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Scope and target readership: The *International Journal of Plant Developmental Biology* deals exclusively with the issues of development and embryogenesis in plants, higher or lower.

The primary topics that are covered include:

- 1) Control of gene expression during development of any cell, tissue or organ;
- 2) Developmental mechanisms leading to a further understanding of the industrial use of plants;
- 3) Gametogenesis and fertilization, and gametophytic embryogenesis;
- 4) Molecular genetics of development;
- 5) Mechanisms of differentiation and dedifferentiation; (programmed) cell death and apoptosis;
- 6) Somatic embryogenesis;
- 7) Uncontrollable developmental processes;
- 8) General plant regeneration and *in vitro* tissue culture, synthetic seeds, cryopreservation.

Hormonal, physiological, environmental, genetic, biophysical, developmental or molecular approaches to the study of the regulation of plant growth and development are all encouraged. Practical *in vitro* regeneration protocols will also be considered.

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Cover photos: Top, left: Multiple shoot regeneration from *Spilanthes mauritiana* shoot tip explant culture (Sharma *et al.*, pp 56-61); Center (top and bottom): *Vanilla planifolia* shoot and plantlet formation *in vitro* (Gantait *et al.*, pp 18-23); Top, right: Somatic embryogenesis on leaf explants of *Coffea arabica* AC1 when cultured with 6-BA (Almeida and Silvarolla, pp 5-9); Bottom, left: *Mukia maderaspatana* shoots (Mahender *et al.*, pp 1-4); Bottom right plate: Sub-cellular structure of rice anther cells (Lin *et al.*, pp 39-46).

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Mahender Aileni, Venugopal Rao Kokkerala, Rajesh Yarra, Pavan Umate, Sadanandam Abbagani (India) High-frequency Regeneration of Shoots from Cotyledon and Leaf Explants of a Medicinal Cucurbit, *Mukia maderaspatana* (L.) M.J. Roem. (pp 1-4)

ABSTRACT

Original Research Paper: The purpose of this study was to develop an efficient protocol for *in vitro* organogenesis from cotyledon and leaf explants of *Mukia maderaspatana* (Linn.) M. J. Roem., a medicinal member of the family Cucurbitaceae. Cotyledon explants isolated from *in vitro* germinated seedlings (5-6 days old) were cultured on Murashige and Skoog (MS) medium containing different concentrations of 6-benzyladenine (BA; 0.89, 1.78, 2.22, 3.11, 4.40 and 6.62 μM) alone, or in combination with indole-3-acetic acid (IAA; 0.57 μM). Cotyledon explants cultured on medium containing BA (2.22 μM) and IAA (0.57 μM) induced a significantly high number of multiple shoots (9.00 ± 0.60) with increased mean shoot length (2.70 ± 0.10) within 5 week of culture. Leaf segments from *in vitro* grown plants (20 days-old) were cultured on MS medium with different concentrations of BA (0.22, 0.44, 0.89, 1.78, 2.22, 3.11 and 4.40 μM) alone, or together with IAA (0.57 μM). Maximum number of shoots (10 ± 0.75) with increased mean shoot length (2.90 ± 0.12) were obtained directly from leaf explants (without intervening callus phase) using a combination of BA (0.89 μM) and IAA (0.57 μM) within 5 weeks of culture. Inclusion of IAA to MS medium with BA triggered a high frequency of regeneration from leaf and cotyledon explants. Elongation of regenerated shoots occurred when cotyledon cultures (3 weeks-old) were transferred to MS basal medium. Leaf cultures with emerging shoots were sub-cultured onto the same treatment medium for further elongation. The elongated shoots (2-3 cm) were excised and rooted on MS medium supplemented with IBA (2.46 μM). Rooted plants were acclimatized in the greenhouse with a 70% survival rate.

Julieta Andrea Silva de Almeida, Maria Bernadete Silvarolla (Brazil) Induction of Somatic Embryos of *Coffea arabica* Genotypes by 6-Benzyladenine (pp 5-9)

ABSTRACT

Original Research Paper: The objective of the present study was to verify the effect of adding 6-benzyladenine (6-BA) on somatic embryogenesis of *Coffea arabica* genotypes AC1, AC2, AC3 and Mundo Novo cv. 'IAC 376-4'. Rectangular foliar explants of these genotypes were inoculated into a single semi-solid culture medium consisting of $\frac{1}{2}$ MS salts supplemented with 10, 15, 20, 30, 40 and 50 μM 6-BA, respectively and kept in the dark at 25°C. The treatments were evaluated with respect to the number of sides of the explant showing formation of structures, an estimate of the size of the structure formed by the explant and the total number of somatic embryos (SEs) produced. The formation of small structures (3 mm) on the borders of the explants of AC and 'Mundo Novo' was observed and these subsequently developed SEs when cultured in medium supplemented with lower concentrations of 6-BA tested (10, 15, 20 or 30 μM). In addition, SEs were also formed directly on the borders of the explants. Another aspect of the present study is the formation of SEs as a response to 6-BA as the sole growth regulator and their development in a single culture medium, in a single phase. This culture method results in a reduction in time, handling and consumables, thus being more advantageous, in addition to opening perspectives for its use with other *C. arabica* genotypes.

