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SAARC Agricultural Information Centre (SAIC)

SAARC Agricultural Information Centre (SAIC)
BARC Complex, Farmgate, Dhaka 1215, Bangladesh

Published : 1995

Cover design : Mafruha Begum

Price : US$ 10.00 for SAARC countries
US$ 15.00 for other countries

Chandel, A S and Kamal, R M
Dynamics of agricultural biotechnology: SAARC bibliographical database. Dhaka: SAARC Agricultural Information Centre, 1995.
ii, 321, liii p.
1. Biotechnology, bibliography. 2. Agricultural biotechnology, bibliography. 3. SAARC Agricultural Information Centre. i. Jt. Author. ii. Title.

Published by : Director, SAARC Agricultural Information Centre (SAIC)
Printed at : Panir Printers, 9 Nilkhet, Dhaka 1205

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A method for producing about 5000 plants in a year from one nodal segment of *R. officinalis var. genuina* forma erectus is described. About 14 shoots differentiated in 30 days from each shoot apex excised from aseptically established plants and cultured in a medium containing 0.2 mg/litre BA. Some 80% of isolated shoots then rooted in 7 days in the presence of 0.25 mg/l indolepropionic acid. The in vitro-raised plants grew normally in soil under greenhouse conditions.

1195 MADHUJAIN; MISRA, P; BANERJI, R; NIGAM, SK; CHATURVEDI, HC; SCHEFFER, JJC; LOOMAN, A; SVENDSEN, AB. 1986. **The essential oils from in vitro grown shoots and from callus of *Rosmarinus officinalis L.* var. *genuina forma erectus***. *Acta Botanica Neerlandica*, 35: 1, 48; 1 ref.

Shoot apices were established on a modified White medium and proliferated on a modified Murashige and Skoog medium, while callus was grown on a modified Schenk and Hildebrandt medium. The essential oil content of cultured shoots increased with culture age from 20 to 40 days; after 40 days it was 1.8% (isolated by solvent extraction), compared with 0.42% for callus. Shoots of 1- year-old field grown plants contained 2.4% essential oil.

1196 MISRA, P; CHATURVEDI, HC. 1984. **Micropropagation of *Rosmarinus officinalis L.***. *Plant Cell, Tissue and Organ Culture*, 3: 2, 163-168; 17 ref.

Single-node stem segments were better explants than shoot tips (c. 2 cm long) for establishment of field-grown plants in aseptic cultures. BA was far more effective than kinetin for shoot induction in shoot tips excised from aseptically-grown plants. Maximum numbers of shoot buds (c. 14) were formed per explant at 0.2 mg/l BA in 30 days. After further growth of isolated shoots and treatment with 0.25 mg/l indolepropionic acid for 7 days, 80% of the shoots produced roots. In vitro raised plantlets were successfully grown in soil to maturity. About 5000 plants could be produced from a single nodal segment in 1 year.

MEDICINAL PLANTS

Azadirachta indica

1197 GAUTAM, VK; NANDA, K; GUPTA, SC. 1993. **Development of shoots and roots in anther-derived callus of *Azadirachta indica A. Juss.* - a medicinal tree**. *Plant Cell, Tissue and Organ Culture*, 34: 1, 13-18.

1198 ISLAM, R; JOARDER, OI. 1992. **Organogenesis and embryogenesis in neem (*Azadirachta indica*), a powerful source of insecticides**. *Proc. COMSTECH-NIAB Workshop on Agroclimatology, Pests and Diseases and Their Control*. (Faisalabad, Pakistan), p. 40.

1199 ISLAM, R; JOARDER, OI; ZAMAN, ATMN; HOSSAIN, M. 1993. **Plant regeneration from nuclear tissues of *Azadirachta indica A. Juss***. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21)

1200 ISLAM, R; JOARDER, OI; ZAMAN, ATMN; KHALEQUZZAMAN, MA; HOQUE, A. 1993. **Plant regeneration from seedling explants of *Azadirachta indica A. Juss***. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21).

1201 ISLAM, R; JOARDER, OI; ZAMAN, ATMN; HOSSAIN, M. 1993. **Somatic embryogenesis and plant regeneration from embryonic tissue of *Azadirachta indica A. Juss***. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21).

1202 JOARDER, N; JOARDER OI; ISLAM, R; BISWAS, BK. 1993. **In vitro response of nucellar tissue and plant regeneration through somatic embryogenesis form cultured cotyledons of neem**. *World Neem Conf.* (Bangalore, India), p. 60.

1203 JOARDER, N; ISLAM, R; JOARDER, OI. 1993. **Micropropagation of *Azadirachta indica A. Juss* through axillary bud culture**. *Proceedings of the World Neem Conf.* (Bangalore, India), p. 56.

1204 JOARDER, OI; ISLAM, R; ZAMAN, ATMN; HOSSAIN, M. 1993. **Micropropagation of neem through axillary bud culture**. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21).

1205 KABIR, A; AZAD, AK; HOSSAIN, SN; JOARDER, OI; HAKIM, L; HOSSAIN, M. 1995. **In vitro regeneration of *Azadirachta indica Adr. Juss.* from immature cotyledon**. *Annual Plant Tissue Culture Conference*. (Dhaka University, Dept. of Botany: 1995: March 19).

1206 KHATUN, R; ARA, M; ISLAM, S; HOSSAIN, MT. 1995. **Tissue culture technology for neem**

propagation. *Annual Plant Tissue Culture Conference*. (Dhaka University, Dept. of Botany: 1995: March 19).

1207 RAMESH, K; PADHYA, MA. 1988. **Isolation of nimbin from *Azadirachta indica* leaves and its callus cultures**. *Indian Drugs*, 25: 12, 526-527; 4 ref.

1208 RAO, MVS; RAO, YV; RAO, YS; MANGA, V. 1988. **Induction and growth of callus in *Azadirachta indica Juss***. *Crop Improvement*, 15: 2, 203-205; 4 ref.

Cotyledons, leaves and stem segments were cultured on MS medium supplemented with different concentrations of various growth hormones. Data indicated that fresh cotyledons were the best source followed by leaves and that the combination of 2,4-D and IAA was the most effective in producing calluses.

1209 ROY, SK; ASADUZZAMAN, M. 1993. **Clonal propagation of *Azadirachta indica* by in vitro culture**. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21).

Calotropis gigantea

1210 DATTA, SK; DE, S. 1986. **Laticifer differentiation of *Calotropis gigantea R. Br. ex Ait.* in cultures**. *Annals of Botany*, 57: 3, 403-406; 14 ref.

The initiation and subsequent development of laticifers were studied in callus tissues grown on Murashige and Skoog medium supplemented with 1 mg IAA/litre. Laticifer development was related to the age of the culture and could be preserved by repeated subculturing on similar medium with IAA. The maximum number was produced after 160 days of culture. BA was also found to stimulate laticifer formation, though to a lesser extent. When BA or kinetin was applied in conjunction with IAA, laticifer differentiation was poorer than with IAA alone. The presence of cardenolides was confirmed in laticifers but they were not detected in the neighbouring parenchyma cells.

1211 ROY, AT; DE, DN. 1990. **Tissue culture and plant regeneration from immature embryo explants of *Calotropis gigantea (Linn.) R. Br.*** *Plant Cell, Tissue and Organ Culture*, 20: 3, 229-233; 16 ref.

Callus cultures were established from immature embryos of this latex-producing plant (under investigation as a possible source of hydrocarbons) on a modified MS basal medium supplemented with 2,4-D at 1 mg/litre. The best results in terms of bud formation (93.8%) were obtained using NAA at 0.1 mg/litre and BA at 1.0 mg/litre. Rooting was induced when shoots were transferred to auxin-supplemented Bonner's solution or half-strength MS basal salt solutions.

Cephaelis ipecacuanha

1212 JHA, S; JHA, TB. 1989. **Micropropagation of *Cephaelis ipecacuanha* Rich**. *Plant Cell Reports*, 8: 8, 437-439; 12 ref.

Shoot cultures of this medicinal plant were established by inoculating seedling nodal explants onto modified MS medium supplemented with 8 mg kinetin, 0.05 mg NAA and 200 mg adenine per litre. Up to 12 new axillary shoots per explant were induced after 12 weeks incubation. Shoot cultures were also established by placing shoot tips on medium containing 0.1-0.25 mg NAA with 8 mg kinetin per litre for 4 weeks and then on shoot multiplication medium for 8 weeks. The multiplication was maintained over several passages. Shoots were rooted using 2 mg IBA/litre and normal plants were re-established.

1213 JHA, S; SAHU, NP; MAHATO, SB. 1988. **Production of the alkaloids emetine and cephaeline in callus cultures of *Cephaelis ipecacuanha***. *Planta Medica*, 54: 6, 504-506; 10 ref.

Callus cultures were established from hypocotyl explants on various media (Murashige & Skoog, Shenk & Hildebrandt, B5 or White) containing various combinations of IBA, IAA and NAA. The best callus growth in terms of both fresh and dry weight was obtained on B5 medium supplemented with 3% sucrose, 8 mg/litre IBA, 4 mg/litre IAA and 4 mg/litre NAA. The highest alkaloid yields were obtained with a medium containing the macronutrient elements of Shenk & Hildebrandt, supplemented with the above combination of growth regulators; cephaeline at 0.93% and emetine at 0.346% were obtained.

1214 JHA, SUMITA; SAHU, NP; SEN, JAYANTI; JHA, TB; MAHATO, SB. 1991. **Production of emetine and cephaeline from cell suspension and excised root cultures of *Cephaelis ipecacuanha***. *Phytochemistry*, 30: 12, 3999-4003; 23 ref.

Production of the ipecac alkaloids, emetine and cephaeline, was studied in cell suspension and excised root cultures of *C. ipecacuanha*. A 2-stage cell suspension culture was developed for enhanced accumulation of the alkaloids. In the first-stage, suspension cultures were established in an MS medium containing 2,4-D and NAA which was suitable for cell growth, and the second-stage culture system was composed of MS medium containing IBA, IAA and 6% sucrose which

favoured alkaloid production. The production of emetine and cephaeline was greatly increased in the 2-stage culture method compared with single-stage culture. Optimal alkaloid synthesis was obtained in excised root cultures in a medium composed of half-strength MS salts, IBA (0.25 mg/l) and 2% sucrose. Cephaeline accumulation in 2-stage cell suspension culture and in excised root culture was higher than that found in 3-year-old roots.

Coleus forskohlii

1215 AKHILA, A; RANI, K; THAKUR, RS. 1990. **Biogenetic relationship of polyoxygenated diterpenes in *Coleus forskohlii***. *Phytochemistry*, 29: 3, 821-824.

1216 SHARMA, N; CHANDEL, KPS; SRIVASTAVA, VK. 1991. **In vitro propagation of *Coleus forskohlii Briq.*, a threatened medicinal plant**. *Plant Cell Reports*, 10: 2, 67-70; 7 *ref.*

Micropropagation was achieved using nodal segments cultured on MS medium supplemented with 2 mg kinetin and 1 mg IAA/litre. Shoots multiplied at a rate of 12-fold every 6 weeks. Rooting occurred when shoots were transferred to MS medium containing 1 mg IAA/litre. Regenerated plants were successfully transferred to the field. *Forskolin* content of tubers of micropropagated plants was the same as for wild plants (0.1%).

Costus speciosus

1217 BANNERJEE, S; SHARMA, AK. 1989. **Quantitation of diosgenin in different cytotypes of *Costus speciosus* and its correlation with the amount of nuclear DNA**. *Cytobios*, 58: 234-235, 141-147; 29 ref.

