

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 photos: Left, top: Protocorm-like bodies of *Aerides maculosum* Lindl. (Malabadi *et al.*, pp 35-39); Left, bottom: Plant regeneration in *Caladium bicolor* cv. 'Bleeding Heart' by somatic embryogenesis pathway in liquid medium (Siddiqui *et al.*, pp 1-9); Right, top: Artificial inoculation of *Colletotrichum acutatum* on stem and leaves of oleander plants (Lahoz *et al.*, pp 62-66); Right, center: Healthy and wilted tuberose flowers in cut flower solution (Motaghayer and Esna-Ashari, pp 59-61). Right, bottom: Histochemical staining of *Gladiolus* plants transformed with GUBQ4-*uidA* showing roots (top) and leaves (bottom) (Kamo *et al.*, pp 10-14).

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Zahid Hameed Siddiqui, Abdul Mujib, Samar Fatima, Rashmi Kapoor (India) Somatic Embryogenesis and Genetic Improvement of Selected Ornamentals (*Chrysanthemum*, *Euphorbia*, *Caladium* and *Cyclamen*) - A Review (pp 1-9)

ABSTRACT

Review: Ornamentals are an important group of plants that include herbs, shrubs and trees of annual and perennial types. Beside propagation of a wide range of ornamentals by plant tissue culture, recently, biotechnological methods have been successfully exploited for their genetic improvement. Various *in vitro* technologies often integrated with conventional breeding methods are somatic embryogenesis (SEG), somatic hybridization by protoplast fusion and genetic transformation which exploits the concept of recombinant DNA technology. In this review, the importance of SEG and the application and events of genetic transformation have been discussed in select ornamentals.

Kathryn Kamo (USA), Young Hee Joung (Korea), Karen Green (USA) GUS Expression in *Gladiolus* Plants Controlled by Two *Gladiolus* Ubiquitin Promoters (pp 10-14)

ABSTRACT

Original Research Paper: Ubiquitin represents a conserved family of genes that is involved in many metabolic processes. The most commonly used promoter for genetic engineering of cereal monocots is the maize ubiquitin promoter because it directs high levels of expression in most plants' tissues, but this promoter results in low levels of expression in *Gladiolus*. Several ubiquitin promoters were isolated from *Gladiolus* to find one that directs higher levels of expression than the maize ubiquitin promoter in *Gladiolus*. Two ubiquitin promoters isolated from *Gladiolus*, GUBQ2 and GUBQ4, are characterized here for their levels of expression and tissue-specific location of expression when transformed into *Gladiolus*. *Gladiolus* cv. 'Jenny Lee' plants were transformed with the *uidA* gene coding for β -glucuronidase (GUS) expression under control of either the GUBQ2 or GUBQ4 ubiquitin promoters. Five plant lines with either the GUBQ2 or GUBQ4 promoter were confirmed to be independently transformed by Southern hybridization. Two plant lines each contained one copy of pGUBQ2, and the other lines with either promoter were multicopy. There was a range in the levels of GUS expression. One of the GUBQ4 lines appeared to be silenced as GUS was not expressed in their young leaves, young roots, and callus derived from the plants. Levels of GUS expression were higher in young roots than in young shoots and callus with the GUBQ2 promoter. Three of the four expressing lines with GUBQ4 showed the highest levels of GUS expression in callus followed by roots. Histochemical staining showed that GUS was expressed throughout the leaves and roots of *Gladiolus* plants transformed with either GUBQ2 or GUBQ4.

Jitender Kumar Kanwar, Surinder Kumar (India) *Agrobacterium*-mediated Genetic Transformation of Carnation for Insect Resistance (pp 15-19)

