

# Dynamics of Agricultural Biotechnology

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# **Dynamics of Agricultural Biotechnology**

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A S Chandel and R M Kamal



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## ALKALOIDAL CROPS

**862** BENJAMIN, BD; ROJA, PC; HEBLE, MR; CHADHA, MS. 1987. **Multiple shoot cultures of *Atropa belladonna*: effect of physico-chemical factors on growth and alkaloid formation.** *Journal of Plant Physiology*, 129: 1/2, 129-135; 13 ref.

Multiple shoot cultures were established from shoot tips and axillary meristems. The cultures were initially raised on agar medium and subsequently maintained on Murashige and Skoog liquid medium supplemented with BA. These cultures were subjected to X-irradiation at 29, 56 or 116 Gy. Recovery from the radiation effects was observed in tissues subjected to 29 Gy (but no higher) during four successive passages. Different growth regulators influenced the growth and morphogenetic responses of cultures, but did not affect the alkaloid contents significantly. BA alone at 1-5 p.p.m. induced profuse shoot production, but 10 p.p.m. retarded growth. BA + NAA promoted shoot bud formation and minimum cell proliferation. Kinetin + NAA and kinetin + IAA both induced rooting. Kinetin + 2,4-D caused rapid tissue proliferation which eventually gave a uniform cell suspension. Addition of precursors of tropane alkaloids to the medium marginally increased alkaloid synthesis during the stationary phase of growth. Shoot cultures established from field-grown plants differing in their alkaloid content (0.2, 0.3 and 0.4%) resembled their parents in growth and morphology but not in alkaloid content which was very low (0.1% or less).

**863** BHAKUNI, DS; GUPTA, S; JAIN, S. 1983. **The biosynthesis of the alkaloids of *Stephania glabra* (Roxb.) Miers.** *Tetrahedron*, 39: 23, 4003-4010; 19 ref.

Tracer experiments using cut branches of young plants are described.

**864** BHATTACHARYYA, P; CHAKRABORTY, A. 1984. **Mukonal, a probable biogenetic intermediate of pyranocarbazole alkaloids from [stem bark of] *Murraya koenigii*.** *Phytochem.*, 23: 2, 471-472; 6 ref.

**865** KHALIDA-KHATOON. 1987. **Flower induction in tobacco cultivar in sterile culture.** *Pakistan Journal of Agricultural Research*, 8: 4, 394-402; 16 ref.

Epidermal explants of tobacco cv. Virginia excised from fruiting branches of the inflorescence produced max. flower buds when cultured on Murashige and Skoog medium containing glycine and vitamins. GA3 incorporation in the flower inducing medium reduced

the percentage of explants producing flower buds and the number of flower buds/explant. The induced flower buds had a green well developed calyx but other organs were absent or less well developed. 10<sup>-3</sup>-10<sup>-5</sup> M adenine inhibited flower bud development from epidermal explants and 10<sup>-3</sup> M adenine proved toxic to both epidermal explants and stem segments in in vitro culture. Flower bud induction was more successful in boiling tubes (88% of explants producing flower buds) than in conical flasks (45-77%) or plastic petri dishes (8%). Continuous light was more effective than continuous darkness or 16 h light + 8 h darkness for flower induction resulting in the greatest number of surviving explants possessing floral buds.

**866** KISHOR, PBK; MEHTA, AR. 1988. **Changes in some enzyme activities during growth and organogenesis in dark-grown tobacco callus cultures.** *Plant and Cell Physiology*, 29: 2, 255-259; 22 ref.

Floral bud callus of tobacco cv. Anand-2 was initiated on Murashige and Skoog's medium supplemented with 0.3 or 2 mg IAA/l and 2 or 3% sucrose to give shoot-, root- and non-organ-forming media and was subsequently harvested at 3-day intervals for 15 days and analysed for growth and enzymes involved in malate metabolism (glutamic-oxaloacetic transaminase, malate dehydrogenase, malic enzyme and phosphoenolpyruvate carboxylase). Specific enzyme activity was higher in organ-forming compared to non-organ-forming callus cultures, particularly during the days preceding root and shoot differentiation. Malate accumulated until day 15 and 6 in non-organ-forming and shoot-and-root-forming callus, resp., while total and reducing sugars accumulated until only day 3 in all cultures. Malate metabolism and the function of malate in organogenesis are discussed.

**867** KUKREJA, AK; MATHUR, AK. 1985. **Tissue culture studies in *Duboisia myoporoides*: 1. Plant regeneration and clonal propagation by stem node cultures.** *Planta Medica*, No. 2: 93-96; 17 ref.

For optimal growth of the axillary bud and its subsequent multiplication, 1.0 mg/litre IAA + 2.0 mg/litre kinetin or BA were required. Rooting was induced by auxin alone (0.5 mg/litre NAA), but only after replacing semi-solid basal Murashige and Skoog (MS) medium with static liquid MS. No synergism was noted between cytokinins and adenine sulphate, while GA3 suppressed shoot growth. The plantlets produced were successfully grown to maturity under field conditions. They showed higher total alkaloid levels (2.17 to 3.90%) than the

parent plant from which the explants were taken (1.82%).

**868 KUMAR, V; YADAV, NR; MAHERCHANDANI, N. 1988. Pattern of acid phosphatase activity during shoot organogenesis in normal and variant callus cultures of *Nicotiana tabacum* L. cv. White Burley. *Current Science*, 57: 11, 612-613; 8 ref.**

In callus cultures from tobacco leaf midrib explants acid phosphatase activity (APA) increased under non-shoot forming (NSF) conditions in normal callus but was low in variant callus cultures. APA was lower under shoot forming than under NSF conditions, and reached a peak 2 d before shoot appearance in normal and variant callus. In view of previously published results it was concluded that the pattern of shoot formation and the preceding events are similar under different sets of conditions.

**869 MATHUR, J. 1993. Somatic embryogenesis from callus cultures of *Nardostachys jatamansi*. *Plant Cell, Tissue and Organ Culture*, 33: 2, 163-169.**

**870 MOHAMED, MTZ; SCRAGG, AH. 1990. A comparison of the growth and alkaloid production by suspension and immobilized *Catharanthus roseus* cells in 6L and 7L bioreactors. *Physiology of Immobilized Cells*/edited by J.A.M. de Bont, J. Visser, B. Mattiasson, J. Tramper. Oxford: Elsevier, p. 335-342.**

**871 MOHAN, J; SHARMA, AK. 1987. *Calotropis* mosaic virus. *Current Science*, 56: 6, 274-275; 4 ref.**  
Details are given of a previously unreported virus occurring on the medicinal weed *C. procera*, causing leaf mosaic, growth stunting and masking of flower colour. The causal virus was sap-transmissible to 6 of 46 plant spp. tested, causing necrotic local lesions in *Chenopodium album*. *Myzus persicae* and *Aphis gossypii* transmitted the virus in a nonpersistent manner from *C. procera* to *Nicotiana glutinosa*, tobacco and *Chenopodium amaranticolor*. The dilution end point was 1:500 and the virus was inactivated by heating sap for 10 min at 60°C and storage at 35° for 2 d or 4° for 6 d. The virus belongs to the potyvirus group and is similar to tobacco etch virus but has a distinct host range and does not produce inclusion bodies in infected plants. It also resembles *Datura* enation mosaic virus but does not infect *D. metel* or *Solanum nigrum*.

**872 SANGAR, RBS; CHANDRA, R; KHURANA, SMP. 1986. Regeneration of TMV-free tobacco**

**plantlets through callus culture. *Indian Journal of Plant Pathology*, 4: 1, 80-82; 5 ref.**

Details are given of a method for obtaining plantlets free from tobacco mosaic virus infection although the original callus cells contained the virus.

**873 SANTHARAM, G; JAYARAJ, S. 1987. Effect of host plants and site of application on the infectivity of nuclear polyhedrosis virus to *Spodoptera litura* larvae. *Journal of Biological Control*, 1: 1, 39-43; 16 ref.**

The infectivity of a nuclear polyhedrosis virus to larvae of the noctuid *Spodoptera litura* on knolkhol [kohlrabi], beetroot, tobacco, cotton (*Gossypium barbadense* and *G. hirsutum*), cauliflower, cabbage and castor [*Ricinus communis*] was studied in pot experiments in Tamil Nadu, India. Maximum larval mortality was obtained on tobacco (96.67%), cauliflower (96.67%) and cabbage (93.33%) with a minimum incubation period of 7.67-8.67 days. Application of the virus to the lower leaf surface of tobacco or cotton caused higher mortality than application to the upper leaf surface.

**874 SELVAPANDIYAN, A; MEHTA, AR; BHATT, PN. 1988. Cellular breeding approach for development of *Fusarium* wilt resistant tobacco. *Proceedings of the Indian National Science Academy, Part B: Biological Sciences*, 54: 6, 391-394.**

Plants regenerated from cells of cv. Anand 2 cultured in the presence of a *F. oxysporum* f.sp. *nicotianae* culture filtrate varied in tolerance to the pathogen. Regenerated plants were screened for resistance by culturing leaf discs in MS medium containing a LD50 concentration of culture filtrate (27% v/v filtrate from a 10-day-old culture). When some selected regenerated plants were inoculated in the field they showed a hypersensitive reaction.

**875 SETHI, U; BASU, A; MUKHERJEE, SG. 1990. Role of inhibitors in the induction of differentiation in callus cultures of *Brassica*, *Datura* and *Nicotiana*. *Plant Cell Reports*, 8: 10, 598-600; 15 ref.**

Hormone-containing media without inhibitors (control) produced 6% shoot formation from calluses of *B. oleracea* (cauliflower), *D. innoxia* [*D. fastuosa*] and *N. tabacum*. Addition of inhibitors such as actinomycin D [dactinomycin], cordycepin, abscisic acid, trigonelline and theophylline greatly enhanced shoot formation suggesting that inhibitors play a regulatory role in the control of differentiation.

