INDUCTION OF ANDROGENIC EMBRYOIDS FROM ANTHER CULTURE OF *MADHUCA INDICA*

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SARKAR, D., MAITY, S. & DAS, P. K. 2004. Induction of androgenic embryoids from anther culture of *Madhuca indica*. The effects of anther development, sucrose and growth regulators on the embryogenic response of *Madhuca indica* anthers were studied. Anthers isolated from 8, 12 and 18 mm flower buds were cultured on Murashige and Skoog's medium supplemented with sucrose (30 and 60 g l^{-1}) and varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (4.5 and 9.1 μM) in combination with N^6-benzyladenine (BA) (4.4 and 8.9 μM) or kinetin (4.6 and 9.3 μM). Difference in response was observed between various developmental stages of anthers. Anthers taken from 12 mm flower buds (stage II anthers) were most responsive in terms of survival as well as callogenic differentiation. Stage III anthers (from 18 mm flower buds) were the least responsive in culture. Cytological observations showed that microspores in both stage I (from 8 mm flower buds) and stage II anthers were predominantly uninucleate. Optimum callus proliferation from stage II anthers occurred in low (30 g l^{-1}) sucrose medium supplemented with 2,4-D and BA. Embryoid induction took place exclusively from stage II anthers at a higher sucrose (60 g l^{-1}) concentration in the presence of high levels of 2,4-D and kinetin. Early embryonic segmentation in stage II microspores indicated androgenic origin of these embryoids. However, these embryoids failed to regenerate into complete plantlets, suggesting restricted morpho-organogenic competence of anthers in *M. indica*.

Key words: Benzyladenine – biotechnology – callus – haploidy – kinetin – 2,4-D – regeneration – sucrose – tropical tree

SARKAR, D., MAITY, S. & DAS, P. K. 2004. Aruhan embrioid androgen daripada kultur cepu debunga *Madhuca indica*. Kesan perkembangan cepu debunga, sukros dan pengawal atur pertumbuhan terhadap respons embriogen cepu debunga *Madhuca indica* dikaji. Cepu debunga yang diasingkan daripada kudup bunga berukuran 8 mm, 12 mm dan 18 mm dikultur dalam media Murashige dan Skoog yang ditambah dengan sukrosa (30 g l^{-1} dan 60 g l^{-1}) dan asid 2,4-diklorofenoasetik (2,4-D; 4.5 μM dan 9.1 μM) berserta N^6-benziladenina (BA; 4.4 μM dan 8.9 μM) atau kinetin (4.6 μM dan 9.3 μM). Terdapat perbezaan respons antara peringkat perkembangan cepu debunga yang berlainan. Cepu debunga daripada kudup bunga berukuran 12 mm (cepu debunga peringkat II) menunjukkan respons paling ketara dari segi kemandirian dan pembezaan kalus. Cepu debunga peringkat III (daripada kudup bunga berukuran 18 mm) menunjukkan respons yang paling sedikit. Pemerhatian sitologi menunjukkan bahawa mikrospora peringkat I (daripada kudup bunga berukuran 8 mm) dan mikrospora peringkat II banyaknya unisel. Pertumbuhan kalus yang optimum daripada cepu debunga peringkat II berlaku dalam kepekatan media sukrosa yang rendah

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(30 g l^{-1}) ditambah dengan 2,4-D dan BA. Aruhan embrioid cuma berjaya dalam cepu debunga peringkat II pada kepekatan sukrosa yang tinggi (60 g l^{-1}) dengan kehadiran 2,4-D dan kinetin berkepekatan tinggi. Pensegmenan embiogen awal dalam mikrospora peringkat II menunjukkan asal androgen embriod tersebut. Namun, embriod ini gagal tumbuh semula menjadi anak tumbuhan lengkap. Ini mencadangkan yang cepu debunga *M. indica* mempunyai kebolehan morfo-organogen yang terhad.

