

Floriculture and Ornamental Biotechnology

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Scope and target readership: *Floriculture and Ornamental Biotechnology* is dedicated to significant advances in ornamental plant science and biotechnology.

Floriculture and Ornamental Biotechnology aims to examine:

- 1) Breeding biotechnology (genetic modification, somatic hybridisation and embryo rescue);
- 2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture);
- 3) Mycorrhizal symbioses (and effects on plant physiology, productivity, reproduction and disease resistance);
- 4) Physiology, molecular biology, structural botany (integrated, pure and applied);
- 5) Phytopathology;
- 6) Post-harvest technology as applies to cut flowers and foliage (deterioration, preservation, shipping, and marketing);
- 7) Production of secondary metabolites, organic and inorganic biochemistry, and phytochemistry;
- 8) Soil dynamics;
- 9) Storage of valuable genetic material (cold-storage or cryopreservation).

For publication in *Floriculture and Ornamental Biotechnology* the research must provide a highly significant new contribution to our understanding of floricultural or ornamental plants and must generally be supported by a combination of either physiological, biochemical, genetic or molecular analyses. All areas of study are welcome and the experimental approaches used can be wide-ranging.

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Cover photo: Top left: Snapdragon (*Antirrhinum majus*) flower; center: Detail of a transection of the pedicel internode showing the tissue composition of a thin cell layer explant at day 0. More details in Teixeira da Silva *et al.*, pp 1-13. Bottom right: Gummosis in tulip bulb (cv. 'Apeldoorn') induced by *Fusarium oxysporum* f. sp. *tulipae* after artificial infection; mycelium growth of the pathogen can be seen on the extruded gums before gums were solidified. More details in Saniewski *et al.*, pp 34-40.

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Jaime A. Teixeira da Silva (Japan), Khiem Tran Thanh Van (France), Stefania Biondi (Italy), Duong T. Nhut (Vietnam) Maria Maddalena Altamura (Italy) Thin Cell Layers: Developmental Building Blocks in Ornamental Biotechnology (pp 1-13)

ABSTRACT

Review: The capacity to control developmental and morphogenetic processes *in vitro* has always been the fascination and primary focus of many ornamental tissue culture studies. Thin cell layers or TCLs were first used to control the development of flowers, roots, shoots and somatic embryos in tobacco pedicels. Since those studies over 30 years ago, TCLs have been successfully used in the micropropagation of many ornamental plant species whose previous *in vitro* morphogenetic pathways were not clearly defined using conventional methods. TCL technology focuses on the size and origin of the explant, which, when appropriately chosen, serves as a fine-scale developmental block for regeneration and transformation. This review highlights the fundamentals of TCLs, and their application in ornamental plant micropropagation and transformation.

Sridevy Srisankarajah (Denmark), Ezz Al-Dein Al-Ramamneh (Jordan), Margrethe Serek (Denmark/Germany) Biotechnology of *Schlumbergera* and *Rhipsalidopsis* (pp 14-19)

ABSTRACT

Invited Mini-Review: Biotechnological tools have been successfully used with several plant species for improving existing cultivars and creating new ones. However, the application of such tools has been limited with ornamental plant species such as cactus. Cacti are known to possess a very slow growth rate and many of them are recalcitrant compared to other cultivated plant species thus creating problems in culturing or improving them. Some early work showed promise in establishing *in vitro* cultures with limited multiplication rates, but research findings recently indicate that *Schlumbergera* (Christmas cactus) and *Rhipsalidopsis* (Easter cactus) can be multiplied more rapidly via both axillary and adventitious shoots, and also via callus and somatic embryogenesis. These studies have discovered key factors that could enhance an understanding of morphogenesis in cacti. Studies were made on endogenous phytohormones and enzymes to elucidate the differences between the recalcitrant cultures and those with increased regenerative capacity of these plants. Recent studies have also shown that *Agrobacterium*-mediated genetic transformation is possible and a transformation efficiency of about 23% in *Rhipsalidopsis* is reported. This transformation efficiency was based on transgenic shoots expressing the activity of the reporter β -glucuronidase gene (GUS) indicated by blue staining of the regenerated shoots. Transgenic shoots regenerated in these studies through callus phase. Transformation was confirmed in the selected transgenic callus lines by GUS staining (for *uidA* gene), ELISA analysis and Southern blot hybridization (for *nptII* gene). The stability of transgenic calli was confirmed through their continuous sub-culturing on media containing the selectable marker kanamycin followed by testing for kanamycin resistance, GUS analysis and Southern hybridization. The regeneration system adopted for transformation was reliable as shown by the stable regeneration of calli and shoots growing on media both on selection and control media. However, due to the slow growth rate of these plants, no attempts were made to verify transformation in the greenhouse-grown plants.