## Datura

876 DATTAGUPTA, S; DATTA, PC. 1984. A search for alkaloids in the callus cultures of black datura by changing the tissue environment. *Bangladesh Journal of Scientific and Industrial Research*, 19: 1/4, 30-36; 30 ref.

Of various media tested, Wood and Braun was the best medium for callus growth of *Datura metel* var. *fastuosa*, but it failed to produce alkaloids in dedifferentiated cells. Different combinations of hormones, precursors and amino acid failed to enhance tissue growth and to produce alkaloids. Increase in KNO<sub>3</sub> content induced better callus but without alkaloids. Vitamin- and kinetin-dependent strains grew fairly well but auxin and kinetin deficiency caused retardation. Streptomycin retarded callus growth whereas colchicine was slightly growth promoting.

877 JAIN, RK; MAHERCHANDANI, N; SHARMA, DR; MISHRA, NR. 1981. Effect of gamma irradiations and gibberellic acid on growth and differentiation in cultured cells of *Datura innoxia*. *International Symposium on Plant Cell Culture in Crop Improvement*. Bose Institute, Calcutta. p. 47.

878 JAIN, RK; MAHERCHANDANI, N; SHARMA, DR; CHOWDHURY, VK. 1980. Effect of gamma irradiation and growth regulators on isoperoxidase in haploid cultured cells of *Datura innoxia*. *Indian Journal Biochemistry and Biophysics*, 17(s): 9.

879 JAIN, RK; MAHERCHANDANI, N; SHARMA, DR; CHOWDHURY, JB. 1984. Effect of gamma radiations and gibberellic acid on growth and shoot regeneration in callus cultures of *Datura innoxia*. *Current Science*, 53: 13, 700-701; 14 ref.

When callus tissues initiated from explants of anther-derived haploid plantlets were exposed to 0.2, 1.0 and 5.0 kR gamma rays whilst growing on modified Murashige & Skoog and B3 media, callus growth was stimulated by the 0.2 kR treatment. Shoot regeneration was stimulated by the 0.2 and 1.0 kR treatments, resulting in 2 and 3 times, respectively, the number of buds produced on unirradiated callus. Differentiation was 10-12 days earlier in irradiated than in unirradiated callus. Addition of GA<sub>3</sub> to the media resulted in a reduced growth rate and prevented organogenesis irrespective of radiation treatment.

880 JAIN, RK; MAHERCHANDANI, N; SHARMA, DR; CHOWDHURY, VK. 1980. Effect of gamma

radiations on haploid cultured cells of *Datura innoxia*. *Natl. symp. plant tissue Culture Genetic manipulations and somatic hybridization of plant cells*. Bhaba Atomic Research Centre, Bombay. p. 17-23.

881 JAIN, RK; MAHERCHANDANI, N; CHOWDHURY, VK; JAIN, SUNITA. 1990. Radiation-induced organogenesis and isoenzyme patterns in long-term callus cultures of *Datura innoxia*. *Annals of Botany*, 65: 6, 659-663; 23 ref.

Callus cultures (8 months old) of *D. innoxia* [*D. fastuosa*] derived from anther cultures were gamma-irradiated with 0.2, 1.0 or 5.0 kR and then sub-cultured onto MS medium containing 2 mg 2,4-D/litre or a B3 shoot regeneration medium (an MS medium containing 2.56 mg kinetin/litre and 4.0 mg IAA/litre). Irradiation with 0.2 or 1.0 kR stimulated shoot regeneration but 5.0 kR totally inhibited it. The specific activities of alpha-amylase and peroxidase in cultured material increased under the shoot regenerating conditions of B3 medium. Irradiation had no effect on alpha-amylase activity but stimulated peroxidase activity and particularly increased peroxidase enzymes with high electrophoretic mobilities. It is concluded that these isoperoxidases could act as useful biochemical markers for shoot differentiation.

882 SHARMA, DR; CHOWDHURY, JB. 1977. Effect of different media on cultured anthers of *Datura innoxia* Mill and comparative morphogenetic potentiality of haploid and diploid tissues. *Indian Journal. Exptl. Biol.* 15: 616-618.

883 SHARMA, VK; KOTHARI, SL. 1990. Somatic embryogenesis from callus culture and protoplast isolation and culture in *Datura innoxia* Mill. *Journal of Phytological Research*, 3: 1 & 2, 137-139; 6 ref.

When excised anthers of *D. innoxia* [*D. fastuosa*] were cultured on MS media under suitable conditions, embryos formed within approx. 20 d. Callus grew over the entire surface of the embryos and differentiated into shoots and plantlets after a further 2-3 weeks. Prolific embryogenesis occurred when the callus was subcultured on medium containing 0.05 mg/litre kinetin + 0.5 mg (but not 3.0 mg) 2,4-D. The isolation of protoplasts from cultured plantlets is described. Protoplast division began within 5-6 d of isolation.

884 TYAGI, AK; RASHID, A; MAHESHWARI, SC. 1984. Streptomycin resistance of a cell line from haploid *Datura innoxia* Mill. is transferred from cell

to plantlet and back in vitro. *Journal of Experimental Botany*, 35: 154, 756-761; 28 ref.

One of 5 anther-derived, haploid cell lines selected for their ability to proliferate on a selection medium containing streptomycin was studied in detail. Resistance was stable in the absence of selection pressure and plantlets were regenerated both in the presence, and absence, of streptomycin. Stability of resistance was also confirmed in callus cultures initiated from stem explants of one of the plantlets.

## Tobacco

**885** APPA RAO, K; RAMAVARMA, KT; VENKATA RAO, C; SUBHASHINI, U; VENKATESWARLU, T. 1984. **Genetic improvement of FCV tobacco: problems and progress.** *Technical Bulletin*, Central Tobacco Research Institute, Rajahmundry, India.

**886** CHARI, MS; RAO, RSN; SREEDHAR, U. 1994. **Integrated management of Tobacco Leaf eating caterpillar *Spodoptera litura* F in India.** *PIS Information Bulletin (India)*. Oct. 9-15.

**887** CHARI, MS; RAO, RSN; GUNNESWARAO, S; SREEDHAR, U. 1994. **Non pesticidal approach to tobacco pest management - a rewarding exercise.** *National Workshop in Non pesticidal approach to pest Management - A New Direction*. (Hyderabad: 1994: Sep. 20-23).

**888** CHARI, MS; RAO, RSN; SREEDHAR, U; RAO, SG. 1994. **Role of trap crops in the management of *Spodoptera litura* F and *Heliothis armigera* in tobacco.** *National Symposium on Emerging trends in pest management*. (Solan, H.P.: 1994: June 28-30).

**889** CHARI, MS; RAO, RSN; MUSHINI, SN. 1993. **Tobacco ecosystem and its interaction with olyphagous pest, *Spodoptera litura* and its natural enemies.** *National Symposium in Advances in Biological Control of Insect Pests*. (5th: Muzaffarnagar: 1993: Oct).

**890** KISHOR, PBK; MEHTA, AR. 1989. **Carbohydrate oxidation during organogenesis in callus cultures of tobacco.** *Indian Journal of Experimental Biology*, 27: 2, 124-127; 22 ref.

Total sugars, reducing sugars and total starch accumulated considerably during the initial 3-9 d in organ forming callus cultures of tobacco. Specific activities of the key glycolytic, Krebs cycle and pentose phosphate

pathway enzymes such as glucokinase, succinate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (but not fructose 1,6-diphosphate aldolase) were higher in the shoot and root forming callus than in the non-organ forming callus. The accumulation and utilization pattern of carbohydrate was essential during growth and differentiation.

**891** KISHOR, PBK; MEHTA, AR. 1988. **Effect of rifamycin and gentamycin on growth, organogenesis and carbohydrate metabolising enzymes in callus cultures of tobacco.** *Phytomorphology*, 38: 4, 327-331; 16 ref.

Growth of tobacco callus was suppressed progressively with increasing concn of rifamycin and gentamycin sulphate (0.01, 0.1, 10 and 100 mg/litre). While rifamycin inhibited roots and shoots at lower concn (0.01 and 0.1 mg, resp.), gentamycin inhibited both at concn above 10 mg/litre. There was a total inhibition of organogenesis at 100 mg/litre of the antibiotics. Acid phosphatase, ribonuclease and key enzymes of carbohydrate metabolism (glucokinase, malate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) were suppressed, especially on the rifamycin especially containing medium (0.1 mg/l) compared with the control and gentamycin medium.

**892** KISHOR, PBK; MEHTA, AR. 1983. **Osmotic involvement and organogenesis in callus culture.** *Proceedings of the Indian National Science Academy, B*, 49: 6, 729-734; 18 ref.

IAA at 0.175-0.3 mg/l and 3% sucrose induced shoots in callus cultures of tobacco. Higher levels of IAA (0.5-2.0 mg/l) favoured root differentiation. Root differentiation was also achieved by increasing sucrose level to 6% in the low IAA (0.3 mg/l) medium. The success in partially replacing the sucrose requirement for shoot and root formation with osmotically equivalent levels of mannitol supported the view that part of the sucrose was acting as an osmoticum, besides providing the C and energy source. The osmotic requirement for root and shoot formation was different. The number of roots and shoots/callus mass increased with increasing mannitol content in the medium.

**893** PENTAL, D; MUKHOPADHYAY, A; GROVER, A; PRADHAN, AK. 1988. **A selection method for the synthesis of triploid hybrids by fusion of microspore protoplasts (n) with somatic cell protoplasts (2n).** *Theoretical and Applied Genetic.*, 76: 2, 237-243; 54 ref.