Ezequiel Marchionni Basté (Argentina), Gustavo R. Rodriguez (USA/Argentina), Guillermo R. Pratta, Roxana Zorzoli (Argentina) Genotype Variations of Early Protein Expression During Tomato *in Vitro* Culture (pp 10-14)

ABSTRACT

Original Research Paper: Polymorphism in total protein patterns is an indicator of variability for genes associated with regeneration ability. The objective was to characterize the early expression of proteins involved in tomato organogenesis from leaflet explants of divergent taxonomic genotypes with different regeneration abilities. *Solanum lycopersicum* cv. 'Caimanta' as the highest regenerating genotype, accession LA722 of *S. pimpinellifolium* as the lowest regenerating genotype and F₁ were assayed. *In vitro* culture was performed according to the standard protocol for tomato. Twenty samples of each genotype were analyzed at different days of incubation (from 1 to 10 days). Uncultured explants were the experimental tester. Total proteins were extracted from these samples in phosphate buffer and then separated by SDS-PAGE. Polymorphism was found for polypeptides of 76.6, 55.5, 52.1, 49.7, 44.5, 27.9, 24.7, 24.1, and 19.5 kDa, which accounted for 56% of the total protein patterns. Polymorphic polypeptides between incubation periods were those of 76.6, 49.7, 44.5, and 24.7 kDa. Polymorphic

polypeptides between genotypes were those of 52.1, 27.9, 24.1, and 19.5 kDa. Polymorphism of total protein patterns during the first days of incubation, and between divergent taxonomic genotypes with different regeneration ability, indicated variability of genetic expression of the *in vitro* response.

Marcos Daquinta, Karomo Brown (Cuba), Jaime A. Teixeira da Silva (Japan), Fernando Sagarra (Cuba) *In Vitro* Propagation of Arrowroot (*Maranta arundinacea* L.) (pp 15-17)

ABSTRACT

Short Communication: Arrowroot (*Maranta arundinacea* L.; Marantaceae) is an excellent source of starch (>85%) used in the food industry. The multiplication of this plant is traditionally from rhizomes, which are also a source of starch. *In vitro* *M. arundinacea* shoot cultures were successfully established from rhizome buds on semi-solid MS medium supplemented with 3 mg.l⁻¹ 6-benzylaminopurine in the dark, which was also the best medium for shoot proliferation. Shoots were acclimatized in zeolite and sugarcane filter substrate (1:1) with a 90% survival percentage.

Saikat Gantait, Nirmal Mandal, Somnath Bhattacharyya, Prakash Kanti Das (India), Sanjib Nandy (Canada) Mass Multiplication of *Vanilla planifolia* with Pure Genetic Identity Confirmed by ISSR (pp 18-23)

ABSTRACT

Original Research Paper: A novel protocol was developed for *Vanilla planifolia* to enhance *in vitro* cloning through multiple shoot induction. Bud induction was recorded from nodal explants as compared to the shoot tip within 10 days in MS with 0.5 mg l⁻¹ NAA and 1.0 mg l⁻¹ BAP. A maximum of 5 buds from a single explant appeared within 11 days after the first bud induction. MS with 2.0 mg l⁻¹ BAP only proved best for multiple shoot proliferation resulting in 7 shoots per inoculated shoot bud within 55 days. Maximum roots per plantlet were observed in MS with 0.25 mg l⁻¹ IAA and 2 g l⁻¹ activated charcoal. Autoclaved sand and intermittent water spraying optimized the primary acclimatization period of 12 days and then larger pots filled with sand, soil, charcoal and coconut fibre ensured 86% acclimatization in next 20 days. Selected ISSR primers were used to ensure genetic clonality of these *in vitro*-generated propagules.

