

# Dynamics of Agricultural Biotechnology

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A S Chandel and R M Kamal



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*Current Science*, 54: No. 4, 184-185; 8 ref.

Callus was derived from leaf explants from 6-month-old plants cultured on MS medium supplemented with 2,4-D. After several subcultures on MS medium with sequentially reduced 2,4-D the callus formed nodular callus masses which were maintained on a supplemented half-strength medium as a continuous source for the induction of embryogenesis. Further subculturing on various MS media led to embryogenesis, shoot formation and rooting.

## ESSENTIAL OIL PLANTS

**1174 GOPALASWAMY, UV; NAIR, CKK.** 1992. **DNA binding and mutagenicity of lindane and its metabolites.** *Bulletin of Environmental Contamination and Toxicology*, 49: 2, 300-305.

**1175 KUMARI, NEENA; SARADHI, PP.** 1992. **Regeneration of plants from callus cultures of *Origanum vulgare* L.** *Pl. Cell Reports*, 11:9, 476-479; 16 ref.

Investigations were undertaken to achieve rapid multiplication and improvement of *O. vulgare* through plant regeneration from callus. Cotyledon hypocotyl and root segment explants excised from 15 d old aseptic seedlings were cultured on B5 medium supplemented with 2,4-D, NAA and BAP individually and in various combinations (0, 10<sup>-7</sup>, 10<sup>-6</sup> and 10<sup>-5</sup> M). Highest callus induction (90%) was noted on medium with 10<sup>-7</sup> M 2,4-D alone. Cotyledonary explants were the best source for compact and nodulated callus. Sub-cultured cotyledonary calluses showed shoot induction when transferred onto media supplemented with BAP alone or in combination with 10<sup>-7</sup> M or 10<sup>-6</sup> M NAA. However, 10<sup>-5</sup> M NAA completely suppressed the shoot inducing ability of BAP. In general, NAA promoted root induction from all explants. Highest shoot induction (95%) was obtained on medium supplemented with 10<sup>-6</sup> M BAP+10<sup>-6</sup> M NAA. Both IBA and NAA at 10<sup>-6</sup> M proved to be equally effective in induction of roots from the cut ends of 15-20 mm long shoots (excised from callus) in half-strength B5 liquid medium. Rooted shoots were successfully re-established in soil under controlled conditions.

**1176 LAVANIA, UC.** 1985. **Nuclear DNA and karyomorphological studies in vetiver (*Vetiveria zizanioides* L.) Nash.** *Cytologia*, 50: 1, 177-185; 17 ref.

Detailed karyotypic information is presented on 20 Indian genotypes (representing wild populations and cultivated forms). All genotypes had chromosome numbers of 2n = 2x = 20, but they varied with respect to haploid chromatin length and 2C DNA content. It is

suggested that these differences may be related to differences in essential oil content and composition.

**1177 QI, SY.** 1990. **Accumulation of secondary metabolites in cell suspension culture of *Aquilaria sinensis* (Lour.) Gilg. (Thymelaeaceae).** *Proc. of the Int. Congress of Essential Oils, Fragrances and Flavours. (11th: New Delhi: 1989: 12-16 November)* /edited by SC Bhattacharyya, N Sen, KL Sethi. London: Aspect Publishing, p. 1-4.

**1178 SETHI, KL; MAHESHWARI, ML; GUPTA, R.** 1990. **Genetic diversity and development of high oil yielding palmarosa strains.** *11th International congress of essential oils, fragrances and flavours, vol. 3: Biosciences.* (New Delhi: 1989: Nov 12-16)/edited by SC Bhattacharyya et al. London: Aspect Publ., p. 89-96.

**1179 WAKHLU, AK; NAGARI, S; BARNA, KS.** 1990. **Somatic embryogenesis and plant regeneration from callus cultures of *Bunium persicum* Boiss.** *Plant Cell Reports*, 9: 3, 137-138; 9 ref.

