyembryonic and monoembryonic) somatic embryogenesis in the cultured nucellus follows a repetitive callus-free pattern[8].

It is apparent from the present study that an inductive phase (through 2,4-D) is essential for establishing embryogenic cultures of monoembryonic mango. A similar requirement has also been reported by Litz and coworkers[2,7]. Despite the crucial role of 2,4-D in inducing somatic embryogenesis, its continued presence deters embryo development into cotyledonary stage in both Amrapali and Chausa cultivars of mango. According to Zimmerman[9], new gene products are needed for the progression from the globular to the heart-stage and these new products are synthesized only when an exogenous auxin is removed.

The present study shows that transfer of somatic embryos in liquid medium is essential for converting them into plantlets. Although Ammirato[10] has stated that the physical state of the medium has little effect on embryogenesis, there are instances in which maturation and germination of SEs are affected by this factor as in *Dioscorea opposita*[11] and *Musa* spp.[12].

Acclimatization is the final and the most important requirement in every micropropagation scheme, albeit difficult. The results of present study indicate that the *in vitro*-raised plantlets of Amrapali cv. have been successfully transferred to the garden soil. However, the two serious problems confronted were low survival and slow growth of the plantlets. Hence, further attention is required to circumvent these after transplantation.

1. Litz, R. E. and Gray, D. J., *Biotechnology of Perennial Fruit Crops* (eds Hammerschlag, F. A. and Litz, R. E.), CAB International, Wallingford, UK, 1992, pp. 3–34.
2. Litz, R. E. and Lavi, U., in *The Mango* (ed. Litz, R. E.), CAB International, Wallingford, UK, 1997, pp. 401–423.
3. Majumder, P. K. and Sharma, D. K., in *Fruits: Tropical and Subtropical* (eds Bose, T. K. and Mitra, S. K.), Naya Prokash, Calcutta, 1990, pp. 1–62.
4. Litz, R. E., *HortScience*, 1984, **19**, 715–717.
5. Jana, M. M., Nadgauda, R. S., Rajmohan, K. and Mascarenhas, A. F., *In Vitro Plant*, 1994, **30**, 55–57.
6. Thomas, P., *J. HortSci. Biotech.*, 1999, **74**, 135–139.
7. Litz, R. E., Mathews, V. H., Moon, P. A., Pliego-Alfaro, F., Yurgalevitch, C. and DeWald, S. G., in *Synseeds: Applications of Synthetic Seeds to Crop Improvement* (ed. Redenbaugh, K.), CRC Press, Boca Raton, 1993, pp. 409–425.
8. Rangan, T. S., Murashige, T. and Bitters, W. P., *HortScience*, 1968, **3**, 226–227.
9. Zimmerman, J. L., *Plant Cell*, 1993, **5**, 1411–1423.
10. Ammirato, P. V., in *Handbook of Plant Cell Culture* (eds Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y.), Macmillan, New York, 1983, vol. 1, pp. 82–123.
11. Nagasawa, A. and Finer, J. J., *Plant Sci.*, 1989, **60**, 263–271.
12. Novak, F. J., Afza, R., VanDuren, M., Perea Dallos, M., Conger, B. V. and Xiaolang, T., *Biotechnology*, 1989, **7**, 154–159.

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Genetic variation and micropropagation in three varieties of *Piper longum* L.

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Genotypic and morphogenetic differences among three female varieties of *P. longum*, one variety each from Assam and Calicut and one released variety, Viswam, were investigated for the development of a common and efficient method of plant regeneration. RAPD analysis, using random oligonucleotide primers, revealed that these varieties are genetically different. Compared to the Assam variety, Viswam and Calicut varieties are genetically closer (95% similarity) among themselves. Morphogenetic potential of node, internode and leaf explants from all the three varieties were compared. Leaf explants from different varieties exhibited maximum regeneration potential. Among the types tested, Viswam variety exhibited best morphogenetic response followed by the varieties from Calicut and Assam. An efficient protocol was developed for regeneration from leaf calli of all the three genotypically different varieties. Callus regenerated plants from leaf explants were subsequently rooted, hardened and established on soil under natural conditions of growth.

P. LONGUM, L. (Piperaceae) is a commercially important dioecious medicinal herb. In traditional and ayurvedic medicines, mature spikes of female plants (long pepper), thick stems, roots and leaves are extensively used in the treatment of bronchial diseases, dispepsia, worms,

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