Asma Ben Ghnaya, Najeh Ben Fadhel, Mohamed Boussaid (Tunisia) High Frequency of Multiple Shoots and Plant Regeneration from Different Explants of *Cucumis melo* L. 'Flexuosus': Histological Study and Biochemical Analysis (pp 24-28)

ABSTRACT

Original Research Paper: Within the framework of genetic improvement of a Tunisian Snake-melon (*Cucumis melo* L.) cultivar by biotechnological methods, we developed a method leading to the regeneration of whole plants by *in vitro* culturing of hypocotyl and cotyledon explants, seeds without one cotyledon or with quartile cotyledons, and the embryonic axis, on Murashige and Skoog (MS) medium with different combinations and concentrations of auxin and cytokinin. The percentage of caulogenesis varied with the source of the explant and the composition of the plant growth regulator. The highest percentage of caulogenesis (64.4%) was observed in embryonic axes cultivated on MS with 0.5 mg l⁻¹ 2,4-D and 1 mg l⁻¹ BAP. Rooting of buds occurred on MS containing 0.5 mg l⁻¹ NAA. As soon as roots appeared, the plantlets were transferred into pots, and 80% survival was recorded. The origin of shoots was investigated by histological observation. In addition, the uniformity of regenerated plants was checked by polymorphism analysis of six isozymes by starch gel electrophoresis.

Neelu Joshi (India) *In Vitro* Growth and Shoot Multiplication in *Nicotiana tabacum* L. - Influence of Gelling Agent and Carbon Source (pp 29-33)

ABSTRACT

Original Research Paper: The present study examined the influence of various gelling agents and carbon sources on *in vitro* growth and multiplication in *Nicotiana tabacum* L. cv. 'Havana 425' (Family: Solanaceae). Shoot multiplication was greatly favoured on medium gelled with reduced concentrations of agar. Complete absence of agar in the medium evoked a better response than that obtained on agar-containing medium. Replacement of agar by guar gum (at 2.0%, w/v) showed a two-fold improvement in *in vitro* growth and multiplication. On this medium ca. 12 elongated shoots were obtained. On medium gelled with Phytigel (0.1%), the rate of shoot multiplication was two times higher than that recorded on control. Incorporation of different carbon sources in the range of 1.5-4.0% (w/v) evoked varied responses in terms of shoot growth and multiplication. Glucose at 4.0% was the most effective carbon source where a maximum number of elongated shoots (>1.6 cm in height) were

produced. Wet and dry weights of such shoots were also highest. The results emphasized the potential of guar gum as a gelling agent and glucose as a carbon source for increasing shoot multiplication and growth of *N. tabacum*.

M. Nasir Khan (Kingdom of Saudi Arabia), Firoz Mohammad, Manzer H. Siddiqui (India) Pre-sowing Seed Treatment and Foliar Application of Gibberellic Acid Improve Seed and Fibre Yield by Inducing Net Photosynthetic Rate and Carbonic Anhydrase Activity of Linseed Genotypes (pp 34-38)

ABSTRACT

Original Research Paper: Linseed (*Linum usitatissimum* L.) is an important oilseed and fibre crop. However, the production of linseed crop is unable to keep pace with the increasing demand of linseed products. Under these circumstances, the best strategy for dual-purpose linseed would be to increase the height of the plant and to improve seed weight, a task which may prove simpler than achieving the synchronization of seed and fibre maturity. To achieve this, the present work was carried out with an aim to find out whether the application of gibberellic acid (GA₃) could improve the performance of linseed crop. The experiment consisted of three GA₃ treatments, viz. 0, 10⁻⁸ and 10⁻⁶ M, with each treatment consisting of a pre-sowing seed treatment followed by foliar spray on plants raised from the treated seeds of five newly released genotypes of linseed namely 'Laxmi 27', 'Parvati', 'Rashmi', 'Shekhar' and 'Shubhra'. Crop performance was assessed in terms of growth characteristics, physiological and biochemical parameters at 60 and 75 DAS and yield and quality characteristics at harvest. Pre-sowing seed and foliar treatment with GA₃ at 10⁻⁶ M proved best for most of the parameters studied. This treatment enhanced, for example, dry weight per plant by 40.5% and P_N by 12.2% at 75 DAS and seed yield per plant by 24.7%, oil yield per plant by 27.1% and fibre yield per plant by 55.9% at harvest as compared with 0 M GA₃ (i.e. the control). However, GA₃ treatments increased lodging, with 10⁻⁶ M GA₃ by 43.7% than the control. The data revealed that genotypes differed critically with regard to parameters studied. Among the genotypes tested 'Shubhra' performed best while 'Laxmi 27' worst.