Muhammad Asif Hanif, Haq Nawaz Bhatti, Raziya Nadeem, Khalid Mahmood Zia, Muhammad Asif Ali (Pakistan) *Cassia fistula* (Golden Shower): A multipurpose Ornamental Tree (pp 20-26)

ABSTRACT

Invited Mini-Review: *Cassia fistula* Linn is a multipurpose, ornamental, fast growing, medium sized, deciduous tree that is now widely cultivated world wide for its beautiful showy yellow fluorescent flowers. This paper reviews the phenolic antioxidants, metal sorption, medicinal and free radical propensities of plant parts and cell culture extracts. This paper also appraises antimicrobial activities and commercial significance of *C. fistula* parts. The main objectives of present review study are to: (1) critically evaluate the published scientific research on *C. fistula*, (2) highlight claims from traditional, tribal and advanced medicinal lore to suggest directions for future clinical research and commercial importance that could be carried out by local investigators in developing regions.

Guohua Ma, Yong Li, Genlin Jiao, Xiaoping Fu, Yourun Lin (China) Adventitious Shoot and Callus Formation *in Vitro* from Young Leaves of *Melastoma affine* (pp 27-29)

ABSTRACT

Short Communication: The genus *Melastoma* contains about 100 species and is distributed mainly in South Asia, the Pacific Ocean and Australia. In China, there are nine species and one variety. They are mainly distributed in areas South of the Changjiang River. Among these, most species in the genus *Melastoma* have varying foliage and beautiful flowers that are used for ornamental and horticultural purposes. In this study, we established an *in vitro* propagation system for *Melastoma affine*. Young leaf lobes from *in vitro* plantlets were induced to form callus on MS medium containing 1.0 mg.l⁻¹ thidiazuron or 1.0 mg.l⁻¹ thidiazuron in combination with 0.5 mg.l⁻¹ 6-benzyladenine (BA). Thereafter, adventitious shoots developed directly from callus on the same medium. The adventitious shoots can develop multiple-shoots on the propagation medium containing 0.2 mg.l⁻¹ BA + 0.02 mg.l⁻¹ NAA and root formation and plantlets regeneration were achieved on ½MS medium with 0.1 mg.l⁻¹ IBA. An efficient *in vitro* propagation and plant regeneration system was successfully established.

Akihide Okamoto, Masataka Yamashita, Yuji Kajitani (Japan) Breeding and Production of Kurume Azaleas (*Rhododendron obtusum* Planch.) (pp 30-33)

ABSTRACT

Invited Mini-Review: "Kurume azalea" is a brand name for evergreen azalea cultivars bred in Kurume, Fukuoka, which is located in northern Kyushu, and belong to *Rhododendron obtusum* Planch. with small to medium sized flowers. It is generally accepted that their foundation stocks are hybrids between *R. kiusianum* Makino and *R. kaempferi* Planch., and *R. sataense* Nakai. However, it was recently clarified that some cultivars show characteristics of *R. macrosepalum* Maxim. and *R. ripense* Makino. Kurume azaleas were created about 170 years ago, in the end of Edo era and appreciated indoors as potted plants (*bonsai*). Originally, they had been grown as a hobby among plant lovers. In the 1900's, breeding improvement, production and marketing were greatly advanced by eager floricultural growers. The production of Kurume azaleas has outstandingly increased since the 1950's because of a great demand as a green plant for parks and other public spaces. Kurume azaleas were introduced to Europe and the United States in the 1870's. In Europe, they were loved as a pot culture, and were thus called Belgian florist azaleas. On the other hand, Kurume azaleas were to be loved in the United States because improvement of florist azaleas in Europe were for reduced height and cold hardiness. They are also used as parents to breed evergreen azaleas with increased cold hardiness and flower quality for gardens.