ABSTRACT

Short Communication: Plant regeneration and genetic transformation techniques have been developed in leaf tissue of carnation (*Dianthus caryophyllus* L. cv. 'Indios'). Callus was induced on Murashige and Skoog (MS) medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). Highest shoot regeneration from callus was obtained with 2 mg/l thidiazuron (TDZ) and 1 mg/l indole-3-acetic acid (IAA). Carnation plantlets were able to regenerate within two months of culture. *Agrobacterium*-mediated genetic transformation was achieved using leaf explants and a synthetic gene encoding the *cry1Ab* δ -endotoxin of *Bacillus thuringiensis*. Highest callus induction was achieved on selective medium containing 100 mg/l kanamycin (Kan) and 500 mg/l cefotaxime (Cef) after 96-hr pre-conditioning following 72-hr co-cultivation. Highest number of shoots per callus was observed when MS medium was supplemented with 2 mg/l TDZ, 1 mg/l IAA, 100 mg/l Kan and 500 mg/l Cef. Shoots were elongated and multiplied on MS medium containing 1 mg/l 6-benzyladenine and solidified with 1% agar. Rooting was accomplished on half-strength MS medium supplemented with 2 mg/l indole-3-butyric acid and 50 mg/l Kan. The putative shoots were hardened with 71% survival in a glasshouse. The transformed shoots were analyzed for the presence of the *cry1Ab* gene by PCR. The expression of the *cry1Ab* gene in the leaves of transgenic shoots was detected by an insect bioassay performed with the larvae of carnation bud borer (*Helicoverpa armigera*).

Zhenbang Chen, Ming Li Wang, Clint Waltz, Paul Raymer (USA) Genetic Diversity of Warm-Season Turfgrass: Seashore Paspalum, Bermudagrass, and Zoysiagrass Revealed by AFLPs (pp 20-24)

ABSTRACT

Original Research Paper: Three major types of warm-season turfgrass, including seashore paspalum (*Paspalum vaginatum* Swartz), bermudagrass [*Cynodon dactylon* (L.) Pers.], and zoysiagrass [*Zoysia japonica* Steud., *Zoysia matrella* (L.) Merr., and *Zoysia tenuifolia* auct.] cover many of the golf courts and sport fields in southern regions of the U.S. Improvement of turfgrass cultivars has been mainly based on the selection from natural mutations or genetic variations resulting from recombination of different ecotypes or species (hybrid bermudagrass and zoysiagrass). Genetic diversity among species and among turfgrass cultivars within species (including 10 seashore paspalum cultivars, 14 bermudagrass cultivars, and 24 zoysiagrass cultivars and elite lines) was assessed using amplified fragment length polymorphism (AFLP) markers. Among species, the polymorphism level of zoysiagrass is higher than bermudagrass and the polymorphism level of bermudagrass is higher than seashore paspalum. Our results demonstrated that AFLP is one of the useful DNA marker systems for quickly revealing the level of genetic diversity among species and assessing the genetic diversity of different turfgrass cultivars within the species. However, some released turfgrass cultivars could not be differentiated in this report by AFLP markers because they were developed from the parents that are closely related genetically. To enhance turfgrass breeding efficiency, different types of DNA marker systems should be used for evaluating turfgrass germplasm. Based on genetic diversity evaluation, more diverged parents should be selected and used to make crosses for developing new turfgrass cultivars.

Kiran Kaul, D. Dhyani, Ram Kumar Sharma (India) Evaluation of DNA Extraction Methods for RAPD, SSR and AFLP Analyses of Wild Rose Species (pp 25-30)

ABSTRACT

Techniques Paper: The current study aims to evaluate a rapid, simple and robust method of DNA extraction for AFLP analysis of 12 wild rose species (*Rosa brunonii*, *R. cathayensis*, *R. moschata*, *R. multiflora*, *R. wichurriana*, *R. indica*, *R. alba*, *R. macrophylla*, *R. tomentosa*, *R. canina*, *R. damascena*, *R. bourboniana* and the F1 progeny of *R. damascena* and *R. bourboniana*). Extraction of quality DNA from wild rose species is difficult as they contain high levels of polysaccharides and polyphenols. Four DNA extraction protocols were compared: two commercial kits from Qiagen and AuPrep, CTAB and a modified CTAB protocol. The protocols were evaluated in terms of yield, purity, restrictability and amplifiability of recovered DNA. The yield and quality of genomic DNA was considerably affected when commercial kits and common CTAB protocol were utilized for DNA isolation. The modified phenol free, CTAB procedure involving a washing step before extraction was the most successful extraction method giving optimum yields (900-1750 µg/g) of quality DNA that was amenable to restriction digestion and polymerase chain reaction (PCR) analyses – RAPD, SSR, AFLP.