Callus was obtained from mericarps cultured on MS medium supplemented with 2.0 mg 2,4-D and 4.0 mg kinetin/litre. Small white clumps of compactly packed cells developed on the callus on medium containing 1.0 mg 2,4-D/litre and no kinetin and differentiated numerous globular embryos. Embryo maturation and germination was achieved on basal medium as well as on that supplemented with 1 mg kinetin/litre. All of the regenerated plants examined were normal diploids (2n = 14).

## Cymbopogon

**1180 BARUAH, ANJANA; BORDOLOI, DN.** 1989. **High frequency plant regeneration of *Cymbopogon martinii* (Roxb.) Wats by somatic embryogenesis and organogenesis.** *Plant Cell Reports*, 8: 8, 483-485; 11 ref.

Embryogenic callus cultures were obtained by repeated subculture of non-embryogenic callus from nodal segments. MS medium supplemented with 1 mg 2,4-D and 0.5 mg kinetin per litre and Linsmaier & Skoog (LS) medium supplemented with 2 mg 2,4-D and 0.4 mg kinetin/litre were used as maintenance media for non-embryogenic and embryogenic cultures, respectively. Plant regeneration occurred through organogenesis in MS basal media containing 2 mg kinetin, 1 mg 6-benzylaminopurine [benzyladenine], 0.2 mg biotin, 0.2 mg Ca-pantothenate and 0.1 mg NAA per litre. Embryogenesis was induced in LS medium supplemented with 1 mg kinetin, 0.5 mg 6-benzylaminopurine and 0.1

mg IAA per litre. Plant regeneration at a high frequency occurred through organogenesis and embryogenesis in different long-term callus cultures.

**1181** SINGH, N; LUTHRA, R; SANGWAN, RS. 1991. Mobilization on starch and essential oil biogenesis during leaf ontogeny of lemongrass (*Cymbopogon flexuosus* Stapf.). *Plant and Cell Physiology*, 32: 6, 803-811.

**1182** SREENATH, HL; JAGADISHCHANDRA, KS. 1988. In vivo and in vitro instability of B chromosomes in palmarosa grass (*Cymbopogon martinii* var. *motia*). *Genome*, 30: 6, 966-973; 39 ref.

Root tips, shoot tips, floral primordia and calluses of diploid palmarosa ( $2n = 20 + 1-2B$ ) were investigated. Meiotic and mitotic instability and elimination of B chromosomes was observed under both in vivo and in vitro conditions. Under in vivo conditions, B chromosomes were totally absent from the roots but preferentially transmitted in the germ line tissues. When present as a pair, the B chromosomes formed bivalents showing almost regular orientation, congression and disjunction. When present singly, the B chromosome formed a univalent and did not pair with any of the A chromosomes and showed nonalignment on the metaphase plate during metaphase I. Immature inflorescences of a race with  $2n = 20 + 2 B$  were cultured on MS medium containing 1 mg/litre 2,4-D to produce embryogenic callus. Cytological analysis of the callus revealed only 20 A chromosomes in nearly all the cells, both the B chromosomes being eliminated. From this callus, plantlets without B chromosomes were regenerated on MS medium without growth regulators and established in the soil. The regenerated plants exhibited 20 A chromosomes with normal meiosis.

**1183** SREENATH, HL; JAGADISHCHANDRA, KS. 1989. Somatic embryogenesis and plant regeneration from inflorescence culture of Java citronella (*Cymbopogon winterianus*). *Annals of Botany*, 64: 2, 211-215; 16 ref.

Embryogenic callus, induced from immature inflorescence segments and maintained for 2 years on MS medium supplemented with 1 mg 2,4-D per litre, retained the original chromosome number of  $2n = 20$ . Somatic embryos germinated into plantlets on MS basal medium or medium with 1 mg IAA, NAA, BAP [benzyladenine] or KN [kinetin] per litre individually. Regenerated plantlets developed a good root-system on full strength solid MS inorganic medium with 1 mg IAA per litre and were similar to the donor plant in morphol-

ogy and had the same chromosome number, but showed some variation in essential oil content.