Marian Saniewski (Poland), Kensuke Miyamoto, Hiroshi Okubo (Japan), Alicja Saniewska, Jerzy Puchalski (Poland), Junichi Ueda (Japan) Gummosis in Tulip (*Tulipa gesneriana* L.): Focus on Hormonal Regulation and Carbohydrate Metabolism (pp 34-40)

ABSTRACT

Invited Mini-Review: This mini-review describes the promoting effects of *Fusarium oxysporum* f.sp. *tulipae*, ethylene and jasmonates on gum induction and its accumulation in tulips (*Tulipa gesneriana* L.). Tulip bulbs infected by *F. oxysporum* f.sp. *tulipae* have shown to produce considerable quantities of ethylene enough to induce gummosis in diseased and/or healthy bulbs of some cultivars. Morphological and histological disease symptoms induced by *F. oxysporum* f.sp. *tulipae* are mostly gummosis, and metabolic activities considered to be regulated by ethylene (i.e. respiration, inhibition of flower bud formation) in tulip bulbs are affected. Interestingly, methyl jasmonate (MeJA) exogenously applied as a lanolin paste also induced gums in tulip bulbs, but also in stem and basal part of leaves. It should be mentioned that under natural conditions for normal growth, gums are not formed in stems and leaves. The simultaneous application of ethylene with MeJA greatly accelerates gum formation in the bulb, stems and leaves compared to the application of MeJA alone. MeJA and jasmonic acid (JA) were successfully identified in stems and the possible mechanism of jasmonates to induce gummosis in tulips is relevant to their effects on sugar metabolism. Not only the content but also the composition of tulip gum polysaccharides has been determined and been shown to consist of glucuronoarabinoxylan (GlcN: Ara: Xyl = 1: 2: 3) with an average molecular weight of ca. 700 kDa. The induction and production of gums are suggested to be regulated by a signal network of jasmonates and ethylene, especially by cross-signals between them.

Hong-Mei Sun (China), Jaime A. Teixeira da Silva (Japan), Yun-Fei Li, Tian-Lai Li (China) Effects of Low Temperature on Dormancy Release in Lily Bulbs (pp 41-45)

ABSTRACT

Invited Mini-Review: The mechanism of dormancy and dormancy release in lily (*Lilium* spp.) bulbs is still incomplete and ambiguous. Based on a summary of dormancy characteristics, we review the effects of low temperature on dormancy release. This paper highlights the metabolic changes in carbohydrates, endogenous hormones, free amino acids and phenols during low temperature storage, and the key stages in dormancy release as well as the effects of different parts of the lily bulb. The possible physiological mechanism of low temperature on dormancy release in lily bulbs and some problems which should be investigated in this field are also discussed in this review.

Xiaopeng Wen, Qiang Xu, Xiuxin Deng, Xiangbiao Ji (China) Chestnut Rose (*Rosa roxburghii* Tratt): a Promising Genetic Resource for Fruit and Ornament Exploitation in China (pp 46-50)