Introduction

High heterozygosity, inbreeding depression and impracticability of controlled hybridisation because of long reproductive cycle restrict the production of haploids in tree species through conventional techniques (Zhang *et al.* 1992). Therefore, the culture of complete anthers containing immature pollen microspores is considered as a practical solution to haploid production in tree species (Bajaj 1986, Bonga *et al.* 1988, Kurt 1998). However, androgenic haploid induction in woody species through anther and/or pollen culture *in vitro*, as compared with that in herbaceous plants, has been found to be somewhat difficult, and hitherto limited to relatively few cases (Zhang *et al.* 1990, Srivastava *et al.* 1991, Venkatesh 1992, Ochatt *et al.* 1996, Wang *et al.* 1998, Jayasree *et al.* 1999). Despite the general recalcitrance of anthers and/or pollens of woody species, a variety of *in vitro* approaches for androgenic induction of haploidy is widely attempted because of their enormous advantages and practical significance in forest tree improvement (Kirby 1982, Radojevic & Kovoor 1986, Bonga *et al.* 1988, Zhukov *et al.* 1994, Kiss *et al.* 1995).

Madhuca indica, commonly known as Indian butter tree, is a multi-purpose tropical tree species belonging to the family Sapotaceae. It yields edible flower, fruit and seed. The protein content of the flower is comparable with that of rice and the total carbohydrate plus digestible nutrient contents are comparable with that of maize and oats (Singh 1982, Dwivedi 1992). In addition, the kernel contains up to 50% edible oil, which has manifold industrial uses. Genetic improvement in this tree species is severely limited due to poor viability and delayed germination of seeds (Vanangamudi & Palanisamy 1989) in combination with restricted seedling vigour and growth. *In vitro* techniques have been successfully used to propagate *Madhuca* spp. (Sarkar *et al.* 1992, Singh *et al.* 1992, Rout & Das 1993, Sarkar 1994, Singh & Bansal 1994, Chibbar & Bansal 1998). In view of the potential advantages of androgenic haploids in the genetic improvement of *M. indica*, an attempt was made to induce androgenic haploidy by anther culture.

Materials and methods

The flower buds were collected from a mature tree and divided into three groups according to their sizes (flower bud length) or developmental stages: 8 mm (stage I), 12 mm (stage II) and 18 mm (stage III). Individual lots of flower buds were washed under running tap water for 20 min followed by washing in Tween-20 solution (2–3 drops in 100 ml distilled water) for 15 min. The flower buds were then treated with 70% ethyl alcohol for 30–45 s, followed by surface disinfestation in 5% H_2O_2 solution for 10 min and subsequent washing in sterile distilled water for three to

four times. Surface-sterilised flower buds were dried over sterile filter papers and pricked open to isolate the anthers.

The anther culture medium was based on MS (Murashige & Skoog 1962) basal medium supplemented with 500 mg l^{-1} casein hydrolysate and 30 or 60 g l^{-1} sucrose. Different combinations of 2,4-dichlorophenoxyacetic acid [2,4-D] (4.5 and 9.1 µM) and N^6-benzyladenine [BA] (4.4 and 8.9 µM) or kinetin (4.6 and 9.3 µM) were used. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 20 min. Twenty anthers were plated on each Petri dish containing 30 ml of medium solidified with 8 g l^{-1} agar. The cultures were maintained under an 8-hour photoperiod (approx. 20 µmol m^{-2} s^{-1} light intensity) at an alternating day and night temperatures of 26 and 22 °C respectively. The embroids, which did not differentiate into plantlets on anther culture medium, were cultured on either growth regulator-free MS medium or MS medium supplemented with 2.2 µM BA or 1.44–2.89 µM gibberellic acid (GA_3) and 30 g l^{-1} sucrose.

The experiment was conducted in a factorial (3 × 2 × 4) completely randomised design involving three different stages of anthers (I, II and III), two sucrose concentrations (30 and 60 g l^{-1}) and four different combinations of growth regulators (GR 1 = 4.5 µM 2,4-D + 4.4 µM BA, GR 2 = 9.1 µM 2,4-D + 8.9 µM BA, GR 3 = 4.5 µM 2,4-D + 4.6 µM kinetin and GR 4 = 9.1 µM 2,4-D + 9.3 µM kinetin). Each treatment had five replications with 20 anthers per replication, and the experiment was repeated once (Experiments I and II). After six weeks' culturing, observations were recorded on percentage survival of anthers and growth characteristics. The percentage survival data were transformed into arc sine square roots and three-way analysis of variance (ANOVA) was calculated combined over individual experiments.