Diosgenin content of 4 different populations collected from the central and eastern Himalayas, representing 3 different cytotypes, was determined by thin-layer chromatography and UV spectrophotometric methods. Significant variation in diosgenin content was recorded amongst these populations, being greatest in one of the diploid (2n = 18) populations (1.65%) and lowest in the tetraploid (2n = 36) population (0.39%). The highest diosgenin content was associated with the lowest total chromosome length and nuclear DNA content, while the lowest diosgenin content corresponded with the highest DNA content and chromosome length. No such trends were observed in the other populations. It is suggested that duplication of chromosome sets does not necessarily promote the genetic control of diosgenin content.

1218 CHATURVEDI, HC; MISRA, P; JAIN, M. 1984. **Proliferation of shoot tips and clonal multiplication of *Costus speciosus* in long-term culture**. *Plant Science Letters*, 35: 1, 67-71; 15 ref.

Shoot tips excised from mature rhizomes of a high diosgenin yielding clone and cultured on a modified Schenk and Hildebrandt (SH) medium supplemented with 0.5 mg/litre BA and 1.0 mg/litre IAA formed on average 10 adventitious shoots/shoot tip within 60 days. About 90 true-to-type plants could be produced from a single shoot tip in one year. Rhizome-like structures developed after 2-3 subcultures and they became a continuous source of material for further propagation. All the regenerated shoots rooted in SH medium supplemented with 1.0 mg/l IAA and were successfully transplanted to soil.

1219 JAIN, M; CHATURVEDI, HC. 1985. **Caulogenesis in rhizome callus of *Costus speciosus***. *Planta Medica*, No. 5: 462-463; 5 ref.

Costus speciosus is a potential source of diosgenin for the manufacture of corticosteroidal drugs. Rhizome tissue explants callused within 30 days of incubation on a modified Schenk and Hildebrandt medium supplemented with 10 mg adenine sulphate, 0.25 mg BA, 0.5 mg IAA and 100 mg malt extract/litre. When the isolated callus was subcultured on the same medium, roots formed in all cultures and were followed by shoot production in about 25% of cultures. However, these organs were not attached to each other. If the shoots were subcultured in a medium containing 2 mg IAA, 15 mg adenine sulphate, 0.25 mg BA and 200 mg malt extract/litre they developed normally and when they were transferred to basal medium containing 3 mg IAA, they rooted and developed into plantlets.

Dioscorea floribunda

1220 AMINUDDIN; CHOWDHURY, AR. 1983. **Production of diosgenin in somatic callus tissues of *Dioscorea floribunda***. *Planta Medica*, 48:2, 92-93.

Shoot-tip and tuber derived callus tissues cultured on revised tobacco medium supplemented with 1-2 mg 2,4-D/l contained 0.295 and 1.33% diosgenin, respectively.

1221 MEENA-SHARMA; CHATURVEDI, HC. 1988. **An in vitro method for production of tetraploid plants of *Dioscorea floribunda* Mart. & Gal**. *Indian Journal of Experimental Biology*, 26: 4, 280-284; 7 ref.

Rooted basal cuttings of 1-year-old plants devoid of shoots were grown in a medium supplemented with 0.1

mg kinetin, 15 mg adenine sulfate and 0.25 mg IAA/-litre to encourage shoot induction and growth. At the same time, cotton wool soaked with colchicine solutions (0.01-0.1%) was applied aseptically to the rooted bases for 1-5 days. A concentration of 0.05% applied for 2 days gave optimal induction of tetraploidy. Within 30 days about 10 tetraploid shoots (2n = 72) developed per culture, with more vigorous growth, stouter stems, larger and thicker leaves, and bigger stomata than the diploid control. Clonal tetraploid plants were produced by rooting single-node stem cuttings of tetraploid shoots in medium with 0.1 mg 2,4-D/litre. During 4 successive regeneration cycles under field conditions, none of 100 tetraploid plants raised in vitro reverted to diploidy.

1222 SENGUPTA, J; MITRA, GC; SHARMA, AK. 1985. **Excised root culture of *Dioscorea floribunda* for diosgenin synthesis**. *Indian Journal of Experimental Biology*, 23: 2, 107-109; 7 ref.

The ability of roots (as opposed to tubers) to synthesize diosgenin was investigated. Continuous culture of excised roots was achieved and maintained successfully by repeated excision of 1-cm long root tips and subculturing them at 40-day intervals for 48 weeks. Cultures were incubated in shaking conditions in darkness at 25° ± 1°C. Half strength Murashige and Skoog basal medium plus NAA (0.025 mg/litre) and BA (0.01 mg/litre) with 2% sucrose was best for normal and continued growth. Laterals were formed in the subapical region of the main axis within 10 days of culture. Cultured root tips showed aneuploid (8%) and polyploid (4%) chromosome numbers in addition to diploid. However, no diosgenin was detected in the cultured roots.

1223 SENGUPTA, J; MITRA, GC; SHARMA, AK. 1984. **Organogenesis and tuberization in cultures of *Dioscorea floribunda***. *Plant Cell, Tissue and Organ Culture*, 3: 4, 325-331; 8 ref.

Callus cultures were established from node and internode segments. Both Murashige & Skoog (MS) and modified White's medium supported callusing as well as organogenesis when supplemented with either 2,4-D or NAA in combination with BA or kinetin. On development of shoot primordia, calluses were transferred to unsupplemented, half-strength MS basal medium. This procedure led to an increase in shoot formation. Several crops of shoots were obtained from single differentiating callus cultures by excising the shoots and subculturing the residual part. Seventy percent of plantlets survived rooting and transfer to soil. When maintained in half-strength MS basal medium and 0.5 mg litre-1 of NAA, 70% of plantlets formed aerial tubers at nodes. These tubers produced both roots and shoots and could be detached from the mother plant.

1224 SENGUPTA, J; MITRA, GC; SHARMA, AK. 1989. **Steroid formation during morphogenesis in callus cultures of *Dioscorea floribunda***. *Journal of Plant Physiology*, 135: 1, 27-30; 20 ref.

Sterols and diosgenin production was studied by chromatographic and spectrophotometric methods at different phases of callus growth of *D. floribunda* using various hormonal regimes. Sitosterol, stigmasterol and diosgenin were the major biosynthetic products in callus during all phases of growth. An increase in the amount of phytosterols in calli was noted during shoot bud formation, but the level of diosgenin decreased during shoot bud formation.

1225 SENGUPTA, JAYANTI; MITRA, GC; SHARMA, AK. 1986. **Chromosomal behaviour in cultured cells of *Dioscorea floribunda***. *Cytologia*, 51: 2, 219-224; 8 ref.

Callus cultures maintained on media supplemented with different combinations of growth regulators were studied for up to a year. Cytological analysis revealed that all cultures contained a mixture of euploid and aneuploid cells. The frequency of polyploid cells increased with the age of the culture. Multipolar anaphases, budded cells with chromosomes migrating from the polyploid mother cell to the bud, and multinucleate cells were observed.

Plantago ovata

1226 BARNA, KS; WAKHLU, AK. 1988. **Axillary shoot induction and plant regeneration in *Plantago ovata* Forssk**. *Plant Cell, Tissue and Organ Culture*, 15: 2, 169-173; 6 ref.

The optimum medium for inducing axillary shoots was found to be MS supplemented with 4.6 μM kinetin and 0.05 μM NAA. Rooting of shoots was best on half-strength MS medium containing 5.0 μM IBA and 0.05 μM kinetin. The regenerated plants were similar to the control plants in karyotypic and phenotypic details.

1227 WAKHLU, AK; BARNA, KS. 1989. **Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk. cv. GI-2**. *Plant Cell, Tissue and Organ Culture*, 17: 3, 235-241; 7 ref.

A technique for callus initiation and growth and plantlet regeneration from cultured hypocotyl explants of

Plantago ovata cv. GI2 (used for medicinal purposes in India) is described. Best initiation and growth of callus were achieved on MS medium containing 2,4-D (1 mg/litre) and kinetin (1 mg/litre). The callus showed maximum shoot differentiation on medium containing kinetin (4 mg/litre) and NAA (0.01 mg/litre). Root formation on shoots was best on half-strength medium supplemented with IBA. The regenerated plantlets were successfully transferred into pots.

Rauvalfia serpentina

1228 BANERJEE, NIRMALYA; SHARMA, AK. 1985. **Nuclear DNA and analysis of heterochromatin in different species and populations of *Rauvolfia L.***. *Proceedings of Indian National Science Academy, Part B: Biological Sciences*, 51: 4, 505-510; 26 ref.

In a study of 18 wild and cultivated Indian populations of *Rauwolfia [Rauvolfia] serpentina*, *R. canescens* and *R. vomitoria* there was significant interspecific and intraspecific variation in nuclear DNA content; that of *R. canescens* (2n = 66) was less than double that of *R. serpentina* (2n = 22). Heterochromatin content, estimated from interphase blocks, bore a similar relation to nuclear DNA content in all populations of *R. serpentina* and *R. canescens*. It is considered that heterochromatin other than that in interphase blocks might have been eliminated when the polyploid *R. canescens* was formed, accounting for its unexpectedly low DNA content.

1229 BRACHER, D; KUTCHAN, TM. 1992. ***Strictosidine synthase* from *Rauvolfia serpentina*: analysis of a gene involved in indole alkaloid biosynthesis**. *Archives of Biochem. and Biophysics*, 294: 2, 717-723.

The gene for strictosidine synthase (str1), the enzyme which catalyzes the stereospecific condensation of tryptamine and secologanin to form the key indole alkaloid 3 alpha(S)-strictosidine has been isolated from genomic libraries prepared from *Rauvolfia serpentina* (India) and from *Rauvolfia mannii* (West Africa). The gene, str1, contained no introns and showed 100% nucleotide sequence homology over 1180 bp, encompassing the entire reading frame, between the two species. Transcription of the *R. serpentina* gene was found to start 81 nucleotides upstream from the AUG (26 nucleotides downstream from the TATA box). Transient expression assays in *Nicotiana plumbaginifolia* protoplasts of the *R. serpentina* str1 5'-noncoding region fused to the beta-glucuronidase reporter gene revealed promoter activity equivalent to 4 +/- 2% of that of 35 S CaMV promoter control. A series of truncated segments of the str1 promoter region indicated the presence of three areas of slight, but reproducible, negative control. Gel retardation assays demonstrated that several regions of the 5'-flanking sequences specifically bound nuclear protein from *R. serpentina* and that at least one region does not bind *R. mannii* nuclear protein. A survey of the expression of str1 in the *R. serpentina* plant suggested that strictosidine synthase poly(A)+ RNA was present predominantly, but not exclusively, in the root. This result correlated well with the distribution of both enzyme activity and indole alkaloids which were also predominant in the root, but, in general, distributed throughout the shrub.

1230 ILAHI, I; AKRAM, M. 1987. **Root callus cultures of *Rauwolfia serpentina Benth***. *Pakistan Journal of Scientific and Industrial Research*, 30: 3, 224-229; 16 ref.

Root explants, obtained from mature plants and juvenile aseptic seedlings, were subcultured for callus formation with 2,4-D, NAA and K [kinetin]. Explants taken from seedling roots gave the best response for callus formation on WRC (White's Root Culture) medium containing 1 mg/litre 2,4-D and 100 ml/litre coconut milk. Callus propagation in subcultures was good both on initiation medium and on AM (Abou-Mandour) medium with BA, K, AS, NAA, 2,4-D and casein hydrolysate at 0.1, 0.3, 4.0, 1.0, 6.0 and 1000 mg/litre, respectively. The alkaloids screened were serpentine, ajmaline, raubasine, raupine and reserpine. The major alkaloid present in cultures was ajmaline. Its maximum percentage was 0.0573% in the cultures grown in the dark on AM medium, corresponding to an increase of 94.61% over cultures kept in 16 h light.