ABSTRACT

Invited Mini-Review: Chestnut rose (*Rosa roxburghii* Tratt) brightens the spring garden with its attractive flowers. Moreover, it shows promising prospects in fruit exploitation due to its high content of vitamin C (2054-3541 mg/100g FW), very high superoxide dismutase activity, attractively senescence-retarding and cancer-preventing effects. Considerable efforts have focused on its research during the past two decades. To satisfy the increasing demand of exploitation of this plant, this overview serves chiefly to demonstrate: 1) the nutritional and medicinal values of chestnut rose fruit, as well as its ornamental features; 2) propagation method; 3) the genetic diversity of germplasms and its evaluation using morphological traits and molecular markers; 4) inheritance tendency of some agronomical traits; 5) immature embryo *in vitro* culture technique, high efficient micropropagation and assessment of the genetic stability of cultures; 6) accumulating process and characteristics of vitamin C during fruit development, and molecular cloning and characterization of L-galactono-1,4-lactone dehydrogenase (GalLDH), a key enzyme catalyzing the terminal step of vitamin C biosynthesis; and 7) screening of molecular markers linked to resistance to rose powdery mildew, and cloning and analysis of resistance gene analogs in chestnut rose.

David C. Zlesak, James M. Bradeen, Neil O. Anderson (USA) The Use of AFLP Markers to Resolve Clonal Origin and Integrity in Rose, Hydrangea, and Lily (pp 51-60)

ABSTRACT

Original Research Paper: Amplified fragment length polymorphism (AFLP) is a reliable and robust marker system which has been useful for various genetic studies including clonal integrity studies differentiating genetically similar germplasm. AFLP was evaluated for its effectiveness to resolve clonal origin and integrity questions in three clonally-propagated, ornamental species. The origins of the rose cultivar BAleam and the hydrangea cultivar Bailday, and relationships among intraclonal selections of Easter lily 'Nellie White' were investigated. A standard AFLP protocol provided repeatable and consistent fingerprints for rose and hydrangea, while repeatable AFLP fingerprints could not be obtained for Easter lily despite exploring modifications to DNA extraction, digestion, preamplification, selective amplification, and polyacrylamide electrophoresis. AFLP data suggest that 'BAleam' may be an apomictic seedling of the maternal parent 'INterlav' resulting from diplospory, and that 'Bailday' is not a sport out of 'Bailmer', as suspected, but differs from the phenotypically similar cultivar 'Variegata' by only one AFLP fragment. AFLP analysis worked well to differentiate genetically similar germplasm for rose and hydrangea. For some organisms like Easter lily, however, factors such as large genome size (~77pg/2C nucleus) and highly repetitive DNA complicates AFLP analysis. Optimization to obtain repeatable, consistent, and scorable fingerprints may not be possible using AFLP to assess genetic variation in species with large genome sizes such as lily.

Judith Viégas, Maria Teresa Rosa da Rocha, Islaine Ferreira-Moura, Dana Leiria da Rosa, Joseane Almeida de Souza, Maria Goreti Senna Corrêa (Brazil), Jaime A. Teixeira da Silva (Japan) *Anthurium andraeanum* (Linden ex André) Culture: *In Vitro* and *Ex Vitro* (pp 61-65)

ABSTRACT

Original Research Paper: In order to optimize the establishment of both *in vitro* and *ex vitro* cultures of *Anthurium andraeanum*, recently expanded leaves from 3- to 4-year-old greenhouse plants were disinfected with 1.43% NaOCl and treated in an antioxidant solution with 150 mg.L⁻¹ citric acid, 100 mg.L⁻¹ ascorbic acid and 200 mg.L⁻¹ cysteine. Callus formation was induced

from disinfected leaf explants ($\pm 1 \text{ cm}^2$) on half Murashige and Skoog (MS/2) medium supplemented with 0.08 mg.L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg.L^{-1} 6-benzylaminopurine (BAP), with and without 1 mg.L^{-1} N6-isopentenyl adenine (2iP), while shoots were induced to root on MS medium with BAP (0.0; 0.5 or 1.0 mg.L^{-1}). Somaclonal variation was not observed. Organic soil, or vermiculite, or both (1:1), or both combined with sand, *xaxim*, sphagnum, pine cone, carbonized rice hull, turf, or sawdust (1:1:1), were used to test the efficacy of *ex vitro* acclimatization. This protocol resulted in the production of regenerants in about 120 days from the *in vitro* incubation of foliar explants to the *ex vitro* establishment of plantlets: callus was formed after 56 days of incubation; aerial buds and roots appeared after 28 days on regeneration medium, and the first leaves, after 33 days; after 70 days, 3 to 4 cordiform and dark green leaves were observed. MS/2 medium + 0.08 mg.L^{-1} 2,4-D + 1 mg.L^{-1} BAP + 1 mg.L^{-1} 2iP for callus induction, and MS + BAP 0.5 mg.L^{-1} for regenerating shoots are important protocol steps. Successful *ex vitro* growth was achieved within 7 months on all substrates with the exception of that containing sawdust.