Mei Zhen Lin, En Ming He, Dong Mei Wei, Hui Qiao Tian (China) ATPase Changes in Rice Anthers (pp 39-46)

ABSTRACT

Original Research Paper: This study investigated ATPase distribution using a lead precipitation technique during rice anther development. The ATPase reaction precipitates were localized in the nuclei of microspore mother cells (MMC), and a few precipitates were detected in the cytoplasm. Low amounts of precipitate were also located in the anther wall, with the exception of tapetal cell nuclei. Following meiosis in the MMC, the precipitates in epidermal cells, endothelium and middle layer cells increased noticeably on the plasma membrane and in the nearby cytoplasm. Numerous precipitates were observed in the pollen wall during pollen development. The pollen wall exine was constructed during microspore development, and the exine precipitates were derived from tapetal cells. The intine was constructed during the bicellular pollen stage, and the intine precipitates originated from the pollen vegetative cell. The vegetative cell contained more precipitates than the generative cell. The amount of precipitate between the two pollen grain sperm cells also differed. The physiological functions of ATPase located in different cells and cellular components during rice anther development were analyzed.

Ravindra B. Malabadi (Canada/India), Jaime A. Teixeira da Silva (Japan), Gangadhar S. Mulgund (India) *In Vitro* Shoot Regeneration by Culture of *Liparis elliptica* (Rees) Lindl. Shoot Tip-derived Transverse Thin Cell Layers Induced by 24-epi Brassinolide (pp 47-51)

ABSTRACT

Original Research Paper: An efficient *in vitro* propagation protocol for *Liparis elliptica* (Rees) Lindl. using transverse thin cell layers (TCLs) was established. The initiation of protocorm-like bodies (PLBs) and the regeneration of shoot buds from PLB TCLs significantly relied on the concentration of 24-epi brassinolide (24-epiBL)-supplemented Mitra *et al.* basal medium. The highest percentage of PLB-TCL explants (93.0%) producing PLBs (71.0 ± 2.1) was recorded on 4.0 µM 24-epiBL in a period of 12 weeks. The cultures were maintained for 6-12 weeks for the initiation of PLBs or proliferating shoot buds. After nearly 12 weeks, small bud-like structures formed healthy shoots. The highest number of shoots with well developed roots was developed on 10.74 µM NAA-supplemented basal medium. This successful protocol will allow for the mass multiplication of *L. elliptica*, fulfilling the timely demand for clonal plantlets. This is the first ever report of *in vitro* culture for this epiphytic orchid.

Mahendar Porika, Radhika Tippani, Praveen Mamidala, Venkataiah Peddaboina, Christopher Thamidala, Sadanandam Abbagani, Rama Swamy Nanna (India) Micropropagation of Red Kino Tree (*Pterocarpus marsupium* Roxb.): A Medicinally

ABSTRACT

Original Research Paper: An efficient protocol is described for the rapid *in vitro* multiplication of an endangered highly valuable medicinal plant, *Pterocarpus marsupium* Roxb., through cotyledonary nodes of immature seeds (IS). High frequency of direct shoot regeneration was induced from cotyledonary nodes of IS on Murashige and Skoog (MS) medium supplemented with 6-benzyladenine (BA). Among the various cytokinins tested (BA, Kinetin (Kn), Zeatin (ZEA)), BA proved to be most effective. The direct shoot regeneration capacity of the IS was influenced by the BA concentrations (0.44-8.87 μM), and the optimal response was observed at 4.44 μM BA, which induced maximum number of multiple shoots (12.9 ± 0.21) with highest shoot length (3.8 ± 0.03) in 100% of the cultures, within 4 weeks. Significant differences were recorded in terms of average number of shoots per explant (1.9-12.9) among the different concentrations of BA investigated. Concentrations of all cytokinins tested reached a level that can be considered above the optimum level, as marked by a reduced frequency of shoot regeneration. A proliferating shoot culture was established by repeatedly subculturing the IS explants on 4.44 μM BA. Rooting of regenerated shoots was achieved under *in vitro* conditions by a two-step procedure employing a pulse treatment with indole-3-butyric acid (IBA) and subsequent transfer to growth regulator free half-strength MS medium. The most effective first-step treatment was found to be 49.00 μM IBA for 24 h, which initiated rooting at a frequency of 68%. *In vitro* raised plantlets were transferred to pots containing sterilized soil and vermiculite mixture (1: 1), and then transferred to the greenhouse. Plantlets established in pots exhibited a 75% survival rate. This procedure is suitable for use in large-scale production of plants and may have potential application to other *Pterocarpus* species.