## Mentha

**1184** AKHILA, A; SRIVASTAVA, R; RANI, K; THAKUR, RS. 1991. Biosynthesis of (-)-mintlactone and (+)-isomintlactone in *Mentha piperita*. *Phytochemistry*, 30: 2, 485-489.

**1185** AKHILA, A; NIGAM, MC. 1983. Biosynthesis of monoterpenes. *Indian Perfumer*, 27: 3/4, 174-196; 104 ref.

A review and discussion on modern methods of separation and structure determination with tabulated data on several genera, mainly *Mentha*, *Pinus* and *Tanacetum* species.

**1186** BHAUMIK, C; DATTA, PC. 1989. Development of Japanese mint tissue culture method. *Indian Perfumer*, 33: 3, 165-168; 12 ref.

Young leaf tissue was used as the primary explant in trials to determine the optimum culture conditions for *Mentha arvensis* subsp. *haplocalyx* var. *piperescens*. Modification of growth hormones and salts of MS medium resulted in MSA and MSB media. MSA and 500 mg/litre phenylalanine at pH 5.4 was used for callus initiation. MSB at pH 5.8, 200 to 400 lux and 20 to 25°C was used for rapid callus growth. Successful cell suspension culture was achieved by omitting coconut milk from the MSB medium.

**1187** KUKREJA, AK; DHAWAN, OP; MATHUR, AK; AHUJA, PS; MANDAL, S. 1991. Screening and evaluation of agronomically useful somaclonal variations in Japanese mint (*Mentha arvensis*). *Euphytica*, 53: 3, 183-191; 23 ref.

A procedure has been standardized for high frequency plant regeneration response from nodal explant cultures of *M. arvensis* var. *piperascens*. MS medium supplemented with IAA or NAA (0.5-2.0 mg/litre) alone, supported axillary shoot elongation while benzyladenine (2.0-3.0 mg/litre) + IAA (1.0 mg/litre) supported multiple shoot production. In vitro-derived shoots readily developed roots when cultured on NAA (1.0 mg/litre) fortified MS medium. Regenerated plantlets were successfully transferred to the greenhouse (90-95% survival rate) and ultimately to the field. Among 280 plants transferred to the field, a wide range of variation was observed for plant height (32.0-92.0 cm), leaf-stem weight ratio (0.53-2.32), herb yield (105.0-870.0g), oil content (0.32-1.10%) and oil yield (0.66-5.22 ml/plant).

Variations were also recorded for 4 major constituents of the essential oil, i.e. menthol (65.2-94.77%), menthone (1.40-20.89%), isomenthone (0.96-5.14%) and menthyl acetate (0.75-8.52%). A positive correlation was found for oil yield with plant height and herb yield, whereas a negative correlation existed between herb yield and oil content. Based on the initial agronomic assessments on an individual plant basis, 27 somaclones were selected and further evaluated in a replicated plant to row trial with parent plant CIMAP/Hy-77 as standard. Somaclones Sc59 and Sc179, selected on the basis of higher herb yield in the initial screening, recorded 55.8% and 64.3% increase in oil yield over the control, respectively. Somaclones Sc93, Sc114, Sc121 and Sc124 that were selected for their better oil content exhibited 47.2%, 50.6%, 57.5% and 48.2% increase in oil yield over the parent variety, respectively.