Results

Variations (Table 1) due to differences in anther stage, sucrose and growth regulators were statistically significant ($p \leq 0.01$). All two-way interactions involving anther stage, sucrose and growth regulators were significant ($p \leq 0.01$). This suggested that the effect of sucrose or growth regulator on percentage anther survival was not uniform over various stages of anthers used in the experiment. There was a significant ($p \leq 0.01$) three-way interaction between anther

Table 1 Analysis of variance for percentage survival of anthers in *Madhuca indica*

Source	DF	MS	F value
Anther stage	2	6261.80	116.65**
Sucrose	1	8673.87	161.58**
Anther stage × sucrose	2	406.41	7.57**
Growth regulator	3	1967.89	36.66**
Anther stage × growth regulator	6	151.33	2.82**
Sucrose × growth regulator	3	269.28	5.02**
Anther stage × sucrose × growth regulator	6	198.16	3.69**
Error	216	53.68	

** = significant at $p \leq 0.01$

stage × sucrose × growth regulator, suggesting that sucrose and growth regulator jointly had a major effect on the expression of variation due to anther stage over and above their individual effects (Table 1).

For stage I anthers, maximum survival (13.0–19.0%) was observed when cultured on medium supplemented with 30 g l^{-1} sucrose and 9.1 μM 2,4-D in combination with either 8.9 μM BA or 9.3 μM kinetin (Table 2). Growth of stage I anthers in these media was associated with callus proliferation from basal ends and abaxial surface of the anthers (Figure 1A). However, survival and callusing frequencies of stage I anthers declined significantly in the presence of 60 g l^{-1} sucrose.

In comparison, stage II anthers were most responsive (36.5% survival) when they were cultured on medium containing 30 g l^{-1} sucrose, 9.1 μM 2,4-D and 8.9 μM BA. In this medium, the anthers became transformed into white compact callus masses (Figure 1B). Callus proliferation from stage II anthers in 30 g l^{-1} sucrose-supplemented medium containing 9.1 μM 2,4-D plus 9.3 μM kinetin was associated with occasional development of meristemoid structures (Figure 1C). Survival of stage II anthers also declined significantly in the presence of 60 g l^{-1} sucrose in combination with either of the hormonal treatments. Cytological observations showed that both stage I and stage II anthers had uninucleate microspores (Figure 1D).

On medium containing 60 g l^{-1} sucrose and 9.1 μM 2,4-D plus 9.3 μM kinetin, embryoids differentiated directly from stage II anthers (Figure 1E) with subsequent development and/or growth in cultures (Figure 1F). About 4% of stage II anthers differentiated directly into embryoids. The frequency of embryoid regeneration from responsive stage II anthers is shown in Table 3. Cytological examinations of

Table 2 Effects of different combinations of growth regulators at two different sucrose concentrations on percentage survival and type of response of anthers of *Madhuca indica*

Growth regulator	Sucrose (g^{-1})			
	30		60	
	% Survival	Type of response	% Survival	Type of response
Stage I anthers				
GR 1	8.5	Callus	0.0	Necrosis
GR 2	19.0	Callus	8.0	Callus
GR 3	2.0	Callus	0.0	Necrosis
GR 4	13.0	Callus	2.5	Callus
Stage II anthers				
GR 1	26.0	Callus	9.5	Callus
GR 2	36.5	Callus	18.5	Callus
GR 3	18.5	Callus	3.0	Callus
GR 4	26.5	Callus	4.0	Embryoids
Stage III anthers				
GR 1	0.0	Callus	0.0	Necrosis
GR 2	13.0	Callus	0.0	Necrosis
GR 3	0.0	Callus	0.0	Necrosis
GR 4	9.0	Callus	0.0	Necrosis