Haploid microspore protoplasts isolated at the tetrad stage from kanamycin-resistant *Nicotiana tabacum* ( $2n=48$ ) were fused with somatic cell protoplasts of wild-type *N. rustica* ( $2n = 48$ ) to produce triploid plants. Hybridization was confirmed by peroxidase and leaf esterase isoenzyme analysis, by neomycin phosphotransferase II assay and by probing digests of total DNA with a cloned 18S rDNA fragment. Root tip squashes of 6 of the hybrids revealed chromosome numbers ranging from 58 to 72.

**894** PENTAL, D; PRADHAN, AK; MUKHOPADHYAY, A. 1989. **Transmission of organelles in triploid hybrids produced by gametosomatic fusions of two *Nicotiana* species.** *Theoretical and Applied Genetics*, 78: 4, 547-552; 33 ref.

Gametosomatic hybrids produced by the fusion of microspore protoplasts of *N. tabacum* Km+Sr+ with somatic cell protoplasts of *N. rustica* were analysed for organelle composition. For the analysis of mtDNA, species-specific patterns were generated by Southern hybridization of restriction endonuclease digests of total DNA and mtDNA with 4 DNA probes of mitochondrial origin: cytochrome-c oxidase subunit I, cytochrome-c oxidase subunit II, 26S rDNA and 5S-18S rDNA. Of the 22 hybrids analysed, some had parental-type pattern for some probes and novel-type for the others, indicating interaction between mtDNA of the 2 parent species. For ctDNA analysis, species-specific patterns were generated by Southern hybridization of restriction endonuclease digests of total DNA with large subunits of ribulose-bisphosphate carboxylase and ctDNA as probes. All the hybrids had *N. rustica* patterns. Hybrids were not resistant to streptomycin, a trait encoded by the chloroplast genome of *N. tabacum*. It is concluded that in gametosomatic fusions of the 2 *Nicotiana* species, mitochondria but not chloroplasts are transmitted from the parent contributing microspore protoplasts.

**895** PRASAD, GSR. 1987. **Cytogenetical and biochemical studies of an interspecific hybrid in the genus *Nicotiana* (*N. benthamiana* Domin X *N. tabacum* L) and transfer of caterpillar resistance.** Ph.D. thesis submitted to Andhra University, Waltiar, 148 p.

**896** PRASAD, GSR; SUBHASHINI, U; VENKATESWARLU, T. 1984. **Tissue Culture for disease resistance in tobacco.** *All India Congress of Cytology and Genetics*. (5th: Bhubaneswar: 1984: Oct. 7-10).

**897** RATNAVATI, CV; USHASRI, V; SUBBARAO, DV; VENKATESWARLU, T. 1988. **Multiple molecu-**

**lar forms of esterases among *Nicotiana* species.** *Tobacco Research*, 14: 58-63.

**898** RAWAL, SK; MEHTA, AR. 1982. **Tissue culture of tobacco. II. Influence of IAA, kinetin and sucrose on organogenesis in *Nicotiana tabacum* callus cultures.** *Indian Journal of Plant Physiology*, 25: 4, 336-347; 42 ref.

Factors influencing the differentiation of shoots and roots from tobacco flower bud callus were investigated. With IAA at 0.3 mg/l in the Murashige and Skoog medium, differentiation of either shoot or root was brought about by altering the sucrose level in the medium. Sucrose at 2-3% induced caulogenesis, whereas at 6% it facilitated rhizogenesis. IAA at 0.5 and 2.0 mg/l in the presence of 2-6% sucrose invariably induced rhizogenesis. IAA and kinetin in a 1:1 ratio could either induce shoots and roots or exclusively shoots. High IAA:kinetin ratio did not induce rhizogenesis. High kinetin:IAA ratio did not favour caulogenesis. IBA, NAA, 2,4-D and kinetin proved ineffective in inducing organogenesis.

**899** SAHA, S; GUPTA, S. 1989. **Role of hormones on propagation of an Indian variety of tobacco (*Nicotiana tabacum* var. *Jayasri*).** *Phytomorphology*, 39: 2-3, 203-208; 12 ref.

Tissue culture of tobacco cv. *Jayasri* was done to standardize the hormonal requirements for the mass propagation of plantlets and to isolate variants as and when required. Unlike other cultivars, regeneration of this cultivar was more dependent on IBA (2 mg/litre) in addition to kinetin (0.5 mg/litre). Growth regulators such as IAA and indole propionic acid could also induce organ formation but at a relatively poor rate. Percentage of differentiation and number of differentiated organs/callus increased up to the 8th subculture and subsequently became stationary. Light treatment of about 4000 lux triggered more organogenesis at different auxin treatments than those in dark and 2 other light intensities. Profuse rooting could be obtained by transferring the shoots to rooting medium containing IBA + kinetin.

**900** SELVAPANDIYAN, A; BHATT, PN; MEHTA, AR. 1989. **Growth inhibition of intact plants and in vitro cultures of tobacco by culture filtrates of *Fusarium oxysporum* f.sp. *nicotianae*.** *Annals of Botany*, 64: 2, 117-122; 22 ref.

The culture filtrate of *F. oxysporum* f.sp. *nicotianae* was able to induce several symptoms on tobacco plants that occur with infection by the pathogen. Positive correlations were established for the degree of culture filtrate

induced symptoms developed in a range of tobacco cultivars and those caused by the live pathogen after inoculation into the same cultivars. Effects of culture filtrate on the growth of intact plants, anthers, leaf discs and cell suspensions were examined to assist in understanding the cellular basis of pathogenicity. The culture filtrate at >25% (v/v) was found inhibitory to the growth of leaf discs and plated cell suspensions. No growth occurred >50% (v/v) level of the filtrate. Similarly, wilt symptoms were observed on the whole plants when the culture filtrate exceeded 25% (v/v). In vitro androgenesis was inhibited at a much lower conc. (12.5% v/v) of culture filtrate.

**901** SUBHASHINI, U; VENKATESWARLU, T; PRASAD, GSR. 1983. **Cytogenetics of the interspecific hybrid in *Nicotiana L.*** *Proc. Inter Symp. Cytogenetics of higher plants, chromosome structure and behaviour.* (Waltair: 1983: Dec. 6-8), p. 99-104.

**902** SUBHASHINI, U; VENKATESWARLU, T; ANJANI, K. 1986. **Embryo rescue in back crosses of *Nicotiana* hybrids.** *National Symp. on tobacco.* (4th: Rajahmundry: 1986: Dec. 13-17). Central Tobacco Research Institute, Rajahmundry - 533 105, A.P., India.

**903** SUBHASHINI, U; VENKATESWARLU, T; ANJANI, K. 1986. **Induction of flowering in a new species *Nicotiana africana*.** *Tob. Res.*, 12: 1, 82-83.

**904** SUBHASHINI, U; VENKATESWARLU, T. 1990. **Maintenance of mammoth bidi tobacco.** *Tobacco Research*, 16: 1, 37-39; 7 ref.

A naturally occurring giant or mammoth mutant of Anand 2, noticed in a field in Mamadapur in 1985, had a large plant size (337 cm tall vs. 150 cm in Anand 2) and many leaves (86 vs. 25). As it did not flower under field conditions it was propagated in vitro on MS medium supplemented with 6 mg NAA and 2 mg benzyladenine/litre. Shoot formation was induced using 2 mg IAA and 2 mg kinetin/l. Flowering and seed set were induced in regenerants by altering the daylength. Regenerants had 75 leaves/plant and were 260 cm tall.

**905** SUBHASHINI, U; VENKATESWARLU, T; NARAYANAN, AI. 1986. **Pollen haploids from mutants in *Nicotiana tabacum*.** *National Symposium on Recent Advances in Plant cell and tissue culture.* (Osmania University, Hyderabad: 1986: July 24-26).

**906** SUBHASHINI, U; VENKATESWARLU, T; ANJANI, K. 1985. **Tissue culture for morphogenesis in *Nicotiana* hybrid embryos.** *National Symposium on*

*Recent advances in Developmental morphology of crop plants.* (Waltair: 1985: July 3-5), p. 60.

**907** VENKATESWARLU, T; SUBHASHINI, U; SREE RAMA MURTHY, CH; NAGESWARA RAO, CR. 1991. **A note on callus proliferation and biogenesis studies in haploid tissues of FCV tobacco.** *Tobacco Research*, 17: 1, 53-54.

**908** VENKATESWARLU, T; SUBHASHINI, U; ANJANI, K. 1989. **Anther culture studies in white burley tobacco.** *Tob. Research*, 15: 2, 120-122; 13 ref. Anthers of the genotype Kentucky 14 were cultured on Nitsch alone (control) and on medium supplemented with 2 adsorbents (activated charcoal and polyvinyl pyrrolidone [polyvidone]), 6 organic supplements and honey. None of the supplements increased embryogenesis. Anthers of 10 genotypes were cultured on the control medium. Kentucky 14 and Harrow Velvet had the highest number of responsive anthers (85.7 and 40.0%, respectively) and mean numbers of plantlets/anther (17 and 16, respectively).

**909** VENKATESWARLU, T; SUBHASHINI, U; PRASAD, GSR. 1984. **Cytogenetics of the interspecific hybrid obtained by tissue culture in *Nicotiana*.** *All India Congress of Cytology and Genetics.* (Bhubaneswar: 1984: Oct. 7-10).

**910** VENKATESWARLU, T; RAO, SV; SATYANARAYANA, KV; JAGADISH CHANDRA, I. 1994. **Direct DNA Transformation in Tobacco.** *Second Asia-Pacific conference on Agricultural Biotechnology.* (Madras: 1994: March 6-10).

**911** VENKATESWARLU, T; SUBHASHINI, U. 1984. **Experimental embryogenesis in Burley tobacco.** *National Symposium on production and exports of burley tobacco.* (Madras: 1984: Oct. 12).