**1188 KUNDU, AK; SHARMA, AK. 1985. Chromosome characteristics and DNA content in *Mentha Linn. Nucleus, India*, 28: 1/2, 89-96; 35 ref.**

*M. spicata*, *M. arvensis*, *M. arvensis* var. *javonica* and three Indian populations of *M. piperita* were shown to have similar karyotypes; in general, the chromosomes were small. Chromosome number varied from  $2n = 48$  in *M. spicata* to  $2n = 120$  in the allopolyploid *M. piperita* population from Lucknow. On the basis of present and previous studies, basic numbers of  $x = 5$  and  $x = 6$  are suggested for the subgenera *Pulegium* and *Menthastrum*, respectively. Microsporocyte degeneration resulting in male sterility was observed in *M. arvensis* var. *javonica*. A 1.4-fold variation in the amount of DNA per nucleus was observed in the 6 taxa. It is suggested that the decrease in the proportion of DNA with increasing ploidy may confer an adaptive advantage.

**1189 NIKOLAEV, AG; PYSOVA, MT; LOLLO, LV. 1990. Monoterpenoids transformation in *Mentha* leaves by means of distant hybridization. *International congress of essential oils, fragrances and flavours, Vol. 3: Biosciences*. (New Delhi: 11th: 1989: Nov 12-16)/edited by SC Bhattacharyya, N Sen, KL Sethi. London: Aspect Publishing, p. 43-48.**

## Ocimum

**1190 KHOSLA, MK. 1988. Breeding in genus *Ocimum*: phytochemical studies of essential oils. *Indian Perfumer*, 32: 3, 236-247; 5 ref.**

F2 populations raised from selfed seed of partially fertile reciprocal F1 crosses of *O. gratissimum* X *O.*

*viride* were studied for morphology, yield and cytology. Heterosis was observed for herb and oil yield. Plants with high oil and herb yields, and altered chromosome and chemical composition of the oil were selected. *Eugenol*, *isoeugenol*, *cineol*, *4-terpenol* and *myrcene* were important constituents of the essential oil.

**1191 KHOSLA, MK; SBOTI, SN. 1986. Cytogenetic studies in the genus *Ocimum*: interspecific hybrids and induced amphiploids of *O. gratissimum* L. ( $2n = 40$ ) X *O. viride* Willd. ( $2n = 40$ ). *Cytologia*, 51: 2, 225-234; 13 ref.**

F1 hybrids between these 2 tetraploids grew vigorously and showed heterosis for vegetative characters, but were sterile (1.4-1.9% seed set). *Colchicine*-induced amphidiploids showed gigantism for vegetative and floral characters, but were shorter than the F1s. The amphidiploids showed normal meiosis and 68-75% seed set. Chromosome pairing studies revealed an homology between one of the genomes of *O. gratissimum* and one of those of *O. viride*; the species are assigned the genome formulae AB and A'C, respectively.

**1192 KHOSLA, MK; SOBTI, SN. 1984. Hybridization between different geographical races of *Ocimum gratissimum* L. *Nucleus, India*, 27: 3, 156-159; 8 ref.**

F1 hybrids, involving 3 geographical races collected from Jammu and Kerala and the USA, were produced. Jammu and US races crossed freely with each other to produce fertile, vigorous hybrids. The hybrids involving the Jammu and US races with the Kerala race were stunted and sterile. Evidence on chromosome length, chromatin length, karyotype formulae and, in sterile F1s, chromosome and meiotic abnormalities showed that the Kerala race has developed isolation barriers with respect to the other 2 races and that these barriers may ultimately result in the evolution of a new species.

**1193 KHOSLA, MK; BRADU, BL; GUPTA, SC. 1990. Polyploidy breeding in *Ocimum* for evolving high yielding, better Quality strains of essential oil importance. *International Congress of Essential Oils, Fragrances and Flavours, Vol. 3: Biosciences*. (11th: New Delhi: 1989: Nov 12-16)/edited by SC Bhattacharyya, N Sen, KL Sethi. London: Aspect Publishing, p. 75-80.**

## Rosmarinus officinalis

**1194 CHATURVEDI, HC; MISRA, P; SHARMA, M. 1984. In vitro multiplication of *Rosmarinus officinalis* L. *Z. Pflanzenphysiologie*, 113: 4, 301-304; 5 ref.**

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 from 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* A. 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**