1231 PERVEEN, R; AKRAM, M; ILAHI, I. 1982. **Differentiation and anatomical changes in callus cultures of *Rauwolfia [Rauvolfia] serpentina Benth***. *Pakistan Journal of Forestry*, 32: 3, 81-85; 12 ref.

1232 ROJA, G; BENJAMIN, BD; HEBLE, MR; PATANKAR, AV; SIPAHIMALANI, AT. 1990. **The effect of plant growth regulators and nutrient conditions on growth and alkaloid production in multiple shoot cultures of Rauwolfia serpentina**. *Phytotherapy Research*, 4: 2, 49-52; 8 ref.

Multiple shoot cultures of *R. [Rauvolfia] serpentina* were established from axillary meristems of the field-grown plants on MS medium supplemented with 1.0 p.p.m. BA and 0.1 p.p.m. NAA. Growth regulators influenced the morphogenetic events of the shoot cultures, namely root initiation in IAA combinations, stunted shoot formation in 2,4-D and slender shoot

formation in kinetin + NAA combinations. The morphogenetic responses were associated with marginal changes in alkaloid production. The cultures produced high levels of alkaloids including ajmaline (0.15%), ajmalidine and 3-epi-alpha-yohimbine in Zenk's production medium. The alkaloid concentrations in the cultures were comparable to those in the roots of the intact plant. The shoot cultures showed stable growth and alkaloid production during 5 years.

1233 ROJA, PC; BENJAMIN, BD; HEBLE, MR; CHADHA, MS. 1985. **Indole alkaloids from multiple shoot cultures of Rauwolfia serpentina**. *Planta Medica*, No. 1: 73-74; 10 ref.

Shoot cultures from axillary meristem explants of field-grown plants contained yohimbine, ajmaline (both of which occur in the roots) and ajmalidine (found in leaves, but not roots).

1234 ROJA, PC; SIPAHIMALANI, AT; HEBLE, MR; CHADHA, MS. 1987. **Multiple shoot cultures of *Rauvolfia serpentina*: growth and alkaloid production**. *Journal of Natural Products*, 50: 5, 872-875; 13 ref.

Leaf and stem explants gave actively growing callus tissues on MS medium supplemented with 2,4-D at 2 mg and BA at 1 mg/litre. Multiple shoots developed from axillary meristem explants on MS medium containing BA (1 mg) in combination with either NAA (0.1 mg) or IAA (2 mg). Sustained growth of the shoot cultures was achieved in MS liquid medium containing BA (1 mg) and NAA (0.1 mg). The shoot cultures yielded significantly high levels of alkaloids (ajmalidine, yohimbine, 3-epi-alpha-yohimbine and ajmaline) and contained compounds found in the roots as well as in the shoots of intact plants.

1235 ROY, SK; HOSSAIN, Z; ALAM, N. 1993. **Mass propagation of *Rauvolfia serpentina* by in vitro shoot tip culture**. *International Plant Tissue Culture Conference*. (Dhaka Univ., Dept. of Botany: December 19-21).

1236 SARKER, KP; ISLAM, A; ISLAM, R; JOARDER, OI. 1993. **Production of secondary metabolites by in vitro grown tissues of *Rauvalfia serpentina Benth***. *International Plant Tissue Culture Conf.* (Dhaka Univ., Dept. of Botany: December 19-21).

1237 SHARMA, N; CHANDEL, KPS. 1992. **Low-temperature storage of *Rauvolfia serpentina Benth. ex Kurz.*: an endangered, endemic medicinal plant**. *Plant Cell Reports*, 11: 4, 200-203; 14 ref.

On a standard MS shoot culture medium supplemented with 1 mg BAP [benzyladenine] and 0.1 mg NAA/litre, nodal cultures of 2 varieties, Delhi local and Indore local, were maintained for 9 months at 25°C by replacing cotton plugs with polypropylene caps as enclosures for culture tubes (33.3% survival). In vitro cultures stored at the lower temperature of 15°C exhibited normal health even after 15 months (66.6% survival). However, storage temperatures of 10°C and 5°C were found deleterious to growth.

1238 SINGH, IS; SINGH, CP. 1987. **Response of excised roots of *Rauvolfia serpentina Benth.* to growth regulatory substances**. *Acta Horticulturae*, No. 208: 141-155; 15 ref.

IAA, IBA, NAA and 2,4-D at low conc. (<0.5 mg/l) promoted root apex growth in a Ca-deficient medium but for lateral root formation auxins were required in relatively higher concentrations. Root tip meristem had an inhibitory effect on lateral root initiation in its proximity. In response to auxins, lateral root formation decreased with successive subculturing. NAA with kinetin and 2,4-D produced prominent subapical swelling in the root apices by activating the division of cortical tissues. Only 2,4-D was effective for callus formation. MH, 2,4,5-T and TIBA inhibited root apex growth.

Rheum emodi

1239 LAL, N; AHUJA, PS. 1993. **Assessment of liquid culture procedures for in vitro propagation of *Rheum emodi***. *Plant Cell, Tissue and Organ Culture*, 34: 2, 223-226.

1240 LAL, NAND; AHUJA, PS. 1989. **Propagation of Indian rhubarb (*Rheum emodi Wall.*) using shoot-tip and leaf explant culture**. *Plant Cell Reports*, 8: 8, 493-496.

Shoot-tip explants of this medicinal plant gave rise to multiple shoots when cultured on MS medium with 2.0 mg 6-benzylaminopurine [benzyladenine] (BAP) and 1.0 mg IBA per litre. Shoot buds developed from leaf explants on MS medium with 2.0 mg BAP and 0.25-1.0 mg IAA or IBA per litre. Roots were induced when shoots were placed on MS medium with 1.0 mg IBA per litre. Both regeneration procedures gave rise to healthy plantlets that were established in soil under greenhouse conditions at 80% frequency after a hardening phase of 2 weeks. Regenerated plants showed a constant chromosome no. of 2n=2x=22. The use of liquid shake cultures minimized the time and culture medium requirements for propagation.

Ruscus hypophyllum

1241 JHA, S; SEN, S. 1985. **In vitro regeneration of *Ruscus hypophyllum L.* plants**. *Plant Cell, Tissue and Organ Culture*, 5: 1, 79-87; 12 ref.

Callus cultures were established from stem explants of *R. hypophyllum* (a source of the sapogenin ruscogenin) on a modified basal Murashige and Skoog medium (MS) supplemented with 1 mg/litre 2,4-D + 0.1 mg/litre BA. The optimal 2,4-D concentration for promoting shoot bud formation and growth was 0.05 mg/litre along with 0.5 mg/litre BA. Sixty percent of rootless shoots produced flowers on the regenerating medium. Rooting was induced when shoots were transferred to half strength MS inorganic salts supplemented with 2 mg/-litre IBA. 80% of plants transferred to soil survived.

1242 JHA, SUMITA; SEN, JAYANTI; SEN, SUMITRA. 1989. **Stable regenerants from long-term callus cultures of *Ruscus hypophyllum L.*** *Cytologia*, 54: 4, 687-691; 8 ref.

Detailed karyological analyses of *R. hypophyllum* (a source of the sapogenin ruscogenin) are presented. Calluses were maintained on media containing 0.5 mg benzyladenine/litre for 5 years. Root tips and flower buds from regenerated plants were utilized. No significant differences between cultured and non-cultured plants were found in morphology or cytology.

Sapindus trifoliatus

1243 DESAI, HV; BHATT, PN; MEHTA, AR. 1986. **Plant regeneration of *Sapindus trifoliatus L.* (soapnut) through somatic embryogenesis**. *Plant Cell Reports*, 5: 3, 190-191; 9 ref.

Callus was induced from leaf explants and grown on Murashige and Skoog medium supplemented with 2 mg/litre 2,4-D and 0.5 mg/litre kinetin. Gradual reduction of the 2,4-D concentration during subsequent subcultures resulted in the formation of embryoids which developed further when transferred to a medium containing BA and kinetin and then to a hormone-free medium. Unless 5-methyl-tryptophan was added and the level of sucrose raised to 4%, the embryoids began to form callus again and failed to form plantlets. Between 350 and 400 plantlets were obtained from a single leaf explant in 19 weeks.

1244 UNNIKRISHNAN, SK; MEHTA, AR; BHATT, PN. 1990. **Abscisic acid induced high frequency embryogenesis from *Sapindus trifoliatus* leaves**. *Acta Horticulturae*, No. 280: 89-94; 14 ref.

The effect of sucrose and mannitol at concentrations of 2-12% on somatic embryogenesis from leaf explants of this tropical tree and medicinal plant species was evaluated. In the sucrose series, frequency of normal somatic embryos was increased to 25% in 8% sucrose. In mannitol, lower concentrations (2 and 4%) favoured the formation of secondary somatic embryos, but at high concentrations cultures died. Low concentration of sucrose in conjunction with abscisic acid (1 μM) induced around 50% normal somatic embryos capable of forming plants. Somatic embryos formed in a medium supplemented with abscisic acid and 2% sucrose accumulated the greatest amount of protein. Protein profiles of zygotic and somatic embryos were compared using SDS-PAGE. Polypeptides of somatic embryos were identical to those of the germinated zygotic embryos and not to the dormant embryos.

Smilax zeylanica

1245 JHA, S; GUPTA, JS; SEN, S. 1987. **Tissue culture of *Smilax zeylanica L.*** *Acta Horticulturae*, No. 208: 273-279; 9 ref.

Callus cultures were established from stem explants on modified MS basal medium (BM) supplemented with 5 mg/litre kinetin + 2 mg/litre NAA + 1 mg/litre 2,4-D + 2.5 mg/litre IBA. Shoot buds developed after 8 weeks in the BM containing 5 mg/litre kinetin + 2.5 mg/litre IBA. A combination of 1 mg/litre BA + 0.05 mg/litre 2,4-D was most suitable for shoot multiplication and development from differentiating calluses. Rooting was induced when shoots were transferred to half-strength MS inorganic salts supplemented with 2 mg/litre IBA. The undifferentiated and differentiating calluses contained more diosgenin than the shoots.

1246 KAR, DK; SEN, S. 1984. ***Smilax zeylanica* Linn. - a new source of diosgenin**. *Current Science*, 53: 12, 661; 9 ref.

Diosgenin was extracted from the roots, leaves and callus of young shoot segments cultured on Murashige and Skoog basal medium supplemented with IBA (1.0 mg/litre) and kinetin (0.5 mg/litre).

Solanum

1247 BARNABAS, NJ; DAVID, SB. 1988. **Solasodine production by immobilized cells and suspension**

cultures of *Solanum surattense*. *Biotechnology Letters*, 10: 8, 593-596; 12 ref.

Cell suspensions derived from leaf callus were cultured in MS medium containing 1 p.p.m. kinetin and 2 p.p.m. NAA. Cells immobilized in calcium alginate beads were cultured in a hormone-free medium or a medium containing kinetin and NAA. Suspension cells showed high intracellular accumulation of solasodine, whereas the immobilized cells released high quantities into the medium. The quantity of solasodine released from immobilized cells was initially largest in the hormone-free medium, but declined in this medium after 19 days of culture whereas it was maintained in the hormone-supplemented medium for up to 27 days of incubation.

1248 DEBATA, BK; PATNAIK, SN. 1988. **Induction of androgenesis in anther cultures of *Solanum viarum* Dunal**. *Journal of Plant Physiology*, 133: 1, 124-125; 8 ref.