**912** VENKATESWARLU, T. 1988. **In vitro production, cytogenetics and bio-chemical studies of two interspecific hybrids in the genus *Nicotiana*.** Ph.D. Thesis submitted to Andhra University, Waltiar, 119 p.

**913** VENKATESWARLU, T; SUBHASHINI, U; REDDY, VR. 1988. **In vitro production and cytogenetics of an interspecific hybrid in *Nicotiana*.** *All India Conference on Cytology and Genetics.* (2nd: Warangal: 1988: Dec. 28-30), p. 90.

**914** VENKATESWARLU, T; SUBHASHINI, U; PRASAD, GSR. 1984. **Interspecific, hybrids in**

*Nicotiana* through fertilized ovule culture. *National Symposium on tobacco*. (5th: Rajahmundry: 1984: Feb. 15-18).

**915** VENKATESWARLU, T; POWER, JB; COCKING, EC. 1992. **Protoplast culture and somatic hybridization in *Nicotiana*: abstract**. *National Symposium in Frontiers in plant Biotechnology*. (IARI, New Delhi: 1992: Nov. 25-27).

**916** VENKATESWARLU, T; SUBHASHINI, U; REDDY, VR. 1986. **Variability in callus derived plants of an interspecific hybrid in *Nicotiana***. *Proc. Symp. Plant cell and tissue culture of economically important plants*. (Osmania University, Hyderabad: 1986: July 24-26), p. 271-276.

**917** VIEGAS, P; MATHEWS, H; BHATIA, CR; NOTANI, NK. 1987. **Monohybrid and dihybrid segregations in the progenies of tobacco transformed for kanamycin resistance with a Ti-vector system**. *Journal of Genetics*, 66: 1, 25-31; 20 ref.

A chimaeric DNA construct, consisting of nopaline synthase promoter, neomycin phosphotransferase (NPT) gene coding sequences and octopine synthase polyadenylation sequences bracketed by T-DNA, was introduced into tobacco leaf discs via *Agrobacterium tumefaciens* containing plasmid pGV3850::1103. Shoots developed directly from leaf discs or from callus following culture on medium containing kanamycin. Hybrid analysis of 9 transformed plants showed that the NPT gene segregated in a monohybrid ratio in progeny of 7 of the plants. Segregation data from the other 2 plants indicated that there were at least 2 NPT insertions on different chromosomes or on the same chromosome at 2 unlinked sites.

## Propagation

**918** BAJAJ, YPS. 1988. **Regeneration of plants from frozen (-196°C) protoplasts of *Atropa belladonna* L., *Datura innoxia* Mill. and *Nicotiana tabacum* L.** *Indian Journal of Experimen. Biology*, 26: 4, 289-292; 12 ref.

Suspensions of protoplasts isolated enzymatically from mesophyll cells and callus cell suspensions were subjected to -196° for 5 min in the presence of a cryoprotectant solution consisting of 10% DMSO, 1 M mannitol and 4% sucrose, followed by thawing in warm water at 35°C and culturing. Initial cell division of protoplasts treated with the cryoprotectant solution with or without freezing was delayed by 3-5 days compared with the control, but all 3 species developed shoots and roots. A.

*belladonna* protoplasts subjected to freezing had a higher frequency of regeneration than *N. tabacum*, but overall regeneration in both species was lower than in control treatments. Regenerated plants produced normal flowers and fertile seed with normal germination when grown in pots. *D. innoxia* [*D. fastuosa*] gave only occasional regeneration.

**919** KHATOON, K. 1985. **Flower induction in *Nicotiana tabacum* cv. *Virginica* in sterile culture**. *Pakistan Journal of Botany*, 17: 2, 195-204; 11 ref.

Flower buds were induced in vitro in whole stem explants of tobacco cv. *Virginica* and in thin epidermal layers excised from stem segments. Cultures were maintained in MS medium supplemented with 10<sup>-6</sup> M IAA and kinetin and adjusted to pH 5.0. The explants supported flower bud induction and growth, with more buds produced on epidermal explants than stem segments. These buds contained viable pollen grains resembling E-type grains (small light-staining grains thought to be involved in the formation of embryoids in anthers) of flower buds produced in vivo. All stages of meiotic and mitotic sequences were recorded.

## *Nicotiana*

**920** CHATTERJEE, S; RAJASEKHAR, EW. 1983. **Microsurgical induction of heterokaryons between plant protoplasts and protozoan cells**. *Proceedings of Indian National Science Academy, Part B: Biological Sciences*, 49: 6, 647-652; 13 ref.

Protoplasts of *Atropa belladonna*, *Nicotiana tabacum* (cv. *Virginia Gold*) and *Phaseolus aureus* [*Vigna radiata*] (cv. *Jalgaon 781*) were introduced into *Amoeba proteus* using microsurgical techniques. Within heterokaryocytes, plant nuclei divided repeatedly during the first 15 days after protoplast introduction. Autoradiography confirmed the heteronucleate nature of the cells. After 4 weeks, the number of plant nuclei/amoeba declined progressively, with concomitant formation of micronuclei.

**921** GANGADEVI, T; RAO, PN; RAO, BH; SATYANARAYANA, KV. 1985. **A study of morphology, cytology and sterility in interspecific hybrids and amphidiploids of *Nicotiana knightiana* X *N. umbratica***. *Theoretical and Applied Genetics*, 70: 3, 330-332; 4 ref.

Interspecific hybrids and amphidiploids of *N. knightiana* (n = 12) X *N. umbratica* (n = 23) resembled either parent in some characters and were intermediate in other characters. The F1 hybrids (2n = 35) showed mostly

univalents during meiosis, while the amphidiploids ( $2n=70$ ) formed bivalents almost regularly. The former were completely sterile and the latter fully male fertile but predominantly female sterile due to disintegration of the embryo sacs leading to collapsed ovules. The few fertile ovules, however, showed normal development of the embryo sac and and embryo. The occurrence of fertile and sterile ovules is believed to be due to segregation of genes governing sterility.

**922 KUMAR, V; MAHERCHANDANI, N. 1988. Differentiation in callus cultures of a tobacco (*Nicotiana tabacum* cv. *White Burley*) variant: some biochemical aspects. *Plant Cell, Tissue and Organ Culture*, 14: 3, 177-185; 17 ref.**

A slow-growing variant plant with distinct foliar and floral morphology was obtained in tissue cultures of White Burley. The root and shoot differentiation in the callus derived from normal plants occurred on the 8th and 12th day, respectively, but took 10 and 14 days, respectively, in the variant callus. Amylase and acid phosphatase activities and starch and soluble carbohydrate contents were studied in non-differentiating callus (NDC), root differentiating callus (RDC) and shoot differentiating callus (SDC). The activities of amylase and acid phosphatase were lower in the variant than normal and their maximum coincided with the appearance of roots or shoots. There was more starch accumulation in normal callus on differentiating media, but the variant showed a less pronounced change. Normal callus under differentiating conditions also showed a greater increase in soluble carbohydrates than the variant, and this was maintained till roots and shoot appeared. The lag exhibited by the variant in differentiating was reflected in slow development of enzyme activities and low starch and sugar concentrations.

**923 PRASAD, GSR; SUBHASHINI, U; VENKATESWARLU, T. 1985. A new technique for mitotic chromosomes from in vitro shoot tips of *Nicotiana* hybrid. *Tobacco Research*, 11: 2, 182-183; 5 ref.**

*N. tabacum* cv. *PCT4* was crossed with *N. plumbaginifolia*, which is resistant to *Cercospora nicotianae*, *Meloidogyne* and *Phytophthora nicotianae* var. *nicotianae*. Fertilized ovules, 17 days old, were germinated on Nitsch medium. The 6 seedlings which germinated were multiplied by in vitro culture on Murashige & Skoog medium supplemented with growth regulators. Shoot tips, 15 days old, taken from the regeneration medium, were treated with colchicine and fixed in Carnoy's fluid for 24 h prior to regressive bulk carmine staining. Examination revealed that the chromosome

number varied in the range  $2n = 30-34$ . The technique is considered useful for mitotic studies on shoot tips obtained in vitro.

**924 RAAGEEVA-BIMAL. 1987. Anther culture of *Nicotiana plumbaginifolia* Viv. *Current Science*, 56: 16, 838-840; 2 ref.**

Plantlets were obtained following culture of anthers at the uninucleate stage of pollen development (taken from surface-sterilized flower buds 1.7 cm long) on MS [Murashige & Skoog] medium supplemented with 1 p.p.m. NAA and 5 p.p.m. BAP [benzyladenine] and incubated at  $25 \pm 5^\circ\text{C}$  and 50-60% RH. The plantlets were rooted on MS medium containing 1.5 p.p.m. IBA. The ontogeny of embryoid formation is described.

**925 SUBHASHINI, U; UNNIKISHNAN, M; SATYANARAYANA, KV. 1978. Directy Style and Stigma neoformations from stigma explants of *Nicotiana tabacum* L. *Current Science*, 14: 508-509.**

**926 SUBHASHINI, U; VENKATESWARLU, T; ANJANI, K. 1986. Embryo rescue in *Nicotiana* hybrids by in vitro culture. *Plant Science, Irish Republic*, 43: 3, 219-222; 11 ref.**

Flowers of *N. benthamiana* were pollinated by *N. tabacum*. Immature fertilized ovules were excised on the 13th day after pollination and cultured on Nitsch medium. The hybrid nature of the F1 progeny was confirmed cytologically, and fertility was restored by colchicine treatment.

**927 SUBHASHINI, U; VENKATESWARLU, T. 1985. Genotypes and their response to in vitro production of haploids in F1 lines of *Nicotiana tabacum*. *Theoretical and Applied Genetics*, 70: 3, 225-226; 11 ref.**

When anthers of 14 genotypes were cultured on Nitsch medium [see *Phytomorphology* (1969) 19, 389-404], Cy37, Cy55, Cy86, Cy85 and Cy89, all hybrids involving at least one mutant parent, gave the best responses (>100 responding anthers/250 anthers cultured).