Shiwali Sharma, Anwar Shahzad, Namreen Jan, Aastha Sahai (India) *In Vitro* Studies on Shoot Regeneration through Various Explants and Alginate-Encapsulated Nodal Segments of *Spilanthes mauritiana* DC., an Endangered Medicinal Herb (pp 56-61)

ABSTRACT

Original Research Paper: This study describes an improved, efficient protocol for adventitious shoot regeneration (through shoot tip and leaf explants) and conservation through synthetic seed (synseed) technology of *Spilanthes mauritiana*, an endangered medicinal herb. MS (Murashige and Skoog 1962) basal medium augmented with 1.0 and 2.5 μM BA was optimum for the induction of multiple shoots formation through shoot tip and leaf explants, respectively. Cytokinin and auxin combinations considerably enhanced the frequency of shoot induction. A maximum of 18.8 shoots/shoot tip were induced on MS basal medium supplemented with 1.0 μM 6-benzyl adenine (BA) and 0.5 μM indole-3-acetic acid (IAA); 15.0 shoots/leaf explants on MS with 2.5 μM BA and 0.5 μM IAA. Microshoots were best rooted on half-strength MS medium supplemented with 2.5 μM NAA. Synseeds, produced by encapsulating axillary buds in calcium alginate gel exhibited a critical response to nutrient concentration for conversion into complete plantlet. On half-strength MS basal medium germination percentage was maximum. A low temperature storage (4°C) experiment was also carried out to understand the explants' ability to revive physiological activity leading to plantlet development. Almost all the synseeds sprouted well and developed into plantlets when cultured on nutrient media after storage, up to three weeks of storage although subsequent storage reduced sprouting capability. Plants retrieved from rooting medium and synseeds were hardened off and successfully established in soil with a 90% survival rate and exhibited normal morphological and growth behavior when compared with *in vivo* grown plants.

Shiwali Sharma, Anwar Shahzad, Aastha Sahai (India) Artificial Seeds for Propagation and Preservation of *Spilanthes acmella* (L.) Murr., a Threatened Pesticidal Plant Species (pp 62-65)

ABSTRACT

Original Research Paper: Synthetic seeds (synseeds) offer several advantages, easy handling, storage, reduced size of propagules and transportability. Germplasm can be effectively stored in the form of synseeds. Nodal segments obtained from *in vitro* raised seedlings of *Spilanthes acmella* were encapsulated in calcium alginate beads. The best gel complexion was achieved using 4% sodium alginate and 100 mM calcium chloride. The maximum frequency ($87.8 \pm 1.15\%$) of conversion of encapsulated nodal segments into plantlets was obtained on Murashige and Skoog (MS) medium containing 1.0 μM 6-benzyl adenine (BA) and 0.5 μM α -naphthalene acetic acid (NAA) after 6 weeks of culture. Encapsulated nodal segments stored at 4°C for 1-8 weeks also showed successful conversion with variable percent in successive weeks of transfer, followed by development into complete plantlets when returned to regeneration medium. Conversion of encapsulated nodal segments into plantlets also occurred when the calcium alginate beads were sown directly into Soilrite™ moistened with quarter-strength MS

salts. Plants regenerated from encapsulated nodal segments were successfully hardened, acclimatized and established in soil, with a success rate of 90%.

Manzer Hussain Siddiqui, Firoz Mohammad, Mohd. Nasir Khan, Mohd. Masroor A. Khan (India) Physio-morphological Response of Erucic acid-Free Genotypes of Rapeseed-mustard to the Application of Graded Combinations of Nitrogen, Phosphorus and Sulphur (pp 66-70)

ABSTRACT

Original Research Paper: A field experiment was conducted to study the effect of five graded combinations of nitrogen (N), phosphorus (P) and sulphur (S) on growth characters, physio-biochemical parameters and yield characteristics as well as fatty acid composition of the oil of three genotypes of rapeseed-mustard (two erucic acid-free, viz. *Brassica napus* L. cv. 'Hyola PAC-401' and *Brassica juncea* L. Czern. & Coss. cv. 'TERI (0E) M21-Swarna', and one the best performing high-yielding, *B. juncea* cv. 'Rohini' as a check). The nutrient combinations with a uniform dose of 30 kg K ha⁻¹ included (i) 0 kg N + 0 kg P + 0 kg S ha⁻¹ (N₀P₀S₀), (ii) N₃₀P₁₀S₁₇, (iii) N₆₀P₂₀S₃₄, (iv) N₉₀P₃₀S₅₁ and (v) N₁₂₀P₄₀S₆₉. Application of N₉₀P₃₀S₅₁ proved best for most parameters studied. 'Hyola PAC-401' surpassed other cultivars in seed and oil yield. The interaction N₉₀P₃₀S₅₁ x 'Hyola PAC-401' (also N₉₀P₃₀S₅₁ x 'TERI (0E) M21-Swarna') proved superior for most parameters, including seed yield, oil yield and erucic acid content.