Anthers of the medicinal plant *S. viarum* at different stages of pollen development (late uninucleate, mitotic and early binucleate) were cultured on MS medium supplemented with 2 mg IAA/litre and 1-3 mg kinetin/litre. Mixed calluses with haploid (n = 12), diploid and polyploid cells were obtained within 5 weeks. The callus proliferated from both pollen and somatic tissues.

1249 JAISWAL, VS; NARAYAN, P. 1985. **Plantlet regeneration from hypocotyl callus of *Solanum torvum Swartz*.** *Journal of Plant Physiology*, 119: 5, 381-383; 8 ref.

Plantlets of this medicinal species were produced after 6 weeks following culture of hypocotyl explants on Murashige & Skoog medium supplemented with 2 mg BA/litre and 1 mg NAA/litre. The plant growth regulators required for shoot and for root initiation are detailed.

Solanum khasianum

1250 BARNABAS, NJ; KARMALAWALA, R; DAVID, SB. 1989. **Stimulation of solasodine production by growth regulators in callus cultures of *Solanum spp*.** *Indian Journal of Experimental Biology*, 27: 7, 664-665; 10 ref.

2,4-D enhanced the solasodine production in stem and leaf callus cultures of *Solanum indicum*, *S. khasianum* and *S. surattense* more than IAA, NAA, kinetin or BAP [benzyladenine]. There was an increase in solasodine production of *S. surattense* in the field when whole plants were sprayed with low concn of 2, 4-D.

1251 BORUA, PK. 1990. **Failure in an interspecific cross between *Solanum khasianum* Clarke and *Solanum mammosum L.*** *Euphytica*, 46: 1, 1-6; 14 ref.

Reciprocal crosses were made between *S. khasianum* (indigenous to India) and *S. mammosum* (an introduction from South America), using long and short-styled forms of each species. Fruit set in the interspecific crosses was extremely low and only detected in a cross between long-styled *S. mammosum* and long-styled *S. khasianum*, with fruits remaining seedless. Pollen viability was higher in the long-styled flowers in both species, (93.6 and 88.7% in *S. khasianum* and *S. mammosum*) compared with 87 and 72% in short-styled counterparts. The identical chromosome numbers (2n = 24) and similar chromosome size found irrespective of style length could not account for the apparent hybridization barrier.

1252 NANDAKUMAR, D. 1985. **Intervarietal hybridization and induction of autotetraploids in steroid-bearing *Solanum* species**. *Mysore Journal of Agricultural Sciences*, 19: 1, 48.

Hybrids between 3 morphological types of *S. khasianum* differed significantly in number of berries per node, fresh and dry berry weight, dry matter in berries and number of spines per leaf. Epistatic gene action was predominant. One hybrid combined high berry yield and high solasodine content with few curved spines. Induced autotetraploids of the 3 types, and of their hybrids and backcrosses, had fewer spines than the diploids and delayed flowering and differed from the the diploids in specific leaf weight, pollen fertility, pollen size and 100-seed weight. There was marked variability in intervarietal hybrids of *S. incanum*; the inheritance of stem, spine, flower and fruit colours was elucidated.

Solanum nigrum

1253 BHATTACHARYYA, N; MALLIK, P; MALLIK, D; CHAUDHURI, RK. 1989. **Small angiosperm genome in *Solanum nigrum Linn*.** *Nucleus Calcutta*, 32: 1-2, 80-82; 15 ref.

Purified nuclear DNA of tetraploid (2n = 48) *S. nigrum* showed 2 prominent fractions, one fast and one slow reassociating. By computing reassociation rates, the genome size of *S. nigrum* was estimated as 0.307 pg (3 X 108 nucleotide pairs), the smallest value reported for an angiosperm other than *Arabidopsis thaliana* (.07 pg).

1254 BHATTACHARYYA, NANDAN; MUKHOPADHYAY, DK; CHAUDHURI, ILA; CHAUDHURI, RK. 1986. **Repetitive DNA amount in *Solanum nigrum* genome**. *Curr. Sci.*, 55: 12, 569-571; 16 ref.

Analysis of *S. nigrum* DNA revealed that it has an unusually low content of repetitive sequences, showing 11.56% hybridization at a C0t value at which plant repetitive DNAs normally reassociate.

1255 CHAUDHURI, RK; MUKHOPADHYAY, DK; BHATTACHARYYA, N; CHAUDHURI, I. 1985. **In vitro labelling of plant DNA with high specific activity**. *Nucleus, India*, 28: 3, 240-242; 8 ref.

The technique described, considered useful for molecular genetic studies, involves enzymatic nicking (nick translation or 3'-end labelling), followed by labelling with alpha-32P-deoxycytidine triphosphate. It was successfully applied to DNA from shoot tips of *Solanum nigrum* and shoot tips and callus of *Daucus carota*.

1256 GANAPATHI, A; RAO, GR. 1985. **Cytogenetics of some diploid species of the *Solanum nigrum* complex**. *Canadian Journal of Genetics and Cytology*, 27: 6, 735-740; 14 ref.

Crosses between *S. douglasii* and (1) *S. americanum*, (2) *S. nigrum* (Indian diploid form) and (3) *S. nodiflorum* were compatible if *S. douglasii* was used as male parent. Normal, fertile F1 hybrids were produced, but the F2 plants showed meiotic breakdown and reduced fertility. The isolation of *S. douglasii* from the other species is attributed to genetic and chromosome structural differences. It is concluded that *S. nodiflorum*, *S. nigrum* (2x) and *S. americanum* represent a single biological species, *S. americanum*, but that the treatment of *S. douglasii* as a separate species is justified.

1257 GANAPATHI, A; RAO, GR. 1985. **Spontaneous triploidy in *Solanum nigrum L.* complex**. *Current Science*, 54: 23, 1242; 5 ref.

From natural hybridization between *S. villosum* (2n = 48) and *S. americanum* (2n = 24), 3 sterile triploid plants were obtained, having irregular meiosis and n = 18 chromosomes. At diakinesis and metaphase I, univalents, bivalents and trivalents were observed. At anaphase I there were laggards, chromatin bridges and unequal chromosome distribution at the poles. Synthetic hexaploids (*S. nigrum*), raised from these triploids, were highly fertile and set fruit and seed. They exhibited normal meiosis characterized by bivalents, and several morphological characters were identical to those in natural hexaploids. Synthetic and natural forms readily crossed to give fertile progeny. It is suggested that in nature the incompatibility between genomes in the triploid hybrids induced chromosome doubling, and that this played a significant role in the origin and evolution of hexaploidy in the *S. nigrum* complex.

1258 KUMAR, A; RAO, GR. 1984. **Cytology of hybrids between the Indian hexaploid *Solanum nigrum L.* and *S. opacum A. Br. & Bouche***. *Journal of the Indian Botanical Society*, 63: 3, 247-251; 12 ref.

The material studied came from natural populations of *S. nigrum* growing in and around Delhi, India, and from a stock of *S. opacum* raised from seed supplied by the Botany School, Cambridge, United Kingdom. F1 hybrids showed fairly normal meiosis with moderate pollen fertility and produced purplish black fruits with viable seeds. It is suspected that the 2 species are genetically closely related.

1259 RAO, GP; KUMAR, A. 1984. **Meiotic studies in species-hybrids of the *Solanum nigrum L.* complex**. *Cytologia*, 49: 1, 33-38; 11 ref.

Information is presented on plant morphology and meiotic behaviour in *S. retroflexum* (2n = 48), *S. nodiflorum subsp. nutans* (2n = 24), the triploid F1 hybrid between them and a synthetic hexaploid obtained by colchicine treatment of the triploid. The triploids showed irregular meiosis, were sterile and did not set fruit. The synthetic hexaploids mostly showed normal meiosis, their pollen fertility (35.5-52%) was higher than that of the triploids and they set fruit and seed spontaneously. It is concluded that differences in ploidy level and structural chromosome differences are the main isolating mechanisms between these species.

1260 RAO, GR; KUMAR, A. 1983. **Chromosome pairing in interspecific hybrids of the *Solanum nigrum* complex**. *Indian Journal of Genetics and Plant Breeding*, 43: 3, 321-323; 2 ref.

Meiosis was studied in the F1 of *S. nigrum* X *S. sarrachoides*. The hybrids were highly sterile, showed irregular meiosis and did not set fruit. Cytological studies suggested that polyploidy, structural hybridity and genic differences are responsible for reproductive isolation and morphological distinctness between the 2 species. Since only 11 metaphase I bivalents occurred in some cells of the hybrids, it is suggested that *S. sarrachoides*, or one of its close diploid relatives, might have contributed a set of 12 chromosomes at the origin or during the evolution of hexaploid species of the *S. nigrum* complex.

1261 RAO, GR; KUMAR, A. 1984. **Cytology of hybrids of the *Solanum nigrum L.* complex**. *Science and Culture*, 50: 5, 164-165; 3 ref.

F1 hybrids between the hexaploids *S. nigrum* and *S. nigrum subsp. schultesii* were also hexaploid (all taxa 2n = 72) and had black fruits, viable seeds and an average

pollen stainability value of 81.24%; this value in the F2 was 74.31%. Most F1s and F2s showed normal meiosis, but univalents and multivalents were observed in some instances, as were lagging chromosomes and chromatin bridges in the former and lagging chromosomes in the latter. These findings, it is concluded, point to 1) allosyndetic pairing by majority of chromosomes in the hybrids and 2) genetic and structural differences between the chromosomes of the parents.

1262 RAO, GR; KUMAR, A. 1983. **Interrelationships among diploid species of the *Solanum nigrum L.* complex**. *Journal of the Indian Botanical Society*, 62: 1, 25-31; 9 ref.

Seven diploid taxa of the complex were studied for crossability and pairing behaviour of the chromosomes. Reciprocal crosses between *S. douglasii* and *S. americanum*, diploid *S. nigrum*, *S. nodiflorum*, *S. nodiflorum subsp. nutans* and *S. sarrachoides* were not successful. It is concluded that *S. douglasii* is distantly related to the other species. Reciprocal crosses between *S. nodiflorum subsp. nodiflorum* and *S. douglasii* produced several fertile hybrids of identical morphology with normal meiosis, whereas reciprocal crosses between *S. nodiflorum subsp. nutans* and *S. douglasii* failed. On the basis of chromosome pairing in hybrids between *S. nodiflorum subsp. nodiflorum* and *S. douglasii*, it is thought that the species have a close genetic relationship.

Syzygium aromaticum

1263 MATHEW, KM; FRANCIS, MS; HARIHARAN, MOLLY. 1987. **Development of callus in cloves (*Syzygium aromaticum (L.) Merr. & Perry*)**. *Journal of Plantation Crops*, 15: 2, 123-125; 6 ref.

Of several media and growth regulators tested, MS medium supplemented with NAA at 2.0-3.5 mg/litre + BA at 0.5-10.0 mg/litre was best for callus development from axillary buds. MS supplemented with high NAA and low BA concentrations promoted callus initiation, while low NAA and high BA promoted further growth and multiplication of callus but without differentiation.

1264 MATHEW, MK; HARIHARAN, M. 1990. **In vitro multiple shoot regeneration in *Syzygium aromaticum***. *Annals of Botany*, 65: 3, 277-279; 10 ref.

A brief summary of the establishment of an in vitro technique for micropropagation of the clove tree *S. aromaticum*. Multiple shoots were induced from nodal segments of 1 month old seedlings on 2 media (Murashige and Skoog's with half strength salts, and Garnborg's supplemented with BAP [benzyladenine] and NAA). Six to 8 shoots were obtained with 3 mg/litre BAP and 0.5 mg/litre NAA in the medium. No significant differences between the 2 media were noted.