**928 SUBHASHINI, U; VENKATESWARLU, T; ANJANI, K; PRASAD, GSR. 1985. In vitro hybridization in an incompatible cross *Nicotiana glutinosa* X *Nicotiana megalosiphon*. *Theoretical and Applied Genetics*, 71: 3, 545-549; 9 ref.**

Immature ovules of *N. glutinosa* were excised 7 days after pollination by *N. megalosiphon* and cultured on Nitsch medium. Callusing, shooting and rooting were achieved on modified Murashige & Skoog media, and

about 50 plants were obtained with chromosome numbers ranging from  $2n = 28$  to  $2n = 32$ . The hybrids resembled *N. glutinosa* at the time of flowering, but the flowers resembled those of *N. megalosiphon*.

**929** SUBHASHINI, U; UNNIKRISHNAN, M. 1981. **Studies on the development of haploid plants from anther cultures of *Nicotiana tabacu*, L.** *Advances and challenges in crop production technology, marketing and processing of tobacco: National Symposium on Tobacco*. (4th: Trivandrum: 1981), p. 42.

**930** UNNIKRISHNAN, M; SUBHASHINI, U. 1992. **Callusing and regeneration from juvenile tissue of *Nicotiana cavicola*.** *New Trends in Biotechnology*, Central Tuber Crops Research Institute, Trivandrum, p. 93-98.

**931** UNNIKRISHNAN, M; SUBHASHINI, U. 1992. **Tissue Culture for overcoming seedling lethality in the interspecific hybrid *Nicotiana debnevi* x *N. tabacum*.** *New Trends in Biotechnology. Section 1: Crop productivity*, Central Tuber Crops Research Institute, Trivandrum, p. 135-140.

**932** UNNIKRISHNAN, M; SUBHASHINI, U; SATYANARAYANA, KV. 1981. **Tissue culture in interspecific hybridization in *Nicotiana*.** *Tobacco Research*, 7: 1, 62-67.

## Tea

**933** ALAM, AFMB. 1994. **Tissue culture in Tea.** *Workshop on Present Status and Future Direction of Biotechnological Research in Bangladesh*. (BARC, Dhaka: 1994: June 25).

**934** ARULPRAGASAM, PV; LATIFF, R; SENEVIRATNE, P. 1988. **Studies on the tissue culture of tea (*Camellia sinensis* (L.) O. Kuntze). 3. Regeneration of plants from cotyledon callus culture.** *Sri Lanka Journal of Tea Science*, 57: 1, 20-23; 6 ref.

Studies were undertaken to investigate conditions that would be suitable for high-frequency somatic embryogenesis and plant regeneration in tea using callus cultures derived from cotyledon tissues. Pieces of cotyledons were first established and made to grow in agitated liquid media and then transferred to solid callusing media, where they showed good growth and callus formation. These cultures, when grown under high light intensity (2000 lx), developed embryoid-like structures which later turned green. In sucrose-enriched

media, the frequency of embryogenesis increased appreciably; however, further development was not observed until they were transferred to the regeneration media, which contained higher levels of cytokinin (BA at 10 mg/litre) and low concentrations of auxins. After 8-10 weeks in the regeneration media, the embryos developed into plantlets with distinct shoots and roots. In some instances, only root formation was observed. The plantlets were successfully acclimatized and planted in soil.

**935** BANO, ZAKIA; RAJARATHNAM, S; MOHANTY, BD. 1991. **Somatic embryogenesis in cotyledon cultures of tea (*Thea sinensis* L.).** *Journal of Horticultural Science*, 66: 4, 465-470; 13 ref.

A procedure to induce somatic embryos from cotyledons of tea seeds is described. Embryogenic culture was obtained from 70% of tender cotyledons (diameter 3-8 mm, with light brown and yellow seed coat) cultured on MS basal medium, supplemented with 2,4-D (0.5 mg/litre) and kinetin (0.05 mg/litre). The ability to produce an embryogenic culture declined as cotyledons matured. When the 2,4-D concentration was increased in the medium, large compact bulging tissues tended to form. However, these tissues when cultured separately on a medium low in 2,4-D and kinetin, produced pro-embryonic cell masses and, eventually, plants. Masses of globular embryos formed in embryogenic culture; these retained their growth and morphogenic ability in a maintenance medium, and on transfer to a growth regulator-free medium or a medium containing kinetin (0.05 µg/litre), 30% matured to form heart- to torpedo-shaped embryos. Of these 40% readily germinated on half-strength MS medium supplemented with kinetin (0.05 mg/litre), glucose (1.5%) and activated charcoal (0.2%) to form complete plants. Morpho-histological evidence suggests that somatic embryos developed directly from cotyledon cells.

**936** BARTHAKUR, MKP; MITRA, GC. 1990. **Nutrient requirements for growth and multiplication of tea plants in vitro.** *Bang. Jrl. of Bot.*, 19: 1, 65-71.

Nodal segments of sterile seedlings and of shoots taken after plucking were cultured on 3 basal media: MS1, MS2 and MS3, which were modifications of MS. These media were supplemented with several combinations of growth regulators. The best result with direct regeneration (9-12 shoots/explant) was obtained with MS2 + 1 mg IAA/litre + 3 mg kinetin/litre. Repeated subculturing of daughter shoots produced a large number of shoots within 16-24 weeks. The best result with regeneration from nodal callus (7-8 shoots/explant) was ob-

tained in MS2 + 1 mg NAA/litre + 3 mg BA/litre. By repeated subculturing of callus pieces containing regenerated shoot buds in MS2 + 1 mg IAA/litre + 3 mg kinetin/litre, a large number of well developed shoots was obtained within a further 10-12 weeks. Shoots produced by direct or indirect regeneration were excised and cultured in half-strength MS or MS2 media with 2-8 mg IBA/litre + 0.5 mg NAA/litre. Roots were formed on 60-70% of shoots in the half-strength MS + 8 mg IBA/litre treatment after 15-25 days. Root development of shoots was variable, but those having one thick root with a few thin ones gave the highest survival rate when transplanted. It is suggested that the use of nodal segments of tender shoots ready for plucking would result in improved regeneration.

**937 CHAUDHURY, REKHA; RADHAMANI, J; CHANDEL, KPS. 1991. Preliminary observations on the cryopreservation of desiccated embryonic axes of tea (*Camellia sinensis* (L.) O. Kuntze) seeds for genetic conservation. *Cryo letters*, 12: 1, 31-36; 6 ref.**

Excised embryonic axes were successfully cryopreserved after desiccation to a moisture content of < 13%. Survival and growth for 20-25 days was achieved on filter paper moistened with water. Survival beyond 25 days and accelerated growth of embryonic axes was obtained by culturing on a Nitsch and Nitsch nutrient medium. Healthy seedlings of about 5-6 cm length could thus be obtained from cryopreserved axes.

**938 HAZARIKA, M; MAHANTA, PK. 1984. Compositional changes in chlorophylls and carotenoids during the four flushes of tea in north east India. *Journal of the Science of Food and Agriculture*, 35: 3, 298-303; 28 ref.**

The quantitative changes of chlorophylls a and b, beta-carotene, lutein, violaxanthine and neoxanthine, were investigated in teas from a deep skiffed area between April and October 1982. The tea flushes with different genetic properties were taken from the Tocklai-released clones TV-1 (*Camellia sinensis*), TV-2 (*C. assamica*), TV-9 (*C. assamica* cv. Cambod) and TV-17 (*C. sinensis*, hybrid). They showed marked changes in these constituents throughout the plucking period, which affected the organoleptic property of the black tea produced. The effects of weather conditions on pigment biosynthesis are discussed.

**939 JHA, TB; JHA, SUMITA; SEN, SK. 1992. Somatic embryogenesis from immature cotyledons of an elite Darjeeling tea clone. *Plant Science Limerick*, 84: 2, 209-213; 11 ref.**

Cotyledons from immature embryos of *Camellia sinensis* cv. T-78 were exposed to various levels of 2,4-D, NAA, IBA, BAP [benzyladenine] and kinetin (1-10 mg/litre). While no embryos were formed in MS basal medium without growth regulators, the auxins tried were also inefficient in stimulating primary embryogenesis. Somatic embryos were induced directly on the cotyledonary explants in MS medium with 10 mg BAP/litre alone. Somatic embryo formation was enhanced by addition of 0.5 mg IBA and 80 mg adenine to 10 mg BAP/litre. Embryo conversion was 2-3% in MS medium with or without growth regulators. Almost 20% of somatic embryos converted into whole plants following transfer to B5 medium containing 3 mg BAP and 2 mg IBA/litre. Embryogenic potential was maintained for over 30 months by secondary embryogenesis through successive generations of embryos. Somatic embryo-derived plants were successfully transferred to potted soil at 70% frequency under greenhouse conditions. Morphologically and cytologically ( $2n = 30$ ) plants were true to type.

**940 JHA, TIMIRBARAN; SEN, SK. 1992. Micropropagation of an elite Darjeeling tea clone. *Plant Cell Reports*, 11: 2, 101-104; 14 ref.**

Shoot cultures of *Camellia sinensis* cv. T-78, an elite Darjeeling tea clone, were established from cotyledonary nodes and shoot tips of germinated seedlings as well as from nodal explants of field grown plants. Cotyledonary nodes produced the highest mean number of micro-shoots/explant (35.12) on half strength MS medium supplemented with 10 mg BA, 2 mg IBA, 10 mg coconut milk and 1000 mg casein hydrolysate/litre, after 18 weeks in culture. Rooting was achieved in 80-90% micro-shoots by either placing them on an inductive medium for 10 d and then transferring shoots to hormone-free medium, or by treating micro-shoots with a chronic dose of IBA (500 mg/litre) for 30-40 min. Rooted plants were established in soil under glasshouse condition at 60% frequency after a hardening phase of 4-6 weeks. The regenerated plants showed a constant chromosome number of  $2n = 30$  and were morphologically true to type. This procedure could be applied to the conservation and utilization of elite clones of Darjeeling tea.