Jitender Kumar Kanwar, Surinder Kumar (India) Direct Adventitious Shoot Regeneration from Leaf and Internode Explants of *Dianthus caryophyllus* (pp 31-34)

ABSTRACT

Short Communication: Direct adventitious shoots were regenerated in *Dianthus caryophyllus* cv. 'Tempo' from leaf and internode explants cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-benzyladenine (BA), kinetin, thidiazuron (TDZ), zeatin, α -naphthalene acetic acid (NAA) and indole-3-acetic acid (IAA) singly, or in combination. Shoot regeneration was highest with 2 mg/l TDZ and 1 mg/l IAA in both explants, whereas the number of shoots per explant was greater with 2 mg/l TDZ and 1 mg/l NAA. Shoots were elongated and multiplied on MS medium supplemented with 1 mg/l BA and solidified with 1% agar to reduce hyperhydricity. *In vitro*-raised shoots were rooted in half-strength MS medium supplemented with 1 or 2 mg/l IAA, NAA and indole-3-butyric acid (IBA). The rooted plants were hardened with 80-82% survival success in pots.

Ravindra B. Malabadi (Canada/India), Jaime A. Teixeira da Silva (Japan), Gangadhar S. Mulgund (India) TDZ-Induced *In Vitro* Shoot Regeneration of *Aerides maculosum* Lindl. from Shoot Tip Thin Cell Layers (pp 35-39)

ABSTRACT

Original Research Paper: Efficient shoot regeneration of *Aerides maculosum* Lindl. was achieved using transverse thin cell layers (tTCLs) of shoot tips in the presence of thidiazuron (TDZ). Protocorm-like bodies (PLBs) or proliferating shoot buds were observed when tTCLs were cultured on Mitra *et al.* (1976) basal medium supplemented with 13.62 µM TDZ. A high percentage (81%) of PLBs survived and ultimately produced healthy shoots with 2-3 leaves. Shoots rooted when cultured on the same basal medium supplemented with 12.25 µM indole-3-butyric acid. The regenerated plantlets grew normally with a 90% survival

rate.

Priyanka Modi, Arunima Sinha, S. L. Kothari (India) Reduction of Hyperhydricity in Micropropagated French Marigold (*Tagetes patula* L.) Plants by Modified Medium Parameters (pp 40-45)

ABSTRACT

Original Research Paper: Adventitious shoot bud regeneration was obtained on Murashige and Skoog (MS) medium supplemented with 4.4 μM 6-benzylaminopurine (BA) and 2.8 μM indole-3-acetic acid (IAA) from cotyledon explants of 12 *Tagetes patula* varieties. In order to reduce the occurrence of hyperhydrated shoots (HS), the effect of various gelling agents, culture vessels and ammonium ions in the medium was investigated in var. 'French Marigold Safari Red'. When the induction medium contained agar (0.9-1.5%) as the gelling agent, the highest organogenic response was observed on 0.9% agar with 11.2 normal shoots (NS) per explant. Among different culture vessels, Petri dishes were found to be most suitable for induction of NS. Vigorous shoots were produced on optimized medium [MS medium containing 10.3 mM NH_4^+ (half the standard MS level of 20.6 mM), BA (4.4 μM) and IAA (2.8 μM)] yielding 25.4 NS per explant. Both chlorophyll content and peroxidase activity were lower in HS than in NS. Full-strength MS medium without any plant growth regulator was effective for elongation and rooting. Plantlets with a well developed shoot and root system obtained on optimized medium were acclimatized with a maximum survival rate of 90% on soil and organic manure in a 1: 1 ratio.

Javier Sánchez (Ecuador), Marcos Daquinta, Iris Capote (Cuba), Jaime A. Teixeira da Silva (Japan), Bryce Chadwick (Ecuador) Frequency of Immersion and Paclobutrazol Application Affect the Propagation of *Zantedeschia* sp. Var. 'Treasure' Shoots in a Temporary Immersion System (pp 46-48)

ABSTRACT

Research Note: Calla lily (*Zantedeschia* sp. Var. 'Treasure'; Araceae family) is an ornamental plant with high commercial demand as pot plants and for cut flowers. Traditional propagation techniques are not able to satisfy the fast introduction of new hybrids to the market. Thus, *in vitro* culture techniques are useful tools for the propagation of new varieties with a wide range of colours. Aiming to establish a more efficient protocol for the *in vitro* propagation of callas in a Temporary Immersion System (TIS), the effect of immersion frequency and paclobutrazol (PBZ) on shoot proliferation and quality were evaluated. This protocol showed greatest biological efficiency when shoots were immersed every 4 hours (or 6 immersions per day) and when 0.3 $\text{mg}\cdot\text{L}^{-1}$ PBZ was included in the culture medium.