Urginea indica

1265 JHA, S; SEN, S. 1986. **Development of Indian squill (*Urginea indica* Kunth.) through somatic embryogenesis from long term culture**. *Journal of Plant Physiology*, 124: 5, 431-439; 20 ref., 14 pl., (1 col.).

U. indica is medicinally important because of its bufadienolide content. One-year-old friable calli, derived from bulb scale explants, were grown in the presence of 2 mg/litre 2,4-D and subcultured at 6 month intervals; 40% formed embryogenic clumps between the 2nd and 4th year after callus induction. Globular embryoids were induced to develop into complete bulbous plants with sequential transfer to Murashige and Skoog (MS) medium containing 0.05-0.1 mg/litre BA (or 15% coconut milk or 0.1 mg/litre BA + 10% coconut milk) for 4-6 weeks and then to MS medium + 0.01 mg/litre NAA + 0.05 mg/litre kinetin for 8 weeks. A final period in liquid MS medium stimulated shoot and root growth sufficiently for successful transplanting in soil. The survival rate was 50%

1266 JHA, S; MITRA, GC; SEN, S. 1984. **In vitro regeneration from bulb explants of Indian squill, *Urginea indica* Kunth**. *Plant Cell, Tissue and Organ Culture*, 3: 2, 91-100; 12 ref.

Callus cultures were established from bulb explants of a high cardiac bufadienolide yielding diploid *U. indica* on a modified Murashige and Skoog (MS) basal medium supplemented with either 2 mg/l 2,4-D + 15% (v/v) coconut milk or 4 mg/l 2,4-D + 2 mg/l NAA + 2 mg/l kinetin + 1 g/l yeast extract. Shoot primordia developed after 2-3 subcultures. Increased growth of shoot primordia was obtained in media containing less auxins and vitamins. The rooted bulbous plantlets obtained were maintained in MS medium with 0.5% sucrose. Adventitious shoots were induced from adaxial epidermal cells of outer scales of regenerated bulbs used as secondary explants in the presence of 1 mg/l 2,4-D with a slightly higher concentration of the three MS vitamins. From each scale leaf approximately 400 bulblets were produced in 18 weeks in liquid culture. Of the plants transferred to potted soil, 90% survived.

1267 JHA, S; SEN, S. 1990. **Induction of mitosis in polytene nuclei and hormonal effect on nuclear**

changes during callus initiation in diploid *Urginea indica Kunth*. (*Liliaceae*). *Genetica*, 80:1, 9-15; 22 ref.

Bulb scale and inflorescence explants of *U. indica* (2n=20) were cultured in vitro on modified MS medium with different combinations of growth regulators. Media containing 2 or 4 mg 2,4-D/litre and 2 mg NAA/litre induced callus in inflorescence explants. Combinations of 2,4-D (4 mg/litre) + NAA (2 mg/litre) + kinetin (2 mg/litre) only induced callus formation in scale explants. Bulb scale explants contained mainly diploid cells while inflorescence explants contained cells with nuclear DNA content ranging from 2C to 64C. The lowest karyological heterogeneity was recorded in callus derived from bulb scale and in callus derived from inflorescences induced with NAA. The highest variability was recorded on media containing 2,4-D alone. Induction of division, probably of the pre-existing polytenic nuclei in the inflorescence explant, was suggested as the cause of origin of polyploid cells in such cases.

1268 JHA, SUMITA; SAHU, NP; MAHATO, SB. 1991. **Callus induction, organogenesis and somatic embryogenesis in three chromosomal races of *Urginea indica* and production of bufadienolides.** *Plant Cell, Tissue and Organ Culture*, 25: 2, 85-90; 10 ref.

Three chromosomal races of Indian squill, *Urginea indica*, were screened for their ability to produce bufadienolide in tissue cultures. The protocols for callus induction, organogenesis and somatic embryogenesis differed in the 3 races with respect to vitamin requirements and growth regulator additions. Bufadienolide contents were determined by HPLC. Undifferentiated calluses and cell suspension cultures did not produce the bufadienolides. Shoot differentiating cultures contained only trace amounts of proscillaridin A while embryogenic cultures and developing embryoids did not contain bufadienolides. All regenerated bulbs (derived from diploid, triploid and tetraploid parents through organogenesis and/or somatic embryogenesis) contained both proscillaridin A and scillaren A, the bufadienolide characteristic of the parent plant.

1269 JHA, SUMITA. 1989. **Cytological analysis of embryogenic callus and regenerated plants of *Urginea indica* Kunth., Indian squill**. *Caryologia*, 42: 2, 165-173; 22 ref.

Friable calluses were indued from bulb scale explants of diploid (2n = 20) *U. indica*. Embryogenic calluses were formed when one-year-old friable calluses were allowed to remain on the high 2,4-D medium for a prolonged period. The original explant, friable calluses, somatic embryos and plants regenerated via somatic embryogenesis were examined cytologically. The original explant had a negligible level of DNA variation. The dedifferentiating calluses showed complete elimination of diploid cells after 6 months and friable calluses were composed of cells with high chromosome number (100-300). Embryogenic calluses contained large and small cells, the latter with deeply stained nuclei, showing variable ploidy (6x-12x). Globular embryoids were also composed of polyploid cells, with chromosome numbers ranging from 40 to 90 with a few highly polyploid cells (>160). The chromosome numbers of 150 of the 1000 plants regenerated from these friable embryogenic cultures were determined. All plants were polyploidy; about 50% were octoploid, 6% were predominantly hexaploid and the rest were of variable ploidy. The results showed that highly polyploid cells are capable of forming somatic embryoids and regenerating.

Other medicinal plants

1270 AKHILA, ANAND; RANI, KUMKUM; THAKUR, RS. 1991. **Biosynthesis of the clerodane furano-diterpene lactone skeleton in *Tinospora cordifolia*.** *Phytochemistry*, 30: 8, 2573-2576.

1271 ARORA, R; BHOJWANI, SS. 1989. **In vitro propagation and low temperature storage of *Saussurea lappa* C.B. Clarke - an endangered, medicinal plant**. *Plant Cell Reports*, 8: 1, 44-47; 16 ref.

A procedure for in vitro multiplication of *S. lappa* (Asteraceae) is described. The dried roots of this plant have been used to treat cough, fever, asthma, rheumatism and skin diseases and also yield costus oil, used in perfumes. On MS medium containing benzylaminopurine [benzyladenine] and gibberellin, 3,5-fold shoot multiplication occurred every 3 weeks. Shoots rooted on MS containing 0.5 μm naphthaleneacetic acid (NAA) with 90% efficiency. Shoot cultures stored at 5°C in the dark for 12 months, without an intervening subculture survived with 100% viability. Shoots cold-stored for 6 months or more showed higher rates of multiplication under culture room conditions than the untreated shoots.

1272 ARUMUGAM, N; BHOJWANI, SS. 1990. **Somatic embryogenesis in tissue cultures of *Podophyllum hexandrum*.** *Canadian Journal of Botany*, 68: 3, 487-491; 19 ref.

When cultured on MS medium supplemented with 2 μM IAA callus derived from zygotic embryos of this medicinal species (a source of podophyllotoxin) formed globular embryos which failed to develop unless the

medium was supplemented with either 6% sucrose or 1-10 μM NAA. Embryogenesis was suppressed by light and temperatures above 25°C. The embryogenic potential of the callus was maintained for over 20 months through subcultures. Plantlets were regenerated on basal MS medium.

1273 BAJAJ, YPS. 1989. **Biotechnology in agriculture and forestry. 7. Medicinal and aromatic plants II.** Berlin: Springer-Verlag, xix + 545 p.

This second volume comprises 29 chapters dealing with the distribution, economic importance, conventional propagation, micropropagation, review of tissue culture studies and in vitro production of important medicinal and pharmaceutical compounds of the following genera/species: *Angelica sinensis*, *Anisodus acutangulus*, *Basella alba*, *Bupleurum falcatum*, *Camellia sinensis*, *Coix lacryma-jobi*, *Coptis*, *Cryptomeria japonica*, *Datura*, *Dioscorea*, *Foeniculum vulgare (fennel)*, *Geigeria aspera*, *Heimia salicifolia*, *Humulus lupulus (hops)*, *Hyoscyamus*, *Jasminum*, *Macleaya*, *Mucuna pruriens*, *Nicotiana tabacum*, *Pimpinella anisum*, *Rauwolfia [Rauvolfia] serpentina*, *Ruta graveolens*, *Salvia miltiorrhiza*, *Saponaria officinalis*, *Solanum*, *Stevia rebaudiana*, *Tabernaemontana* and *Zingiber officinale*.

1274 BEJOY, M; VINCENT, KA; HARIHARAN, M. 1990. **In vitro shoot regeneration of *Coleus parviflorus benth*.** *Indian Journal of Plant Physiology*, 33: 2, 175-176.

1275 BRACHER, D; KUTCHAN, TM. 1992. **Polymerase chain reaction comparison of the gene for strictosidine synthase from ten *Rauvolfia* species.** *Plant Cell Reports*, 11: 4, 179-182; 12 ref.

The gene for strictosidine synthase, str1, was analysed in *R. cambodiana* (originating from Indochina), *R. canescens* [*R. tetraphylla*] (India), *R. chinensis* (China), *R. heterophylla* (Central America), *R. mannii* (West Africa), *R. nitida* (West Indies), *R. praecox* (Brazil), *R. serpentina* (India), *R. sumatrana* (Indonesia) and *R. verticillata* (Indochina). Restriction endonuclease analysis of the gene fragments, produced with genomic DNA from each of the species as template, revealed that str1 was highly conserved in the *Rauvolfia* species investigated. These results suggested that there is a stringent selection pressure on the gene for this key enzyme of indole alkaloid biosynthesis.

1276 CHAKRAVARTY, BIPASHA; SEN, SUMITRA. 1989. **Regeneration through somatic embryogenesis from anther explants of *Scilla indica (Roxb.) Baker*.** *Plant Cell, Tissue and Organ Culture*, 19: 1, 71-75; 12 ref.

Somatic embryogenesis was induced from anther calluses grown on MS medium supplemented with NAA and coconut milk. The calluses produced embryos in growth regulator-free MS medium which developed into plantlets capable of transfer to field conditions. A histological study revealed the involvement of a group of cells in the initiation of embryogenesis. Embryos with distinct root and shoot apices were observed.

1277 CHOWDHURY, AR. 1983. **Atropine biosynthesis in *Atropa belladonna* cultures.** *Herba Hungarica*, 22: 2, 15-22; 23 ref.

Root suspension cultures were grown on Street and McGregor nutrient medium alone or supplemented with ornithine, phenylalanine, citrulline and arginine separately or in combination. Chromatographic analysis of the alkaloid fraction of the cultures revealed the presence of atropine (0.54%) which was confirmed by spectral studies. The supplements, and in particular 3 mg/litre ornithine, increased atropine content up to 0.7%

1278 DATTA, K; DATTA, SK. 1984. **Auxin induced clonal multiplication of *Holarrhena antidysenterica* by tissue culture.** *Journal of Tree Sciences*, 3: 1/2, 45-52; 20 ref.

H. antidysenterica is a small tree occurring throughout India which has been used medicinally since ancient times. Rooted plantlets were obtained after 45 days from nodal explants (1-1.5 cm) of a 20-yr-old tree cultured on MS medium with added IAA at 1 mg/litre. Regenerated shoot buds obtained on MS medium with added auxins (IAA, IBA, NAA) and/or cytokinins (BA, kinetin) were subcultured on MS/2 medium (a lower concn. of salts) with IAA or NAA with or without cytokinin. Only cultures with IAA produced rooted shoots. Shoot tips did not regenerate as well as nodal explants.