**941 LASKAR, MA; DAS, SC; DEKA, PC; BHATTACHARYYA, S. 1993. Tissue Culture of tea: Anthers Culture for haploid plant production. *Teatech*, p. 194-201.**

Anthers from two clones of tea namely TV1 and TV13 were used for the production of haploid callus. Uninu-

cleate stage of the pollen were found to be the best material for haploid calli induction. Bud size can be used for initial screening and selection of anthers. However the mean pollen diameter and the colour of the anthers should be used as the final indicator for selection of right stage (uninucleate) of pollen for anther culture. Highest frequency (10% for TV1 and 27% for TV13) of haploid calli induction observed, 3-4 weeks after inoculation, in a modified media containing MS nutrient salts, vitamins of N6 medium and with 1.5 mg 1-1 2, 4-D, 2.5 mg 1-1 BAP and 6% sucrose.

**942 PHUKAN, MK; MITRA, GC. 1984. Regeneration of tea shoots from nodal explants in tissue culture. *Current Science*, 53: 16, 874-876; 5 ref.**

Nodal explants from seedlings, shoot tips and mature twigs with axillary buds were cultured on a supplemented Murashige & Skoog medium. In the presence of NAA (1 mg/litre) and BA (3 mg/litre), the explants from twigs produced callus showing the initiation of shoot buds (indirect regeneration); but in a medium lacking NAA and BA but containing IAA (2 mg/litre) and kinetin (8 mg/litre) shoot buds were initiated directly from the explants without the production of callus (direct regeneration). The latter shoot buds developed further only in media with low contents of auxins, cytokinins and salts. It was observed that direct regeneration usually resulted in clonal plants but that genetic variability occurred among plants produced by indirect regeneration.

**943 SAHA, DK; BHATTACHARYA, NM. 1988. In vitro development of shoot apices from pollen callus in the anther cultures of tea (*Camellia sinensis* L. O. Kuntz). *Cell and tissue culture in field crop improvement: Proceedings of a Seminar*. (Tsukuba, Japan: 1987: October 4-9)/edited by J Bay-Petersen. Food and Fertilizer Technology Center for the Asian and Pacific Region Taipei, Taiwan. p. 135-145; 34 ref.**

Flower buds of different sizes were collected from the varieties TV1 and TV23 and the Tocklai generative clone (TGC) 124/488 and cultured on 6 different media. Histological studies were made on differentiating callus tissue. Only anthers from TGC 124/488 responded uniformly and a higher proportion of callus was induced (26.33%) on B5 medium. Anthers containing uninucleate microspores had the highest response; these were from buds 0.77-0.83 cm in diameter. Organogenesis was achieved in 12.6% of anthers on the same B5 medium but with increased sucrose (7%) and added glutamine (400 mg/litre).

**944 SARATHCHANDRA, TM; UPALI, PD; WIJEWARDENA, RGA. 1988. Studies on the tissue culture of tea (*Camellia sinensis* (L.) O. Kuntze). 4. Somatic embryogenesis in stem and leaf callus cultures. *Sri Lanka Journal of Tea Science*, 57: 2, 50-54; 9 ref.**

Callus formed at the basal ends of nodal explants of clone TRI2025 when cultured on Vacin & Went medium supplemented with green coconut water and on MS medium supplemented with 0.1 mg IBA and 1.0 mg BAP (benzyladenine)/litre, and on cut margins of leaf pieces in a callusing medium; the pH of the 3 media was 5.7. High sucrose enhanced the multiplication rate of calluses from nodal explants, although better callus formation was observed when whole leaves were employed. Embryoid-like structures formed in nodal explants after 8 weeks.

**945 SATYANARAYANA, N; SHARMA, VS. 1982. Controlled hybridization in tea (*Camellia* spp.). Preliminary studies on compatibility between clones. *Genetics, plant breeding and horticulture: Proceedings of the fourth annual symposium on plantation crops (Placrosym IV)*. (Mysore: 4th: 1981: Dec 3 - 5)/edited by S Vishveshwara. Div. of Botany, UPASI Tea Research Institute, Tamil Nadu, India. p. 302-307; 6 ref.**

In 1979-81, 29 crosses were made involving 12 clones of the Assam (*C. assamica*), China (*C. sinensis*) and Cambod (*C. assamica* subsp. *lasiocalyx*) types. Fruit set in crosses within types was highest in Assam. Assam types as female parents also gave the highest fruit set in crosses with the other two types, followed by China. Cambod was the best pollen parent in crosses between types. F1 plants were more vigorous than plants raised from cuttings of their respective clonal parents.

## Propagation

**946 ARULPRAGASAM, PV; LATIFF, R. 1986. Studies on the tissue culture of tea (*Camellia sinensis* (L.) O. Kuntze). 1. Development of a culture method for the multiplication of shoots. *Sri Lanka Journal of Tea Science*, 55: 1, 44-47; 8 ref.**

In vitro proliferation of shoots of tea, clones TRI 2025, CY 9 and PK 2 was achieved on amended Murashige and Skoog medium (details given). Shoot tips 4-5 cm in length from actively growing shoots and nodal segments were used as explants. Better shoot proliferation occurred with BA at 2 than at 1 mg/litre. No attempt was made to induce rooting.

**947 SARWAR, M.** 1985. **Callus formation from explanted organs of tea (*Camellia sinensis* L.).** *Sri Lanka Journal of Tea Science*, 54: 1, 18-22; 15 ref.

Browning of explants, which occurred when different parts of tea plants were cultured, was reduced by lowering the salt concentration to 1/20th of the normal contents in Murashige and Skoog medium. Stem pieces produced better callus when high concentrations of auxins, 2,4-D and NAA (2 X 10<sup>-5</sup>M) and low concentrations of cytokinins, BA and kinetin (10<sup>-5</sup>M) were present in the medium. 2,4-D (4.5 X 10<sup>-5</sup>M) and yeast extract (0.2%) together were conducive to callus growth. Bud growth occurred on BA (10<sup>-5</sup>M) when the cultures were incubated in the dark for the first 10 days before maintaining under light. Callus production was highest in leaf petioles.

**948 SENEVIRATNE, P; LATIFF, R; ARULPRA-GASAM, PV.** 1988. **Studies on the tissue culture of tea (*Camellia sinensis* (L.) O. Kuntze). 2. Rooting of shoots produced in culture.** *Sri Lanka Journal of Tea Science*, 57: 1, 16-19; 12 ref.

Following successful establishment and multiplication of tea in culture using shoot tips and nodal segments as explants [see *Sri Lanka Journal of Tea Science* (1986) 55, 1, 44-47], the work carried out in rooting the shoots produced in vitro are reported in this paper. Shoots of clones TRI 2025 and CY9, and shoots regenerated directly from cotyledons in culture, were successfully induced to produce roots on basic MS medium with IBA at 0.1, 0.2, 1 and 2 mg/litre, without cytokinins. Differences in genotypic responses to root initiation were observed with different clones requiring different concentrations of auxins for root initiation. The rooted plantlets were transferred to soil and were acclimatized in a humid chamber. No rooting was induced in clones of the 3000 series (TRI 3011 and TRI 3017).

## Insect pests control

**949 BORTHAKUR, MC; RAGHUNATHAN, AN.** 1987. **Biological control of tea looper with *Bacillus thuringiensis*.** *J. of Coffee Research*, 17: 1, 120-121.

The results of an evaluation study of the use of *Bacillus thuringiensis* subsp. *thuringiensis* strain HB III for the biological control of the geometrid *Buzura suppressaria* were presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems held in India in 1986. Fifty percent mortality of 3rd-, 4th- and 5th-instar larvae occurred at spore concentrations of 62.4, 135.8 and 177.8 mg/l after 148,

120 and 96 h exposure, respectively. It was concluded that *Bacillus thuringiensis* could arrest the feeding of *Buzura suppressaria* even at sublethal dosages and could be effectively used to minimize crop loss in tea.

**950 DANTHANARAYANA, W; VITARANA, SI.** 1987. **Control of the live-wood tea termite *Glyptotermes dilatatus* using *Heterorhabditis* sp. (Nemat.).** *Agriculture, Ecosystems and Environment*, 19: 4, 333-342; 19 ref.

*Glyptotermes dilatatus* is the main pest of lowland-grown tea in Sri Lanka, affecting some 42 000 ha. The concealed way of life of the pest makes it difficult to control by chemical, classical biological or other conventional methods. It was recently found that the nematode *Heterorhabditis* sp., which was isolated in Darwin, Australia, and mass-produced by CSIRO entomologists, infects the termite and is able to kill it and breed in the cadaver both in the laboratory (at 22°C and 100% RH) and under extreme climatic conditions in the field (mean temperature 28.3°C; temperature range 19.5-38.4°C; only 7 days low rainfall between 20 December 1981 and 28 February 1982). Within each cadaver, up to about 3500 juveniles were produced which were able to infect healthy termites in the laboratory and in the field, creating a chain of infection leading to the eradication of a colony. In the laboratory, using the dosage mortality equation, an LD<sub>50</sub> of 3670 ± 564 nematodes/ml was predicted, which suggested that a very high application rate would be needed to obtain 99.9% mortality. In the field, experimental application of infective larvae at 4000 and 8000 nematodes/ml and dosages of 40 and 30 ml/bush, respectively, gave complete control within 60-95 days. The cost of control was US\$2.39 (50.20 Sri Lankan rupees)/1000 infected bushes (1987 values).