GR 1 = 4.5 μM 2,4-D + 4.4 μM BA, GR 2 = 9.1 μM 2,4-D + 8.9 μM BA,
GR 3 = 4.5 μM 2,4-D + 4.6 μM kinetin and GR 4 = 9.1 μM 2,4-D + 9.3 μM kinetin

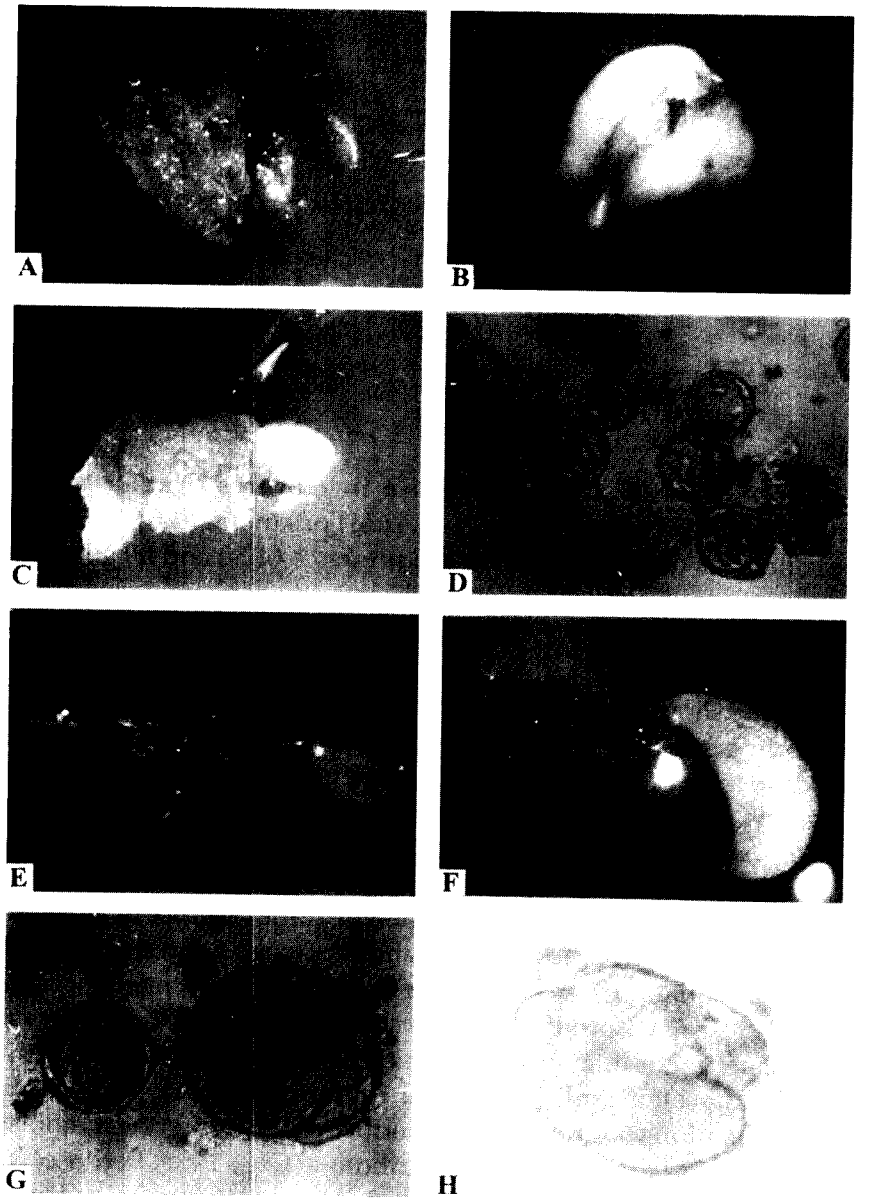

Figure 1 Anther culture of *Madhuca indica*. Callus developing from stage I (A) and stage II (B) anthers. Meristemoid developed in a stage II anther callus (C). Uninucleate microspores of stage II anthers (D). Embryoid differentiating from stage II anthers after three (E) and six (F) weeks of culture. Early (G) and late (H) embryonic segmentation leading to proembryoid differentiation in a microspore of stage II anther.

stage II anthers growing in this medium showed early embryonic segmentation in microspores (Figure 1G) leading to proembryoid formation (Figure 1H). However, these embryoids failed to differentiate further into plantlets, even when they were transferred to growth regulator-free MS medium or MS medium supplemented with 2.2 μM BA or 1.4–2.9 μM GA_3 and 30 g l^{-1} sucrose.