1279 DHANKHAR, BS; KISHORE, NAND; SHARMA, DR; PANDITA, ML. 1982. **A note on the susceptibility of *Solanum khasianum* to fruit and shoot borer *Leucinodes orbonalis*.** *H.A.U. Jour. Res.* 12: 523-524.

1280 DHIR, SK; SHEKHAWAT, NS; PUROHIT, SD; ARYA, HC. 1984. **Development of laticifer cells in callus cultures of *Calotropis procera (Ait.) R. Br.***

Plant Cell Reports, 3: 5, 206-209; 20 ref.

Tissue cultures were established from stem explants of *C. procera* (a possible source of hydrocarbons) on Murashige and Skoog medium supplemented with 1.5 mg/litre 2,4-D + 0.5 mg/litre kinetin and polyvinylpyrrolidone. Laticifer cells were not present in young callus but were observed in the 5th week of culture and were distinguished from surrounding cells by the presence of characteristic cytoplasm and thin walls. A group of cells with extensive branching was developed after 8 weeks of callus growth; these cells were thick-walled and contained latex particles in coagulated masses. A positive Liebermann-Burchard test indicated the presence of terpenoids in these laticifers.

1281 GIRI, A; AHUJA, P SINGH; KUMAR, PV AJAY. 1993. **Somatic embryogenesis and plant regeneration from callus cultures of *Aconitum heterophyllum Wall***. *Plant Cell, Tissue and Organ Culture*, 32: 2, 213-218.

1282 GULATI, ANU. 1988. **Tissue culture of *Coccinia grandis***. *Current Science*, 57: 22, 1232-1235; 7 ref.

Shoot tip, hypocotyl, internode, leaf and nodal explants of 20-day-old seedlings were cultured on MS medium supplemented with 10-7 to 10-5 M IBA and kinetin. All the treatments induced callus formation. Shoot tip explants produced shoots on medium with 10-6 M concentrations of both growth regulators and rooting was induced on medium with 10-6 M kinetin and 10-5 M IBA. Nodal explants cultured on medium supplemented with 10-5 M of both growth regulators produced roots and shoots when transferred to the same medium after 4 weeks.

1283 HARSH, ML; NAG, TN. 1984. **Antimicrobial principles from in vitro tissue culture of *Peganum harmala***. *Journal of Natural Products*, 47: 2, 365-367; 19 ref.

The active principles were identified as flavonoids, including quercetin and kaempferol.

1284 HEBLE, MR; CHADHA, MS. 1988. **Recent developments in the biotechnological application of plant tissue, cell and organ cultures**. *Acta Horticulturae*, No. 188A: 67-74; 28 ref.

The subject is surveyed with reference to (1) the production and transformation of plant drugs by tissue, cell and organ cultures and (2) medicinal plant germplasm preservation.

1285 JHA, S; SEN, S. 1985. **Regeneration and rapid multiplication of *Bowiea volubilis Harv.* in tissue culture**. *Plant Cell Reports*, 4: 1, 12-14; 11 ref.

The most important reason for propagating this plant was to maximize the production of high drug (cardiac glycoside) containing clones. Callus cultures were raised from segments of the inflorescence axis on a modified basal medium of Murashige and Skoog (MS) supplemented with 1 mg/litre 2,4-D + 15% v/v coconut milk. Shoot primordia developed after 2-3 subcultures when auxin concentration was lowered. Rooted bulbous plants were obtained in MS medium without any hormone. Shoots were induced directly on scales of regenerated bulbs used as secondary explants on modified MS medium supplemented with 2 mg/litre 2,4-D. These shoots grew and multiplied rapidly in shake culture using liquid MS medium. From each scale 400-600 bulblets could be produced in 16-20 weeks. Eighty percent of plants survived on transfer to pots.

1286 JHA, S; JHA, TB; MAHATO, SB. 1988. **Tissue culture of *Artemisia annua L.* - a potential source of an antimalarial drug**. *Current Science*, 57: 6, 344-346; 4 ref.

1287 JHA, TB; ROY, SC. 1982. **Regeneration and cytological investigation on plantlets from protoplasts of *Nigella sativa***. *Phytomorphology*, 32: 1, 51-54; 8 ref.

Protoplast-derived calluses were maintained on Murashige and Skoog medium containing 0.5 mg/l NAA, 0.1 mg/l BA or kinetin, 0.25 mg/l 2,4-D and 500 mg/l casein hydrolysate. Plantlets were obtained when callus was transferred to White's medium containing 0.5 mg/l NAA and casein hydrolysate, with or without 0.25 mg/l BA or kinetin. In the calluses, 73% of cells were diploid and the rest showed variation from aneuploidy to octoploidy. Roots of the regenerated plants showed mixoploidy.

1288 JIT, S; NAG, TN. 1985. **Antimicrobial principles from in vitro tissue culture of *Tribulus alatus***. *Indian Journal of Pharmaceutical Sciences*, 47: 3, 101-103; 16 ref.

Extracts from unorganized callus of *T. alatus*, raised and maintained by frequent subculturing on Murashige and Skoog medium supplemented with 5 p.p.m. kinetin and 1 p.p.m. 2,4-D, were screened for antimicrobial activity. Some of the active principles were isolated separately and identified as flavonoids (quercetin and kaempferol).

1289 KUMAR, A; GHOSH, K; SINHA, NK; DUTTA, SK. 1986. **Bark anatomy of *Xylia xylocarpa Roxb.* with histo-pharmacognostic evaluation**. *Journal of Plant Anatomy and Morphology*, 3: 1, 49-54; 14 ref.

The salient diagnostic characters of the bark were rhomboidal crystals, olive grey fluorescence of aqueous yellowish red extracts, and presence of alkaloids, glycosides and sterols.

1290 KUMAR, ANJANI; DATTA, SK. 1989. **Plantlet regeneration from hypocotyl tissue of *Strychnos nuxvomica L.*** *Current Science*, 58: 14, 812-813; 9 ref.

Seeds of *Strychnos* nux-vomica were germinated on Murashige and Skoog (MS) medium supplemented with 1 mg/litre gibberellic acid, and hypocotyls excised from the resulting seedlings were inoculated onto MS medium supplemented with 0-3.0 mg/litre kinetin, 0-2.0 mg/litre NAA or 0-1.0 mg/litre IAA in various combinations. Shoot formation was promoted by kinetin alone and root formation by NAA alone, but whole plantlets were formed in the presence of kinetin and NAA. The best plantlet growth occurred with 2.0 mg/litre kinetin and 0.5 or 1.0 mg/litre NAA.

1291 KUMAR, G. 1992. **Modification of gamma irradiation [in seed] induced genetic damage by dimethylsulphoxide in two *Solanum* species**. *Journal of Genetics and Breeding*, 46: 1, 1-8.

The pre- and post-treatment of Dimethylsulfoxide (DMSO) on gamma-irradiated seeds of two medicinally important species of *Solanum* (*S. incanum L.* and *S. surattense* Burm. f.) at LD50 and LD90 (sublethal doses) were investigated. Pretreatment with DMSO improved growth of seedlings and their survival and was coupled with higher mitotic index and reduced frequency of chromosome aberrations. Further, it led to greater incorporation of 3H-thymidine into root-tip cell DNA while post-treatment caused inhibition. Moreover, pretreatment initiated early flowering and fruiting and produced large number of vigorous fruits and seeds. The pollen sterility was also lower in pre-treated seedlings as compared to irradiated ones. DMSO post-treatment at LD90 caused male sterility in either species; which, however, produced fruits and seeds after cross pollination with controls.

1292 KUMAR, KS; BHAVANANDAN, KV. 1989. **Regeneration of plants from leaf callus of *Plumbago rosea L.*** *Indian Journal of Experimental Biology*, 27: 4, 368-369; 9 ref.

Callus was induced from *P. rosea* [*P. indica*] leaf explants on MS medium supplemented with 1.5 mg 2,4-D and 0.1 mg kinetin/litre. Shoots were produced from calluses on MS medium with 3 mg benzyladenine + 1 mg kinetin+1 mg IAA/litre. Roots were initiated on a medium containing 1.5 mg IBA/litre. Regenerated plants were sucessfully transferred to a sand and soil mixture.

1293 MATHUR, J; MUKUNTHAKUMAR, S; GUPTA, SN; MATHUR, SN. 1991. **Growth and morphogenesis of plant tissue cultures under mineral-oil**. *Plant Science Limerick*, 74: 2, 249-254; 17 ref.

When cell and tissue cultures of 6 plant species were grown with and without a mineral oil (MO) overlay on MS medium supplemented with appropriate growth regulators, the rate of growth and morphogenesis in cultures covered with MO was greatly reduced compared to the controls. This lengthened the subculture period from 35-45 days to 150-240 days and resulted in significant savings in effort, material and time. Subsequently, the shelf life of encapsulated propagules of *Selinum candolii*, *Nicotiana tabacum* and *Valeriana wallichii* was enhanced from 25-30 days to 150-240 days. Plants were regenerated from the cultures overlaid with MO in each case.

1294 MATHUR, J; AHUJA, P. 1991. **Plant regeneration from callus cultures of *Valeriana wallichii DC.*** *Plant Cell Reports*, 9: 9, 523-526; 9 ref.

Petiole explants of *V. wallichii*, a threatened medicinal plant, were used for callus induction. Optimum callus formation was observed on MS medium supplemented with 3.0 mg NAA and 0.25 mg kinetin (Kn)/litre. Shoot regeneration was achieved by transferring the callus to medium containing 1.0 mg Kn and 0.25 mg NAA/litre. Complete plantlets were obtained on the same medium or upon transfer of the regenerated shoot buds to medium containing 5.0 mg Kn and 1.0 mg IAA/litre. Nearly 1000 callus-regenerated plants were successfully transferred to the field following previously standardized hardening procedures.

1295 MATHUR, J. 1992. **Plantlet regeneration from suspension cultures of *Valeriana wallichii* DC**. *Plant Science Limerick*, 81: 1, 111-115; 15 ref.

Suspension cultures were initiated from petiole explants in liquid MS medium containing 16.1 μM NAA and 0.93 μM kinetin. Cell aggregates showed rhizogenesis within 2-3 weeks, giving rise to clumps of 4-6 roots. Isolated roots developed a green callus or a flattened leaf like structure at one end. Such bipolar structures when transferred to solid MS medium containing either 0.89-2.22 μM BAP or 0.93-4.6 μM kinetin developed shoots and grew into complete plantlets.

1296 MHATRE, M; BAPAT, VA; RAO, PS. 1984. **Plant regeneration in protoplast cultures of *Tylophora indica*.** *Journal of Plant Physiology*, 115: 3, 231-235; 10 ref.

Protoplasts isolated from callus tissue derived from freshly cultured stem segments rapidly divided and resulted in callus. Subsequent embryoid/shoot bud differentiation in callus was observed on Murashige & Skoog medium supplemented with auxins and cytokinins. On transfer to basal medium without growth substances, plantlets were obtained. Protoplasts isolated from callus tissue maintained for over 5 years showed divisions and subsequent callus formation but failed to regenerate plants.

1297 MUKHOPADHYAY, S; SHARMA, AK. 1990. **Chromosome number and DNA content in callus culture of *Costus speciosus (Koen.) Sm.*** *Genetica*, 80: 2, 109-114; 25 ref.

Karyological changes in callus tissue of 3 populations of *C. speciosus* at different ages were analysed both by counting chromosome number and measuring DNA content through cytophotometry. Cultures were established from the tuber and maintained in MS basal medium supplemented with 2,4-D, NAA and benzyladenine. Abnormalities in chromosome behaviour leading to the formation of hypo- and hyperdiploid cells together with the diploid cells were observed. These reached an optimum at different callus ages followed by a decline which differed amongst populations. The frequency of hyperdiploid cells was higher than that of the hypodiploids. Evidence for the roles of endomitotic replication and non-disjunction of chromosomes with resulting variation in chromosome number was provided by nuclear DNA values in successive passages. The average amount of nuclear DNA differed with increase in callus age and also differed amongst populations.