**951 MURALEEDHARAN, N.** 1986. **Pest management in tea. A guide to the field study and control of tea pests in South India.** 88 p.

The 12 chapters in this booklet on pest management on tea in southern India include a general account of concepts in pest management and separate chapters dealing with the incidence, injuriousness, biology and control (including notes on biological control where relevant) of mites, thrips, Lepidoptera, Coleoptera, Hemiptera, other insect pests, nematodes and rats. Detailed recommendations for pesticides and their application are brought together in a final chapter. Some illustrations to aid in recognizing pests and their damage are provided.

952 SELVASUNDARAM, R; MURALEEDHARAN, N. 1987. **Natural enemies of certain leaf folding caterpillar pests of tea in southern India.** *Journal of Coffee Research*, 17: 1, 118-119.

The results of surveys of natural enemies of the main lepidopterous pests of tea carried out in Tamil Nadu and Kerala, India, since 1983, were presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems. The larvae of the tortricid *Cydia leucostoma* were parasitized by the braconids *Apanteles aristaeus*, *Fornicia sp.* and *Apanteles sp.* and the ichneumonids *Eriborus sp.* and *Plectochorus sp.*, of these species the 1st was the most common. The main predators of *C. leucostoma* included the carabid *Calleida nilgirensis*, the coccinellids *Jauravia pubescens* and *Menochilus sexmaculatus*, the lygaeid *Pseudopachybrachius guttus* and the hemerobiid *Micromus timidus*. The gracillariid *Caloptilia theivora* was parasitized by the eulophids *Sympiesis dolichogaster* and *Mestocharella javensis*. The tortricid *Homona coffearia* has been controlled by the introduction of the braconid *Macrocentrus homonae* and was also attacked by several indigenous natural enemies.

## Coffee

### Breeding

953 RAGHURAMULU, Y. 1989. **Anther and endosperm culture of coffee.** *Journal of Coffee Research*, 19: 2, 71-81; 19 ref.

Anthers cultured on a modified MS medium supplemented with 0.2 mg IAA and 0.2 mg benzyladenine (BA)/litre at microsporogenesis (3 days after pollination) produced callus profusely. However, the calluses failed to respond to further subcultures. There were genotypic differences in response to anther culture. When immature endosperms from 6-month-old beans of Robusts hybrids were cultured, calluses formed which became embryogenic upon transfer to MS medium supplemented with 2 mg BA and 1 mg IAA/litre. However, no further response was observed.

954 RAGHURAMULU, Y; SREENIVASAN, MS; RAMAIAH, PK. 1989. **Regeneration of coffee plantlets through tissue culture techniques in India.** *Journal of Coffee Research*, 19: 1, 30-38; 9 ref.

Soft internodal sections from orthotropic shoots of *Coffea arabica* and *C. canephora*, and immature fruits of *C. arabica* cv. *San Ramon* were collected, washed and surface sterilized with 0.5% HgCl<sub>2</sub> (+ a few drops

of lactic acid for stem sections) for 5 min (stems) or 10 min (fruits) and then rinsed with sterile water. Explants 2-3 mm in length were taken from the stem sections and dipped in a 1% L-cysteine HCl solution for 5 min before inoculation into culture tubes containing MS medium supplemented with 1mg IAA and 4.3 mg kinetin/litre. The cultures were incubated in the dark. Torpedo-shaped embryos were removed from the immature fruits and transferred to culture tubes containing MS medium supplemented with 1mg BA, 1mg IAA and 6g activated charcoal/litre; the cultures were kept under high light intensity. The sterilization procedures for stem explants and embryos were 80-100% and 100% successful, respectively. Sub-culturing of calluses obtained from stem explants resulted in formation of somatic embryos which were cultured further to give 25-30 plantlets per culture. Following a 3-month hardening-off period in sterile vermiculite medium, approximately 90% of plantlets were established on transfer to a soil medium. Root and shoot growth occurred in all the embryo cultures, leaves appearing after 7-8 weeks. Establishment of plantlets in sterile vermiculite medium was good.

955 RAM, AS; SREENIVASAN, MS. 1982. **A chemotaxonomic study of *Coffea arabica* L.** *Genetics, plant breeding and horticulture: Proceedings of the Annual symposium on plantation crops.* (4th: Mysore: 1981: Dec 3-5)/edited by S Vishveshwara. Central Coffee Research Institute, Coffee Research Station 577 117, Chikmagalur District, Karnataka, India. p. 368-374; 13 ref.

Tests for phenols, tannins, flavonoids, steroids and other compounds were made on *C. arabica*, four putative relatives and a *C. liberica* X *C. eugenioides* hybrid. Evaluation of data using the coefficient of similitude indicated a close relationship between *C. stenophylla* and both *C. arabica* and *C. congensis*. The hybrid proved to be closer to *C. arabica* than to either of its parent species.

956 RAM, AS; RAMACHANDRAN, M; SREENIVASAN, MS. 1982. **Pollen fertility of the interspecific hybrids of coffee.** *Genetics, plant breeding and horticulture: Proceedings of the Annual symposium on plantation crops.* (4th: Mysore: 1981: Dec 3 - 5)/edited by S Vishveshwara. Central Coffee Research Institute, Coffee Research Station Chikmagalur, Karnataka, India. p. 406-412; 11 ref.

The pollen stainability and (shown in brackets) germinability are as follows: *Coffea congensis* X *C. eugenioides* hybrid S2361, 89.6% (76.2%); *C. congensis* X *C.*

*canephora* hybrid S885, 81.1% (27.6%); *C. canephora* X *C. eugenioides* hybrid S1713, 96.2% (85.3%); *C. canephora* X *C. liberica* hybrid S1712, 82.0% (53.6%); *C. excelsa* X *C. eugenioides* hybrid S1715, 59.6% (18.9%); *C. racemosa* X *C. canephora* hybrid S3678, 13.5% (1.7%) and *C. liberica* X *C. eugenioides* hybrid S1721, 15.5% (14.0%).

**957 REDDY, AGSM; RAJU, KVVS; DHARMARAJ, PS.** 1982. **Fruit set in coffee under different modes of pollination in Andhra Pradesh.** *Genetics, plant breeding and horticulture: Proceedings of the annual symposium on plantation crops.* (4th: Mysore: 1981: Dec 3-5)/edited by S Vishveshwara. Regional Coffee Res. Sta., Chinthapalli, 531 133, Andhra Pradesh, India. p. 252-256; 13 ref.

The 6 *Coffea arabica* derivatives and four interspecific hybrids studied all set fruit under natural cross pollination (with emasculation), although to a lesser extent than on selfing. S2464, a spontaneous tetraploid from a sterile diploid F1 of *C. liberica* X *C. eugenioides*, was both cross compatible with its diploid parents and self compatible.

**958 SREENIVASAN, MS.** 1982. **Aneuploids in Robusta X Arabica hybrids.** *Genetics, plant breeding and horticulture: Proceedings of the Annual symposium on plantation crops.* (4th: Mysore: 1981: Dec 3-5)/edited by S Vishveshwara. Central Coffee Res. Inst., Coffee Res. Sta., 577 117, Karnataka, India. p. 38-43; 10 ref.

Of five plants which did not set fruit in a BC2F1 population (S1156) from a *Coffea canephora* X *C. arabica* hybrid, one was a nullisomic, one a trisomic and three were monosomics. The aneuploids were morphologically similar to their normal counterparts.

**959 SREENIVASAN, MS.** 1982. **On megaspore tetrads of coffee.** *Genetics, plant breeding and horticulture: Proceedings of the Annual symposium on plantation crops.* (4th: Mysore: 1981: Dec 3 - 5)/edited by S Vishveshwara. Central Coffee Res. Inst., Coffee Res. Sta., 577 117, Karnataka, India. p. 9-10; 6 ref.

As well as the usual tetrad types, other functional forms designated isobilateral, decussate and cruciform were observed in *Coffea canephora* and *C. arabica*.

## Insect pests control

**960 Entomology/nematology.** *Thirty eighth annual detailed technical report 1984-85,* Central Coffee Res. Inst., Chikmagalur, Karnataka, India, p. 91-123; 16 ref.

The results are presented of investigations in India on the biology and control of pests of coffee, especially the insects *Xylotrechus quadripes*, *Xylosandrus compactus*, *Planococcus citri*, *Coccus viridis*, *Holotrichia spp.* and the nematode *Pratylenchus coffeae*. Special attention is paid to chemical control, natural enemies of insect pests and the biological control of *Planococcus citri*. A list is presented of about 40 pests and natural enemies that were identified.

**961 Plant protection.** *Thirty eighth annual detailed technical report 1984-85,* Central Coffee Res. Inst., Chikmagalur 577 117, Karnataka, India, p. 183-189.

The results are presented of investigations on the biology and control of insect pests of coffee at the Regional Research Station, Kalpetta, Kerala, for the Coffee Board Research Department, India. Special attention is paid to the biological control of *Planococcus citri*; the biology and natural enemies of *Tropicomyia sp.*; the biology of the predacious coccinellid *Cryptolaemus montrouzieri*; and studies on the biology and natural enemies of several minor pests of coffee. A list of about 15 species of insect pests and their natural enemies that were identified is presented.

**962 Plant protection.** *Thirty ninth annual report 1985-86,* Central Coffee Res. Inst., Coffee Res. Sta., 577 117, Chikmagalur District, Karnataka, India. 197-199.

The investigations on coffee pests reported from Kerala, India, include studies on the biological control of *Planococcus spp.* using the predatory beetle *Cryptolaemus montrouzieri* and the parasitoid *Leptomastix dactylopii*; the role of natural enemies (4 species of *Hymenoptera*) in suppressing populations of *Tropicomyia*; and a species of *Eupelmus* acting as a natural enemy of *Xylosandrus compactus* by preying on rather than parasitizing this scolytid.