Table 3 The frequency of embryoid regeneration from stage II anthers of *Madhuca indica*

Replication	Experiment I		Experiment II	
	Number of anthers cultured	Number of anthers regenerated into embryoids	Number of anthers cultured	Number of anthers regenerated into embryoids
1	20	1	20	0
2	20	0	20	1
3	20	2	20	0
4	20	0	20	2
5	20	2	20	0

Anthers were cultured on medium supplemented with 60 g l^{-1} sucrose and 9.1 µM 2,4-D plus 9.3 µM kinetin

Stage III anthers were least responsive in cultures (Table 2). At lower concentrations of 2,4-D (4.5 µM) plus BA (4.4 µM) or kinetin (4.6 µM), the anthers turned necrotic after two weeks both in the presence of 30 and 60 g l^{-1} sucrose.

Discussion

The present study showed that the stage of development of the anther was most critical for androgenic differentiation in *M. indica*. Both stage I and II anthers isolated from 8 and 12 mm flower buds, respectively contained uninucleate microspores, and consequently they were more responsive in cultures than stage III anthers. However, stage II anthers were found to be superior to stage I anthers in terms of survival and callusing. The influence of flower bud length on androgenesis induction has also been reported in anther culture of *Aesculus carnea* (Marinkovic & Radojevic 1992). The amenability of stage I and stage II anthers to androgenic differentiation *in vitro* may be due to the presence of uninucleate microspores (Radojevic & Kovoor 1986, Hofer & Hanke 1994). Of the two cytokinins tested in the experiment in combination with 2,4-D, BA was more effective than kinetin in inducing callus proliferation from cultured anthers. The effectiveness of 2,4-D in combination with BA for callus induction on anthers was reported in a number of tree species (Tsay *et al.* 1990, Canhoto & Cruz 1993, Perl *et al.* 1995).

The study clearly demonstrated that sucrose had a major role in survival and subsequent differentiation of anthers *in vitro*. Optimum survival and callus proliferation occurred when the anthers were cultured on medium containing 30 g l^{-1} sucrose. Higher concentration of sucrose (60 g l^{-1}) significantly reduced the survival rate of anthers with concomitant suppression of callus proliferation. It can be assumed that microsporal division was favoured at a higher concentration of sucrose at the expense of callus proliferation from somatic anther tissues. This may perhaps explain why a reduction in survival and callusing frequency was observed in anthers grown in the presence of 60 g l^{-1} sucrose. Harn & Kim (1972) reported a positive correlation between restricted callogenic response and androgenic origin of differentiated callus tissues in *Prunus armenica*. However, in the absence of detailed cytological analysis, true androgenic origin of callus in high sucrose medium, as reported in the present study, cannot be established.

Interestingly, embryoids were directly differentiated from stage II anthers without intermediary callus phase in a high-sucrose (60 g l^{-1}) medium at higher concentrations of 2,4-D and kinetin. The result clearly showed that kinetin in combination with a high level of sucrose was effective in inducing embryoid formation. This is in agreement with reported mode of action of BA and/or kinetin in combination with low level of sucrose (30 g l^{-1}) in the present study. The superiority of stage II anthers for embryoid differentiation may be because they contained microspores at the mid-uninucleate stage (Hofer & Hanke 1994, Arrillaga *et al.* 1995). Direct embryoid formation from anthers was also reported in a number of tree species including *Aesculus hippocastanum* (Radojevic 1978) and *Hevea brasiliensis* (Chen *et al.* 1982). Early embryonic segmentation in the microspores of stage II anthers preceding embryoid differentiation confirmed the androgenic origin of these embryoids. However, the inability of these embryoids to regenerate further into plantlets indicated their recalcitrance, as commonly observed in tree species in general (Radojevic & Kovoor 1986). The general recalcitrance of *Madhuca* cell cultures due to poor morpho-organogenic competence in relation to ploidy instability and nuclear DNA variation has been reported earlier (Sarkar 1994, Sarkar *et al.* 2001). In future, research should be directed towards establishing ideal culture conditions for the germination of androgenic haploids of *M.indica.*

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References

Arrillaga, I., Lerma, V., Perez-Bermudez, P. & Segura, J. 1995. Callus and somatic embryogenesis from cultured anthers of service tree (*Sorbus domestica* L.). *HortScience* 30: 1078–1079.