1298 MUKHOPADHYAY, SANDIP; BANERJEE, NIRMALYA; SHARMA, AR. 1989. **Chromosome behaviour in regenerants from excised leaf discs of *Solanum sarrachoides* Sendt.** *Caryologia*, 42: 2, 147-152; 22 ref.

Chromosome behaviour was studied in *S. sarrachoides* plants regenerated in vitro through direct adventitious bud formation from leaf segments. The highest number of adventitious shoots was obtained in MS semisolid medium supplemented with 2 mg 2iP [isopentenyladenine]/litre as the only growth regulator. Addition of IAA was necessary in the later phase of culture to obtain better growth and development of shoots with well-defined leaves, roots, flowers and fruits. Analysis of the PMCs and root tip cells of regenerants showed n= 12 and 2n = 24 chromosomes, respectively, and meiosis was normal. Hyperdiploids along with normal diploid cells were also recorded at a low frequency.

1299 NAIR, AS. 1989. **Micropropagation of *Scilla hyacinthiana (Roth) Macbride*.** *Proceedings of the Indian National Science Academy, Part B: Biological Sciences*, 55: 2, 121-123; 7 ref.

A 3-stage method was developed for micropropagation of *S. hyacinthiana* [*S. indica*] (tetraploid 4n=60) using young leaves as explants. (The bulbs contain the glycoside scilladienolide which is used as an expectorant, cardiac stimulant and diuretic.) Stage I consisted of regeneration using MS basal medium, while stage II involved rapid shoot multiplication using the same medium supplemented with NAA (1 mg/litre) and kinetin (5 mg/litre). In stage III, liquid MS basal medium containing 2% sucrose was used for rooting the regenerated shoots. The rooted shoots (plantlets) were successfully transferred to soil with about 75% survival.

1300 NANDWANI, D; RAMAWAT, KG. 1991. **Callus culture and plantlets formation from nodal explants of *Prosopis juliflora (Swartz) DC.*** *Indian J. of Experimental Biology*, 29: 5, 523-527; 11 ref.

Multiple shoot formation from nodal explants of *P. juliflora* was observed in several treatments consisting of a cytokinin in combination with an auxin incorporated in Murashige and Skoog medium. The initial growth of shoots was slow and elongation was obtained by transferring them as such in subsequent passages. Benzyladenine was found better than kinetin and cytokinin was essential for shoot formation. Explants grown on medium supplemented with benzyladenine at 5.0 mg/l and IAA at 0.1 mg/l produced the maximum number (7) of shoots/node. Shoots (2.5 cm or more long) obtained in vitro could be rooted by transferring them to MS medium containing NAA or IBA. Callus formation was observed in various explants by medium manipulations. In most cases explants produced cottony and white callus. Immature inflorescence produced pale yellow, fragile and watery callus. In all cases, callus did not survive after subculture on any of the media tried. Phenoxy acids were found most effective in callus induction but were not effective for callus maintenance. Cotyledons tolerated a concn as high as 30 mg/litres of these phenoxy acids up to two passages.

1301 NATARAJA, K; PATIL, JS. 1984. **Responses of isolated floral buds and anthers of *Abutilon indicum* in vitro.** *Current Science*, 53: 14, 757-759; 4 ref.

Flower buds at the uninucleate pollen grain stage did not form callus on several media but 25% of the explants showed callusing in 2 weeks when cultured on Bourgin & Nitsch medium with growth adjuvants + 2% sucrose (BN)+1 ppm IAA. In response to 1 ppm IAA+15% coconut water, sepals and cut ends of the pedicel showed proliferation within a week but the callus failed to differentiate organs upon subculture to the same medium. However, some rooting occurred on BN+0.5 ppm IAA+0.5, 1 or 2 ppm kinetin, or BM+2 ppm IAA+2 ppm kinetin. Callus proliferation, failure to undergo organogenesis and some rooting, in presence of NAA were obtained from anthers cultured on similar media.

1302 PAL, DHRU; GUPTA, SK; SINGH, CHARAN. 1990. **Organogenesis and plant regeneration in leaf callus culture of *Nerium oleander Linn*.** *Advances in Plant Sciences*, 3: 1, 61-65; 10 ref.

In a comparison of MS media supplemented with 0.5, 1 or 1.5 mg NAA or BA/l, callus formation and growth from leaf tip explants of *N. oleander* were greatest with 1.0 mg NAA/l. When callus from this treatment was subcultured on an MS medium containing 0.2 mg BA + 0.5 mg IAA/l, roots developed after about 90 h. Subculturing on media containing IAA or BA alone did not result in root development. Roots were produced directly on leaf tip explants cultured on an MS medium containing 1.0 mg BA/litre.

1303 PANDA, AK; BISARIA, VS; MISHRA, S. 1992. **Alkaloid production by plant cell cultures of *Holarrhena antidysenterica*. II. Effect of precursor feeding and cultivation in stirred tank bioreactor.** *Biotechnology and Bioengineering*, 39: 10, 1052-1057.

Precursor feeding strategy for increasing the yield of conessine, a steroidal alkaloid of *Holarrhena antidysenterica*, was established in cell suspension culture. A total of 50 mg/L added cholesterol was converted into 43 mg/L of alkaloid, 90% of which constituted the conessine. By applying the precursor feeding policy to the cell suspension culture in modified MS medium, a total of 143 mg/L of alkaloid was produced in 8 days. In this way the alkaloid content of the cells was increased more than six times compared to that obtained in the standard MS medium. The steps leading to biotransformation of cholesterol into alkaloids were unaffected by phosphate. The shake flask data were successfully transferred to a bench scale 6-L stirred tank bioreactor in which the specific biosynthetic rate of alkaloid production was 110 mg/100 g dry cell weight per day, about 160 times higher than that of whole plant.

1304 PANDA, AK; BISARIA, VS; MISHRA, S; BHOJWANI, SS. 1991. **Cell culture of *Holarrhena antidysenterica*: growth and alkaloid production.** *Phytochemistry*, 30: 3, 833-836; 16 ref.

Callus and suspension cultures of *H. antidysenterica* [*H. pubescens*] were established for production of steroidal alkaloids, especially conessine. The doubling time and specific growth rate of cells in suspension culture were computed to be 47.5 h and 0.35 per day, respectively. A maximum of 300 mg alkaloids per 100 g dry cells in 40 days and 130 mg per 100 g dry cells in 8 days were obtained in the callus and suspension cultures respectively. Alkaloid production in suspension culture was a combined growth- and non-growth-associated phenomenon. Spectral data confirmed that about 90% of the total alkaloids produced in cell culture was conessine.

1305 PANDEY, R; CHANDEL, KPS; RAO, SR. 1992. **In vitro propagation of *Allium tuberosum Rottl. ex. Spreng.* by shoot proliferation**. *Plant Cell Reports*, 11: 7, 375-378; 13 ref.

Halved shoot bases of 200 field-grown plants proliferated both axillary and adventitious shoots on B5 medium supplemented with either 0.5 mg BAP [benzyladenine] or 0.1 mg NAA and 0.5 mg 2iP/litre. In vitro shoots proliferated further numerous shoots upon subculture to fresh medium, and these shoots rooted spontaneously. Plantlets were transplanted successfully to soil and retained the diploid condition of the parents.

1306 PRAMANIK, TK; DATTA, SK. 1986. **Plant regeneration and ploidy variation in culture derived plants of *Asclepias curassavica L.*** *Plant Cell Reports*, 5: 3, 219-222; 20 ref.

Clonal propagation of this medicinal plant was achieved by culturing excised nodes on Murashige and Skoog medium supplemented with growth regulators. BA and kinetin were equally effective for shoot initiation, IAA and NAA were suitable for root induction, and combinations of kinetin and NAA induced roots and shoots. Chromosomal variation was observed in the roots of in vitro regenerated plants. Regenerants with a higher chromosome number (e.g. 33 compared with 2n = 22), obtained with 9.2 muM kinetin + 10.7 muM NAA, showed vigorous growth and higher propagation rates in culture than the plants possessing less than the diploid chromosome number (2n-2 = 20, 2n-4 = 18).

1307 RANJAN, KS; PRASAD, GAJENDRA; SINHA, AK. 1991. **Evaluation of some bitter plant extracts against aflatoxin production and growth of *Aspergillus flavus*.** *Nat. Acad. Sci. Letters*, 14: 6, 241-243.

The effect of plant extracts on aflatoxin production and growth of *A. flavus* on SMKY liquid medium was investigated. Of the 15 plant extracts tested, extracts of *Streblius asper*, *Artemisia indica*, *Adhatoda zeynatica*, *Emblica officinalis* [*Phyllanthus emblica*], Citrus aurantium and *Morus indica* inhibited production of aflatoxin B1 and B2 by >50%. A correlation between inhibition of fungal growth and aflatoxin production was not observed.

1308 ROY, SC; SARKAR, A. 1991. **In vitro regeneration and micropropagation of *Aloe vera L.*** *Scientia Horticulturae*, 47: 1-2, 107-113; 13 ref.

A. vera [*A. barbadensis*] was rapidly propagated by inducing shoot formation on in vitro callus cultures produced from axillary shoot segment explants taken from the underground rhizomatous stem. The presence of 1 g polyvinylpyrrolidone/litre in the culture medium reduced the secretion of phenolic substances by the explants. MS basal medium containing 1 mg 2,4-D and 0.2 mg kinetin/litre gave the best callus induction. Shoot initiation was greatest from callus grown on media containing 0.02 mg 2,4-D and 1 mg kinetin/litre.

1309 SARKAR, DD; DATTA, KB. 1988. **Callus initiation and differentiation in *Herpestis monnieria* H. B. & K. (Scrophulariaceae).** *Experimental Genetics*, 4: 1, 1-3; 5 ref.

Young leaf explants of this medicinal plant produced callus and differentiated shoot buds on MS medium with added NAA and cytokinins, best results being obtained with NAA at 0.01 mg/litre and benzyladenine at 0.2 mg/litre.

1310 SEHGAL, CB; KHURANA, S. 1985. **Morphogenesis and plant regeneration from cultured endosperm of *Emblica officinalis Gaertn.*** *Plant Cell Reports*, 4: 5, 263-266; 17 ref.

Mature endosperm of *E. officinalis* (*Phyllanthus emblica*) formed a continuously growing callus on Murashige and Skoog (MS) medium supplemented with an auxin (2,4-D or IAA) and a cytokinin (kinetin or BA). Subculture of callus on MS with BA (0.2 mg/litre) and IAA (0.1 mg/litre) resulted in the formation of shoots and embryo-like structures in 50 and 8% of cultures, respectively. Regeneration of shoots was more frequent when both BA (0.2 mg/litre) and IAA (0.1 mg/litre) were present than on BA (0.2 mg/litre) alone. The embryo-like structures produced plantlets.

1311 SEN, J; SHARMA, AK. 1991. **In vitro propagation of *Coleus forskohlii Briq.* for forskolin synthesis.** *Plant Cell Reports*, 9: 12, 696-698; 8 ref.