Chhaya Sharma, Sunita Chandel, Rajinder Kaur (India) *In Vitro* Callus Multiplication and Shoot Regeneration of Resistant Calli of Carnation cv. 'Raggio-de-Sole' against *Rhizoctonia solani* Kuhn (pp 49-52)

ABSTRACT

Original Research Paper: In a procedure of *in-vitro* callus multiplication and shoot regeneration of resistant callus to *Rhizoctonia solani* Kuhn., it was observed that callus cultures were established only from leaf explants of carnation cv. 'Raggio-de-Sole' treated with 0.2% bavistin for 6 min and 0.1% HgCl_2 for 2 min and not from petals or internodes segments. Pale, friable growth of callus was obtained after 40-45 days on MS basal medium containing 2.0 mg/l NAA and 0.5 mg/l 2,4-D and screened *in-vitro* for resistance to Millipore-filtered purified culture produced by the fungus *R. solani* for creating resistance to rot disease in carnation. Calli were challenged by different concentrations of culture filtrate of *R. solani*. A cell survival rate of 20.66% at a 20% selective dose of culture filtrate was achieved in unselected calli while at >25% concentration of culture filtrate, 100% calli died after 4 weeks. However, calli survived within a range of 40-93.33% subjected to 15-2% fungal culture filtrate indicating low survival rate at higher concentration and high survival rate at lower concentration. The selected calli multiplied better after screening on the callus multiplication medium. Regeneration of shoots from the resistant calli were obtained successfully after 4 weeks on MS medium containing 0.5 mg/l NAA and 0.5 mg/l IAA.

Jaime A. Teixeira da Silva, Michio Tanaka (Japan) Culture Vessel Affects Hybrid *Cymbidium* Protocorm-like Body and Callus Formation (pp 53-55)

ABSTRACT

Short Communication: The number of protocorm-like bodies (PLBs) and embryogenic callus formed in hybrid *Cymbidium* Twilight Moon 'Day Light' is affected by the culture vessel (CV) used. Borosilicate test tubes (CV1), plastic and glass Petri

dishes (CV2 and CV3), Whatman filter paper No. 1 membrane rafts (CV4), Milliseal[®]-covered jam jars (CV5), the Vitron[™] (CV6) and 100-ml glass Erlenmeyer flasks (CV7, control) were tested. CV7, which is the vessel conventionally used for the sub-culture and micropropagation of *Cymbidium* PLBs, resulted in 15.6 ± 1.15 PLBs per CV. CV2 and CV3 were as effective as CV7 (14.9 ± 0.95 and 15.8 ± 1.07 , respectively) in PLB proliferation. Even though PLBs that formed in CV4 had higher fresh and dry weights, much fewer PLBs per CV were formed. In general, aerated CVs (CV5 and CV6) resulted in greater responsiveness of PLBs to callus formation, but differences were not significant. Although some laboratories have their established protocols for PLB proliferation, tests on the use of different CVs should be conducted prior to mass propagation since the choice of CV can affect material and running costs, the ease of multiplication and the quantitative output.

Jaime A. Teixeira da Silva, Michio Tanaka (Japan) Impact of Gelling Agent and Alternative Medium Additives on Hybrid *Cymbidium* Protocorm-like Body and Callus Formation (pp 56-58)

ABSTRACT

Short Communication: The gelling agent and a selection of alternative medium additives impacted the number of protocorm-like bodies (PLBs) and percentage callus formed in hybrid *Cymbidium* Twilight Moon 'Day Light'. Gellan gum resulted in greater PLB production and callus formation than all other gelling agents tested which included agar, Bacto agar, phytigel, oatmeal agar, potato dextrose agar, guar gum, isubgol and corn starch. All of the alternative medium additives (full fat milk, Coca-cola[®], coffee, green and Darjeeling teas) negatively impacted PLB production and almost completely suppressed callus formation, although tissue browning appeared to have been reduced by the presence of teas and coffee.

Mahroo Sadat Motaghayer, Mahmood Esna-Ashari (Iran) Effect of Different Concentrations of Four Preservative Solutions on Tuberose (*Polianthes tuberosa* L.) Cut Flower Vase-Life (pp 59-61)

ABSTRACT

Short Communication: The effect of different concentrations of 4 preservatives, including 8-Hydroxyquinoline sulfate (8-HQS), citric acid, silver nitrate and sucrose in 3 kinds of water (Hamedan city tap water, Hamedan cooled boiled water and double distilled water) on vase-life of tuberose (*Polianthes tuberosa* L.) 'Gol Dorosht' cultivar cut flower was studied. The best preservative solution for tuberose cut flower was 2% sucrose in double distilled water, which performed significantly better than other treatments.