Bajaj, Y. P. S. 1986. Biotechnology of tree improvement for rapid propagation and biomass energy production. Pp. 1–23 in Bajaj, Y. P. S. (Ed.) *Biotechnology in Agriculture and Forestry Vol. 1: Trees I.* Springer-Verlag, Berlin.

Bonga, J. M., von Aderkas, P. & James, D. 1988. Potential application of haploid cultures of tree species. Pp. 57–77 in Hanover, J. W. & Keathley, D. E. (Eds.) *Genetic Manipulation of Woody Plants.* Basic Life Sciences. Volume 44. Plenum Press, New York.

Canhoto, J. M. & Cruz, G. S. 1993. Induction of pollen callus in anther cultures of *Feijoa sellowiana* Berg. (Myrtaceae). *Plant Cell Reports* 13: 45–48.

Chen, Z. G., Qian, C., Qin, M., Xu, X. & Xiano, Y. 1982. Recent advances in anther culture of *Hevea brasiliensis* Muell-Arg. *Theoretical and Applied Genetics* 62: 103–108.

Chibbar, T. K. & Bansal, Y. K. 1998. Callusing ability in mahua (*Madhuca latifolia* Macb.). *Advances in Plant Sciences* 11: 275–279.

Dwivedi, A. P. 1992. *Principles and Practices of Indian Silviculture.* Surya Publications, Dehradun.

Harn, C. & Kim, M. Z. 1972. Induction of callus from anthers of *Prunus armenica. Korean Journal of Breeding* 4: 49–53.

Hofer, M. & Hanke, V. 1994. Anther culture of apple and cherry: effect of the stage of pollen development—correlative relations with morphological characters of the flower. *Gartenbauwissenschaft* 59: 225–228.

JAYASREE, P. K., ASOKAN, M. P., SOBHA, S., AMMAL, L. S., REKHA, K., KALA, R. G., JAYASREE, R. & THULASEEDHARAN, A. 1999. Somatic embryogenesis and plant regeneration from immature anthers of *Hevea brasiliensis* (Muell.) Arg. *Current Science* 76: 1242–1245.

KIRBY, E. G. 1982. The use of *in vitro* techniques for genetic modification of forest trees. Pp. 369–386 in Bonga, J. M. & Durzan, D. J. (Eds.) *Tissue Culture in Forestry*. Martinus Nijhoff, Hague.

KISS, J., RITTER, A., GERGACZ, J. & HESZKY, L. 1995. Use of *in vitro* androgenesis in poplar breeding. *Erdeszeti Kutatasok* 85: 135–148.

KURT, O. 1998. Anther culture. *Ondokuzmayis Universitesi Ziraat Fakultesi Dergisi* 13: 207–231.

MARINKOVIC, N. & RADOJEVIC, L. 1992. The influence of bud length, age of the tree and culture media on androgenesis induction in *Aesculus carnea* Hayne anther culture. *Plant Cell, Tissue and Organ Culture* 31: 51–59.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473– 497.

OCHATT, S. J., ZHANG, Y. X., JAIN, S. M., SOPORY, S. K. & VEILLEUX, R. E. 1996. *In vitro* haploidization of fruit trees. Pp. 193–210 in Jain, S. M. & Sopory, S. K. (Eds.) In vitro *Haploid Production in Higher Plants Volume 3: Important Selected Plants.* Current Plant Science and Biotechnology in Agriculture No. 25. Kluwer Academic Publishers, Dordrecht.