**963 BHAT, PK.** 1987. **A review of entomological research in coffee with reference to major insect pests.** *Journal of Coffee Research*, 17: 1, 77-79.

The impact of major insect pests of coffee in India is reviewed in a paper presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems. Appropriate chemical and biological measures were recommended for the control of the cerambycid *Xylotrechus quadripes*, the scolytid *Xylosandrus compactus*, the pseudococcid *Planococcus spp.*, the coccid *Coccus viridis*, the scarabaeid *Holotrichia nilgiria* and *Eupterote spp.*

**964 BHAT, PK. 1987. Current status and possibilities of integrated pest management in coffee with special reference to white stem-borer.** *Journal of Coffee Research*, 17: 1, 126-128.

The use of integrated pest management for the control of insect pests of coffee in India is discussed in a paper presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems held in India in 1986. Cultural, chemical and biological methods for the control of several species were reviewed with emphasis on a cerambycid, the white stem-borer [*Xylotrechus quadripes*].

**965 BOOPATHY, R. 1989. The overall economics of anaerobic digestion on a coffee estate. 1. Design calculation.** *Indian Coffee*, LIII: 3, 3-6; 5 ref.

Design calculations are presented for 2 identical biogas plants, each producing 720 m<sup>3</sup> gas/d, 1 for generating electricity and 1 for cooking and lighting for 400 houses. Coffee waste and cattle manure are used as substrate. For the electricity generating plant, the calculations cover house and street lighting, water pumping, gas requirements for power generation, total organic waste and cattle requirements, plant layout and digester calculation. Total gas, coffee waste and cattle manure requirements, plant layout and vol. of daily charge are discussed for the cooking and lighting gas plant.

**966 CHACKO, MJ. 1987. Biological control with exotic as well as indigenous natural enemies.** *Journal of Coffee Research*, 17: 1, 109-113.

The basic principles of biological control are outlined in a paper that was presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems, held in India in 1986. On coffee, the coccinellid predator *Cryptolaemus montrouzieri* and the encyrtid parasitoid *Leptomastix dactylopii* have been effective for the control of the pseudococcid *Planococcus citri*, while on tea the tea tortrix [*Homona coffearia*] has been controlled by the braconid *Macrocentrus homonae*.

**967 MATHEW, G. 1987. Cossid pests of plantation crops in India and the prospects of their management.** *Journal of Coffee Research*, 17: 1, 137-140.

The behaviour patterns and pest management of cossids in plantation crops in India were reviewed at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems, held in India in 1986. Most cossid species are polyphagous, *Zeuzera coffeae*, a pest of tea and coffee, has over 40 food

plants. Many species also have a high reproductive rate, *Xyleutes persona* and *X. ceramica* lay about 60 000 and 1 000 eggs per female, respectively. Multiple infestations of trees are common. Chemical control methods are generally not practical in plantations but the use of insecticide implants is being investigated. Ants and birds are important predators and the ichneumonid parasitoid *Nemeritis tectonae* provides effective control of *X. ceramica*. *Beauveria bassiana* causes mortality in *Cossus cossus* and *X. ceramica* but an appropriate method of application is needed. Other control measures include the removal of infested trees for the control of *X. ceramica* in teak [*Tectona grandis*] plantations and the use of pheromones for mass trapping.

**968 NARASIMHAM, AU. 1987. Scale insects and mealybugs on coffee, tea and cardamom and their natural enemies.** *Journal of Coffee Research*, 17: 1, 7-13; 25 ref.

Species of coccoids which have been recorded as pests of coffee, tea and cardamom in India are reviewed with reference to their status and natural enemies. The main pest species on coffee are *Planococcus citri*, *P. lilacinus*, *P. ficus*, *Coccus viridis* and *Saissetia coffeae*. The encyrtid *Leptomastix dactylopii* has been introduced for the control of *P. citri*. Forty-four species of coccoids have been recorded on tea, of which *Fiorinia theae*, *Eriochiton theae* and *Phenacaspis manni* [*Pseudaulacaspis manni*] are known to be pest species. The 1st of these species has been controlled by its parasitoids in Assam. *S. coffeae* and *Planococcus sp.* also occur as pests of cardamom, the latter being parasitized by *Aphidencyrtus sp.*

**969 PRAKASAN, CB. 1987. Biological control of coffee pests.** *Journal of Coffee Research*, 17: 1, 114-117.

The biological control of major pests of coffee in India is discussed in a paper presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems. The lycaenid *Spalgis epius*, the coccinellid *Pullus pallidicollis* [*Pseudoscymnus pallidicollis*] and the cecidomyiid *Triommata coccidivora* are predators of the pseudococcids *Planococcus citri* and *P. lilacinus*. The coccinellid predator *Cryptolaemus montrouzieri* and the encyrtid parasitoid *Leptomastix dactylopii* have been introduced for the control of *P. citri*. The coccid *Coccus viridis* is attacked by several indigenous natural enemies including the coccinellid predator *Chilocorus nigrita* and the entomogenous fungi *Verticillium lecanii* and *Empusa lecanii* [*Entomophthora lecanii*]. The cerambycid *Xylotrechus quadripes* is

parasitized by the braconid *Allorhogas pallidiceps*. The natural enemies of *Eupterote canaraica* and *E. fabia* include the entomogenous fungus *Beauveria bassiana*. The pseudococcids *Ferrisia virgata* and *Nipaecoccus viridis* and the agromyzid *Tropicomyia* are naturally controlled by different parasitoid species.

**970 RAMESH, PK. 1987. Observations on crop loss in robusta coffee due to mealybug and shot-hole borer. *Journal of Coffee Research*, 17: 1, 94-95.**

The results of studies on losses in robusta coffee due to the pseudococcid *Planococcus spp.* and the scolytid *Xylosandrus compactus* in India in 1984 and 1986 were presented at a workshop on insect pest management strategies in coffee, cardamom and tea cropping systems. The encyrtid *Leptomastix dactylopii* gave effective control of *Planococcus* and the crop loss due to this species varied from 14.38 to 17.08%. The losses due to *X. compactus* were 21.07% on 45-year-old plants and 23.49% on young plants.

**971 SRIVASTAVA, LS; VERMA, RN. 1987. New records of Rhizoctonia blight diseases of coffee and water hyacinth from India. *Bangladesh Journal of Botany*, 16: 2, 214-215; 2 ref.**

*R. solani* is reported causing much damage to coffee plants in Manipur nurseries and on water hyacinth [*Eichhornia crassipes*]. The possibilities of biological control of the latter weed is discussed.

## Papaver somniferum

**972 HANSEN, E BROCHMANN. 1984. A second pathway for the terminal steps in the biosynthesis of morphine. *Planta Medica*, 50: 4, 343-345; 21 ref.**

[2-3H] Oripavine was administered, using morphinone as a carrier, to mature *Papaver somniferum* plants from Tasmanian and Indian strains, the first of which contained oripavine. Incorporation of oripavine into morphine amounted to 28.1 and 21.4%, respectively. Morphinone generally decomposed during isolation. Thebaine and codeine were not radioactive showing that the 3-O-demethylation of thebaine was not reversible. The results suggest that oripavine represents a step in a secondary pathway from thebaine to morphine.

**973 HEBLE, MR. 1985. Multiple shoot cultures: a viable alternative in vitro system for the production of known and new biologically active plant constituents. *Primary and secondary metabolism of plant cell cultures*/edited by KH Neumann, W Barz, E Reinhard.**

Heidelberg, German Federal Republic: Springer-Verlag, p. 281-289; 34 ref.

The initiation and growth of multiple shoot cultures and their production of alkaloids and steroids is reviewed with reference to research on *Dioscorea composita*, *Digitalis*, *Catharanthus roseus*, *Papaver spp.*, *Cinchona spp.*, *Rauwolfia serpentina*, *Chrysanthemum cinerariaefolium* [*Tanacetum cinerariifolium*], *Atropa belladonna* and *Withania somnifera*.

**974 KHANNA, KR; SHUKLA, S. 1986. HPLC investigation of the inheritance of major opium alkaloids. *Planta Medica*, No. 2: 157-158; 9 ref.**

Opium collected from capsules of 2 *Papaver somniferum* lines, *P. setigerum* and their F1 and F2 interspecific hybrids was analysed using high pressure liquid chromatography. Some F1 plants showed heterosis for codeine and thebaine concentration. In some F2 plants concentrations of morphine, thebaine, narcotine and papaverine exceeded the concentrations found in the parents and F1; codeine, however, did not show this effect. Narcotine, which was absent from *P. setigerum*, was absent or present in small amounts in the F1 and F2, suggesting that its absence is almost completely dominant to its presence.

## SPICE CROPS

### Cuminum cyminum

**975 CHATTOPADHYAY, D; SHARMA, AK. 1990. Chromosome studies and estimation of nuclear DNA in different varieties of *Cuminum cyminum* L. and *Carum copticum* Benth and Hook. *Cytologia*, 55: 4, 631-637; 21 ref.**

Somatic chromosome number was determined to be  $2n=14$  in 3 varieties of *C. cyminum* and  $2n = 18$  in 2 varieties of *C. copticum* [*Trachyspermum ammi*]. Most chromosomes of *C. cyminum* possessed submedian or subterminal constrictions. Karyotype analysis showed gross morphological similarity in *C. cyminum* despite the evolution of different varieties. No marked variations in length and volume of chromosomes were observed among the varieties. Study of DNA content also revealed varietal constancy, with slight variation in the different varieties of *C. cyminum*. A proportionate increase in DNA content was recorded with the increase in chromosomal length and volume in the varieties. A gross homogeneity in chromosome morphology was also found in the 2 varieties of *C. copticum*. The majority of chromosomes possessed either a nearly median or a