Shoot multiplication was obtained in vitro within 20-25 days from shoot tip explants of 30-day-old aseptically germinated seedlings of *C. forskohlii*, using 2 mg benzyladenine (BA)/litre. Shoot multiplication was further enhanced with the gradual decrease in the level of BA, and its final omission after 4 months. Different auxins supplemented at the level of 0.05 mg/litre plus BA did not yield better results. Seven regenerated plants showed only diploid cells in their root tips, while 3 plants did not. Of these, 2 were diploid with occasional aneuploid cells. In one plant 32 chromosomes were observed. The potential of shoot culture in vitro and use of micropropagated plants for the production of forskolin has been demonstrated.

1312 SENGUPTA, J; JHA, S; SEN, S. 1988. **Karyotype stability in long-term callus derived plants of *Crepis tectorum L.*** *Biolog. Plant.*, 30: 4, 247-251.

In vitro growth of *C. tectorum* gave 100% callusing and 40% plantlet regeneration. The root and leaf tissues used as explants showed the normal diploid (2n = 8) chromosome constitution. In 1-month-old cultures, 95% of callus cells were diploid. Callus maintained in 2,4-D for 2 years showed 62% diploid, 5% tetraploid and 33% hyperdiploid cells. Differentiation of shoots occurred in 2-year-old calluses after subculturing in medium containing benzyladenine and the potentiality of regeneration was retained for more than a year. Leaf tips of regenerated plants were homogeneous and identical to the donor plant both in number and morphology of chromosomes.

1313 SHARMA, DK; HALL, IH. 1991. **Hypolipidemic, anti-inflammatory, and antineoplastic activity and cytotoxicity of flavonolignans isolated from *Hydnocarpus wightiana* seeds.** *Journal of Natural Products*, 54: 5, 1298-1302.

1314 SHARMA, RK; MUKAT BEHARI. 1991. **Screening of the compounds isolated from the leaves of *Adenocalymna alliceum* for antibacterial activity.** *Bulletin Pure and Applied Sciences*, 8-10: 1-2, 1-5.

1315 SHARMA, RK; MUKAT BEHARI. 1991. **Screening of the compounds isolated from the *Amaranthus tricolor* for antibacterial activiry.** *Acta Ciencia Indica*, 17, C-4: 357-362.

1316 SHARMA, RK; MUKAT BEHARI. 1992. **Screening of the compounds isolated from the leaves of *Annona squamosa* for antibacterial activity**. *Acta Ciencia Indica*, 18, C-4: 249-252.

1317 SUDARSHANA, MS; SHANTHAMMA, C. 1991. **In vitro regeneration from excised leaves of *Flaveria trinervia (Sprengel) C. Mohr***. *Plant Cell, Tissue and Organ Culture*, 27: 3, 297-302; 24 ref.

Flaveria trinervia leaves are used for the treatment of jaundice and fever. Regeneration of plantlets was achieved from leaf callus cultures. Bud formation was greatly stimulated by BA in concentrations ranging from 2 to 5 mg/litre but not at very low concentrations (0.2-1.0 mg/litre). Roots developed on the regenerated shoots over a range of treatments, but were most prolific in the medium containing 1 mg/litre IAA. Histological observations revealed that cultured spongy mesophyll cells greatly enlarged and underwent repeated cell divisions leading to the formation of hard nodular callus from which shoot buds differentiated. The shoots obtained were readily rooted and transplanted into glasshouses. Cytological studies of the callus showed abnormalities such as bridges, endomitosis and multinucleolate conditions. Root tip squashes of the regenerated plants showed no variations and were diploid in chromosome number.

1318 SUDARSHANA, MS; SHANTHAMMA, C. 1988. **Plant regeneration from inflorescence culture of *Boerhavia diffusa L.*** *Current Science*, 57: 5, 268-270; 7 ref.

Immature inflorescences, after surface sterilization, were cultured on MS medium and Nitsch medium (NM), containing 3% sucrose and 1% agar. Various concentrations of zeatin (0.1, 0.2, 0.3, 0.4, 0.5 mg/litre) alone and in combination with BAP (benzyladenine) and NAA (2+1, 3+1, 4+1, 5+2 mg/l) were used. Cultures were maintained at 22°C. Callus proliferation was initially observed on the pedicel then the flower buds on both MS and NM supplemented with 3 mg/litre BAP and 1 mg/litre NAA after 1 week. Many shoots (7-8) were noted on NM supplemented with 0.3 mg/l zeatin and on MS fortified with 3 mg/l BAP and 1 mg/l NAA. Shoots were subcultured on this medium supplemented with GA3; best response was obtained with 2 mg/l. Histological analysis of 4-week-old callus showed shoot bud differentiation directly from the meristem region. This technique could be exploited for biosynthesis of the alkaloid punarnavine.

1319 TANDON, P; RATHORE, TS. 1992. **Regeneration of plantlets from hypocotyl-derived callus of *Coptis teeta***. *Plant Cell, Tissue and Organ Culture*, 28: 1, 115-117; 9 ref.

Coptis teeta is found in the temperate zone of the Mishmi Hills of Arunachal Pradesh in North-East India. The rhizome of this plant contains berberine and is used in the treatment of fever, malaria, backaches and also as a tonic. Callus cultures of *C. teeta* were established from hypocotyl segments (excised from aseptically germinating seeds) on MS medium containing 2,4-D and Kinetin. Microshoots were produced within 6-7 weeks of subculturing this callus in 1/2 strength MS medium supplemented with kinetin alone. Excised microshoots were rooted in 1/2 strength MS medium containing IBA. The complete plantlets were hardened off and established in soil.

1320 TURAKHIA, DV; KULKARNI, AR. 1988. **In vitro regeneration from leaf explants of *Ladebouria hyacinthiana, Roth.* (*Scilla indica Bak.*)**. *Current Science*, 57: 4, 214-216; 11 ref.

Explants from mature leaves of *S. indica*, a commercial substitute for the medicinal plant Indian squill [*Urginea indica*], were cultured on MS medium unsupplemented or containing 5 mg each of IAA and BAP [benzyladenine] per litre. Bulbil primordia were directly initiated from all explants, except leaf bases, on media with and without growth regulators but bulbils per explant was higher on hormone supplemented medium. Callus was produced when the IAA was replaced with 2-20 mg 2,4-D per litre and most callus was obtained when 2 mg 2,4-D per litre was used.

1321 UPADHYAY, RAJENDER; ARUMUGAM, N; BHOJWANI, SS. 1989. **In vitro propagation of *Picrorhiza kurroa Royle ex Benth.* - an endangered species of medicinal importance**. *Phytomorphology*, 39: 2-3, 235-242; 12 ref.

Shoot cultures initiated from stem cuttings of plants collected in the Western Himalayas multiplied 36-fold every 4 weeks on MS medium containing 1 X 10-6 M benzyladenine. Normal shoots were recovered from vitrified and fasciated shoots which occurred during the shoot multiplication cycles. On MS medium supplemented with 1 X 10-6 M NAA, 89% of shoots formed roots. Plants were also regenerated from 1 mm long shoot tip explants. When the shoots were stored for 10 months in the dark at 5°C, 70% remained viable, indicating that in vitro germplasm storage is possible in *P. kurroa*.

1322 VINCENT, KA; BEJOY, M; HARIHARAN, M; MATHEW, MK. 1991. **Plantlet regeneration from callus cultures of *Kaempferia galanga L.* - a medicinal plant**. *Ind. Journal of Plant Physiology*, 34: 4, 396-400.

GUM AND RESIN PLANTS

1323 ASOKAN, MP; SOBHANA, P; SUSHAMA-KUMARI, S; SETHURAJ, MR. 1988. **Tissue culture propagation of rubber (*Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.) clone GT (Gondang Tapen). 1. Ind. J. of Nat. Rubber Res.***, 1: 2, 10-12.

An in vitro propagation method for this clone is outlined. The optimal growth regulator range for shoot and root development was 1.5-3.0 mg/litre IAA + 0.5-1.5 mg/litre kinetin. Rooted plantlets were successfully transplanted in the field.

1324 DHAR, AC; KISHOR, PBK; RAO, AM. 1989. **In vitro propagation of guayule (*Parthenium argentatum*) - a rubber yielding shrub**. *Plant Cell Reports*, 8: 8, 489-492; 9 ref.

Nodal explants (0.5 to 0.8 cm long) isolated from 2-year-old plants when cultured on MS medium supplemented with different concentrations of kinetin, BAP [benzyladenine], 2,4-D, 2,4-D + BAP, NAA and NAA + BAP produced callus tissues and shoots simultaneously at different frequencies. Shoots were regenerated at a high frequency (80-88%) from callus on MS medium containing NAA + BAP with or without glutamine. Addition of glutamine to these media improved considerably the number of shoots formed. Shoots could be regenerated from 200-day-old callus cultures at a very high frequency but the organogenetic capacity declined thereafter. Increases in the concentration of sucrose (up to 4%) significantly enhanced the shoot forming ability of callus, but higher concentrations (6%) suppressed it. Rooting was only induced in the dark when IAA, IBA and NAA were used, but 2,4-D could induce root formation in the light and dark.

1325 GUNATILLEKE, ID; SAMARANAYAKE, CHANDRA. 1988. **Shoot tip culture as a method of micropropagation of Hevea**. *Journal of the Rubber Research Institute of Sri Lanka*, 68, 33-44; 26 ref.

Shoot tips of Hevea from aseptically grown seedlings were established in culture on a liquid MS medium with half strength salts, supplemented with 0.5 mg BA + 0.005 mg IBA/litre or on a solid MS medium with full strength salts, both with and without 0.5 mg BA + 0.005 mg IBA/litre. BA was better for axillary bud proliferation and growth than the other cytokinins tested (kinetin and 2iP). Proliferation of buds improved with successive subcultures. A multiplication rate of 30 + 2 shoots per explant was obtained with 3 subcultures in 165 days.

1326 SETIA, RC. 1984. **Traumatic gum duct formation in *Sterculia urens Roxb.* in response to injury**. *Phyton, Austria*, 24: 2, 253-255; 6 ref.

Gum ducts normally occur in the pith and cortex of young stems, but after mechanical injury to both young and old stems gum ducts were formed in the xylem within 30-40 min. These so-called traumatic ducts were formed as a result of breakdown of xylem cells. A traumatic duct shows an irregular lumen without any distinct epithelial cells. Gum produced in these cells was shown histochemically to be similar to that in normal ducts.

1327 SHAH, JJ. 1983. **Gum, resin and gum-resin secretion in plants**. *Acta Bot. Indica*, 11: 2, 91-96.

A brief review of Indian gum and resin plants, discussing familiar distribution, structure of secretory ducts, their development and mode of gum/resin secretion. Experiments showed that ethephon applied to the stem markedly increased gum/resin exudation on injury in mangoes, *Bombax ceiba*, *Sterculia urens*, *Anogeissus latifolia* and *Ailanthus excelsa*.

1328 VENKAIAH, K; SHAH, JJ. 1984. **Distribution, development and structure of gum ducts in *Lannea coromandelica (Houtt.) Merril***. *Annals of Botany*, 54: 2, 175-186; 35 ref.

Gum ducts were present in leaves, stems and fruits and were most abundant in the bark of the stem of *L. coromandelica [grandis]*. They were absent in roots. Ducts developed schizogenously in the primary phloem, pith and xylem rays and lysigenously in the secondary phloem and phelloderm. Ducts were classified according to their position and arrangement as vertical, horizontal (radial and tangential) and irregular ducts. There was an integrated ramifying duct system in the bark of the trunk. Gum was formed in 2 stages. Secretion of gum occurred from the epithelial cells into the duct lumen and subsequently the disintegration of epithelial as well as neighbouring cells followed, i.e. gummosis occurred.

FRUIT CROPS

Apples

1329 BHARDWAJ, SV; KHOSLA, PK; THAKUR, PD; SHARMA, DR. 1994. **Detection of apple mosaic**