PERL, A., SAAD, S., SAHAR, N. & HOLLAND, D. 1995. Establishment of long-term embryogenic cultures of seedless *Vitis vinifera* cultivars—a synergistic effect of auxins and the role of abscisic acid. *Plant Science* 104: 193–200.

RADOJEVIC, L. 1978. *In vitro* induction of androgenic plantlets in *Aesculus hippocastanum. Protoplasma* 96: 369–374.

RADOJEVIC, L. & KOVOOR, A. 1986. Induction of haploids. Pp. 65–86 in Bajaj, Y. P. S. (Ed.) *Biotechnology in Agriculture and Forestry Vol. 1: Trees I.* Springer-Verlag, Berlin.

ROUT, G. R. & DAS, P. 1993. Micropropagation of *Madhuca longifolia* (Koenig) MacBride var. *latifolia* Roxb. *Plant Cell Reports* 12: 513–516.

SARKAR, D. 1994. Morpho-organogenic evaluation and histochemical studies in some forest plants *in vitro.* Ph.D. thesis, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur.

SARKAR, D., DAS, P. K. & MAITY, S. 1992. *In vitro* propagation of *Madhuca* spp. *SABRAO Journal* 24: 133–135.

SARKAR, D., MAITY, S. & DAS, P. K. 2001. Morpho-organogenic competence in relation of ploidy instability and nuclear DNA variation in callus cultures of *Madhuca indica* J. F. Gmel. *The Nucleus* 44: 72–80.

SINGH, R. V. 1982. *Fodder Trees of India.* Oxford and IBH Publishing, New Delhi.

SINGH, M. & BANSAL, Y. K. 1994. Regeneration of plantlets from cotyledons of *Madhuca latifolia* Macb. cultured *in vitro. Indian Journal of Forestry* 17: 15–19.

SINGH, H. P., SINGH, S., SAXENA, R. P., SINGH, R. K. & SINGH, S. 1992. Pretreatment of nodal segments for *in vitro* establishment and bud activation of *Madhuca latifolia. Plant Physiology and Biochemistry* 19: 116–122

SRIVASTAVA, H. K., PRAKASH, J. & PIERIK, R. L. M. 1991. Current status of forest biotechnology. Pp. 169–176 in Prakash, J. (Ed.) *Proceedings of the International Seminar on New Frontiers in Horticulture, "Horticulture—New Technologies and Applications".* 25–28 November 1990. Bangalore.

TSAY, H. S., YEH, C. C. & HSU, J. Y. 1990. Embryogenesis and plant regeneration from anther culture of bamboo (*Sinocalamus latifolia* (Munro) McClure). *Plant Cell Reports* 9: 349–357.

VANANGAMUDI, K. & PALANISAMY, V. 1989. Viability of Illupai (*Madhuca longifolia* var. *latifolia*) seed. *Seed Research* 17: 186–187.

VENKATESH, C. S. 1992. Biotechnology in tree and bamboo improvement. *Indian Forester* 118: 249–255.

WANG, Z. X., ZHANG, L. & LIU, Y. Q. 1998. Anther culture *in vitro* and variation of chromosome ploidy of jujube. *Plant Physiology Communications* 34: 180–182.

ZHANG, Y. X., LESPINASSE, Y. & CHEVREAU, E. 1990. Induction of haploidy in fruit trees. *Acta Horticulturae* 280: 293–305.

ZHANG, Y. X., LESPINASSE, Y., HAMMERSCHLAG, F. A. & LITZ, R. E. 1992. Haploidy. Pp. 57–75 in Hammerschlag, F. A. (Ed.) *Biotechnology of Perennial Fruit Crops.* Biotechnology in Agriculture Series No. 8. CAB International, Wallingford.

ZHUKOV, O. S., SAVELYEV, N. I., OLENYNIKOIVA, O. J., SCHMIDT, H. & KELLERHALS, M. 1994. Prospects for application of anther culture in selection of fruit plants. Pp. 411–413 in Schmidt, H. (Ed.) *Proceedings of the Eucarpia Fruit Breeding Section Meeting, "Progress in Temperate Fruit Breeding".* 30 August–3 September 1993. Wadenswil-Einsiedeln.