Ernesto Lahoz, Rosa Caiazzo, Angela Carella, Felice Porrone, Renato Contillo (Italy) *Colletotrichum acutatum* Simmonds as Agent of Anthracnose and Stem Blight on *Nerium oleander* in Italy (pp 62-66)

ABSTRACT

Original Research Paper: Oleander is an evergreen shrub or small tree in the genus *Nerium* and is extensively grown as an ornamental plant in parks, and along roadsides. Many diseases are reported to cause damage and death of plants, thus transforming the landscape. In the present work plants with anthracnose-like symptoms were observed and Koch's postulates were applied to determine the aetiological agent of the disease. Morphological and cultural observations indicated that *Colletotrichum acutatum* J.H. Simmonds should be the agent, but these observations could not confirm the assignment of our isolates to this species. Sequencing of ITS-rDNA fragments showed significant homology (99%) with many isolates of *Colletotrichum acutatum* present in the NCBI gene bank, solving the classification problem and indicating that molecular tools are necessary for correct classification of *C. acutatum*. In addition, the results of cluster analysis demonstrated that, according to many authors, some sub-groups may exist in *C. acutatum* species, but the common origin and/or characteristics of isolates belonging to the same sub-group are not well described yet. Data of pathogenicity tests demonstrated that oleander isolates failed to infect pepper and this finding could be useful for studying resistance mechanisms in pepper and/or host specificity.

Harender Raj, Ashok Kumar (India) Corm Treatment and Soil Solarization for the Management of Wilt (*Fusarium oxysporum*) in Gladiolus (*Gladiolus grandiflorus*) (pp 67-70)

ABSTRACT

Original Research Paper: Aqueous extracts of leaves, seed and cloves of seven different plants [eucalyptus (*Eucalyptus* hybrid), aonla (*Phyllanthus emblica*), ginger (*Zingiber officinale*), aloe (*Aloe barbadensis*), neem (*Azadirachta indica*), darek (*Melia azedarach*) and garlic (*Allium sativum*)], three commercial neem (*Azadirachta indica*) formulations, five *Trichoderma*

species and two bacterial antagonists (*Bacillus subtilis*, *Pseudomonas fluorescens*) were evaluated against the wilt pathogen (*Fusarium oxysporum*) of gladiolus (*Gladiolus grandiflorus*), var. 'Peter Pears'. Among these, neem formulation Neemazal, oil of *E. hybrid*, *Trichoderma viride*, *B. subtilis* and *P. fluorescens* were found to be effective with 100, 68.9, 62.0, 59.2 and 57.0% inhibition, respectively of the mycelial growth of the wilt pathogen. These effective treatments were used as corm treatment and integrated with soil solarization for the management of gladiolus wilt. Soil solarization with transparent polyethylene mulch (25 µm thick) for 40 days resulted in an increase of 8.3°C at 5 cm soil depth and the average maximum soil temperature during the period was 40.3°C. Corm treatment with *T. viride* formulation followed by their plantation in solarized plots resulted in a 72.2% reduction in wilt incidence in comparison to untreated corms sown in unsolarized soil. This treatment also resulted in improved growth as well as quality parameters with an increase of 32.3, 40.6, 84.4, 96.3, 38.4 and 42.2% in plant height, spike length, number of florets per spike, number of cormels per corm, corm size and corm weight, respectively and flowering was also recorded in 17.4% fewer days.

Chhaya Sharma, Sunita Chandel (India) Biotechnological Approaches for Treating Viral Diseases in Orchids (pp 71-74)

ABSTRACT

Research Note: Viral diseases of orchids cause major losses in productivity and quality. Host plant resistance offers an effective means of controlling plant diseases caused by viruses. It minimizes the necessity for the application of pesticides. However, in most orchids no natural disease resistance is available. Genetic engineering allows the introduction of specific, in some instances broad spectrum, disease resistance derived from other species or even from the pathogen itself into plant genotypes that have been selected for desirable horticultural properties. This manuscript broadly provides an overview of these themes.