A.S. Lukatkin, S.V. Bikunova (Russia), Jaime A. Teixeira da Silva (Japan) Cultivation of *Dioscorea nipponica* Makino *in Vitro* and *ex Vitro* (pp 66-69)

ABSTRACT

Original Research Paper: The patterns of introduction of *Dioscorea nipponica* Makino to *in vitro* culture as well as the cultivation of cuttings *in vitro* and multiplication have been investigated. The optimal culture conditions were determined.

Marcos Daquinta, Osbel Mosqueda, Maria Teresa González, Reinerio Benega (Cuba), Jaime A. Teixeira da Silva (Japan) Shoot Proliferation of *Caladium x hortulanum* in a Temporary Immersion System (pp 70-72)

ABSTRACT

Short Communication: Caladiums are highly prized ornamental plants whose high cost derives from their beautiful leaves. Within this group of plants *Caladium x hortulanum* commands a prominent market position. In this study, the petioles of young leaves were used to establish *in vitro* cultures. We were able to multiply this species on semi-solid Murashige and Skoog (MS) medium supplemented with 2.0 mg/L 6-benzylamino-purine (BAP). Moreover, a unique procedure for the mass propagation of caladium plants using a temporary immersion technique is described. This procedure involved an initial sprouting phase in an automated temporary immersion system followed by an elongation phase using conventional culture methods. To establish this protocol, *in vitro* shoots developed from petioles cultured on a semi-solid medium were used as starting materials. When using temporary immersion the multiplication rate was more than 12 times higher than under a conventional propagation system after 45 days. The highest multiplication rate was found when explants were cultured in sprouting medium (MS + 2.0 mg/l 6-BAP) in the temporary immersion system for four weeks. The highest number of competent (i.e. ready for acclimatization) and uniform plants was achieved when bud clusters were subcultured for four weeks on MS medium without plant growth regulators. Plantlets could be effectively acclimatized (92%) on a 1:1 zeolite : sugarcane filter (i.e. derived from the sugar milling process) substrate. Although these results are preliminary, the methodology is already being employed at a commercial level.

Adnan Younis, Muhammad Aslam Khan, Asif Ali Khan, Atif Riaz, M. Aslam Pervez (Pakistan) Effect of Different Extraction Methods on Yield and Quality of Essential Oil from Four *Rosa* Species (pp 73-76)

ABSTRACT

Original Research Paper: In the present study rose oil was extracted from the petals of four *Rosa* species i.e. *R. damascena*, *R. centifolia*, *R. borboniana* and *Rosa 'Gruss an Teplitz'* through solvent extraction through hexane, solvent extraction through ether and steam distillation. *R. damascena* yielded (0.145%) of absolute oil, *R. centifolia* yielded 0.11% whereas *R. 'Gruss an Teplitz'* yielded the least (0.035%) absolute oil. Solvent extraction through hexane yielded more absolute oil (0.11%) than steam distillation (0.075%) and solvent extraction (0.07%) through ether on petal weight basis. Gas-chromatography of the rose oil was carried out for the qualitative and quantitative analysis of the oil constituents. Major compounds identified were citronellol, methyl eugenol, geraniol, geranyl acetate, phenyl ethyl alcohol, linalool, benzaldehyde, benzyl alcohol, rhodinyl acetate, citronellyl acetate, benzyl acetate and phenyl ethyl formate. Both techniques (solvent extraction and steam distillation) yielded oil with differences in the percentage composition of each component, but solvent extraction through hexane proved better (i.e. higher yield and more components) than steam distillation for extraction of essential oil from roses.