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Improved propagation of ornamental shrubs

Key external stakeholders:

Nursery stock growers, consultants, specialist plant propagators

Practical implications for stakeholders:

- A loss in vigour and dieback of shoots may indicate the presence of systemic bacteria in stock plants used for vegetative propagation of ornamental shrubs.
- Meristem culture can regenerate bacteria free plants and can re-invigorate/rejuvenate nursery stock plants for further propagation on large commercial scales.
- Methods for micropropagating 10 cultivars of *Buddleia* by Mersitem culture and *Salvia greggii* 'Variegata', *Tulbaghia violaceae* 'Silver Lace' and *Erysimum linifolium* 'Variegatum' have been described for the first time.

Main results:

Commercial cultivars of *Buddleia* were shown to be systemically infected with several bacteria which could be cultured on bacteriological medium but the bacteria did not show on medium used for regular micropropagation. Meristem culture was successfully used to produce bacteria free plants which grew more vigorously and could be used as clean stock for commercial propagation by nursery propagators.
Micropropagating conditions were determined for shoot production, rooting and weaning of *Tulbaghia, Erysimum and Salvia* and conditions to minimise the loss of leaf variegation were described.
The concentration range of paclobutrazol was determined for reducing the height of plants in vitro and in pots for *Salvia* and *Erysimum*.

Opportunity/Benefit:

- Micropropagation systems for *Buddleia, Tulbaghia, Erysimum and Salvia*, are described.
- Meristem culture was successful in producing bacteria-free shoot cultures of *Buddleia* and these stock plants were supplied to commercial nurseries.
- Nursery stock producers may use the propagation information to commission specialist producers to build up stocks of these plants and the methods described should be applicable to related and/or new cultivars of these species.
- Paclobutrazol was effective in producing more compact marketable plants of *Salvia.*
- The capacity to regenerate plants from cells of *Buddleia* offers the potential for genetic modification to generate new varieties.

Collaborating Institutions:

UCD
Fitzgerald nurseries Ltd

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1. Project background:

Vegetative propagation is the preferred means of propagating many ornamental shrubs and herbaceous plants. However, stock plants as sources of cuttings, can lose their vigour and become infected systemically, by bacteria; similarly for stocks of plants which are much handled and intensively propagated by recycling of mini cuttings. Micropropagation in sterile conditions offers the possibility to reduce or eliminate the load of microorganisms which may cause disease such as dieback of shoot tips. Micropropagation can be an effective means of bulking up scarce material and rejuvenating plant material so that cuttings derived from micropropagated plants can root more easily and grow more vigorously.

Nursery growers reported that plants of *Buddleia davidii* produced by conventional cuttings suffer from shoot-tip dieback, a disorder that reduces plant quality and may be the result of a build up of systemic bacteria. Our objective was to develop methods for micropropagation by Meristem culture and determination of the bacterial status of regenerated plants for nine cultivars of *B. davidii*: 'Black Knight' (deep violet), 'Royal Red' (red/purple), 'Nanhoensis' (pure blue), 'Border Beauty' (lilac purple), 'Ile de France' (dark violet), 'Empire Blue' (lavender blue), 'Pink Delight' (pink), 'White Ball' (white), 'White Profusion' (pure white with yellow eye) and one hybrid *B. davidii* x *B. fallowiana*, i.e. B. 'Lochinch' (lavender). The potential for genetically modifying ornamentals such as *Buddleia* depends on a capacity to regenerate whole plants from somatic cells, therefore we investigated this potential by determining the optimal tissues and the growth regulators that can induce cells to regenerate into whole plants.

We also investigated the use of micropropagation to rapidly build up stocks of novel cultivars which are in short supply with growers or which have difficulties in large scale propagation. They included variegated material such as *Salvia greggii* 'Variegata', *Tulbaghia violaceae* 'Silver Lace' and *Erysimum linifolium* 'Variegatum'. We aimed to test the effectiveness of several nutrient media formulations and growth regulators to support the growth and development of shoots and adventitious roots as well as survival of the plantlets after weaning in the greenhouse. The effectiveness of the chemical growth retardant paclobutrazol on producing more compact plants of *Salvia* and *Erysimum* was also studied in the micropropagation stage and on weaned plants in the glasshouse.

2. Questions addressed by the project:

- Do cultivars of *Buddleia* harbour endogenous bacteria?
- Can *Buddleias* be regenerated from Meristems and adventitious buds and later micropropagated to provide healthy stock plants?
- Can micropropagation protocols be developed for the ornamentals specified?
- Can leaf variegations be retained in micropropagated *Tulbaghia, Erysimum*, and *Salvia*?

3. The experimental studies:

Shoot cultures of 10 *Buddleia* cultivars were established in vitro on MS medium containing 0.22 µM BAP and for rooting we used half strength medium with 19.7 µM IBA for four days. Shoot apical Meristems used were approximately 0.1-0.2 mm in diameter consisting of the apical dome. Meristems were excised from shoot-tips of established shoot cultures and were cultured on MS medium containing 20% (w/v) sucrose, 6% (w/v) agar, 4.6 mM kinetin, and 2.9 mMGA3.

Tulbaghia was cultured on MS medium with either 3 mg·L-1 BAP,(M3) or 5 mg·L-1 Kinetin (M4), all media contained 0.1 mg·L-1 NAA. Shoot cultures of *Erysimum* were established using MS and WPM media with various growth regulators. *Salvia* was cultured for micropropagation on MS medium with 30 g·L-1 glucose, 3.3 g·L-1 phytagel, 0.1 mg·L-1 BAP, and 0.01 mg·L-1 IBA; half strength MS basal medium was used for rooting. Full details on culture media and conditions are in the papers cited below.

Micropropagated *Erysimum* and *Salvia* plants were potted into compost in two-L pots prior to drenches with paclobutrazol (Bonzi). Plants were randomly assigned to three blocks with each block containing 6 treatment groups (including control plants) with seven replications per treatment. Paclobutrazol drenches were applied at various concentrations of active ingredient (a.i.) per 2 L compost and growth was measured after six weeks.

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4. Main results:

Shoot cultures of *Buddleia* were established and two-node explants produced higher micropropagation rates compared to those of single nodes or shoot-tips. Rooting and weaning was 100%. Shoot cultures appeared sterile on micropropagation media, however, bacteria were detected by culturing internodal explants on Tryptic Soy Broth (TSB) and were found also in cuttings from stock plants, shoots from plants cultured *in vitro* and in plants weaned to the greenhouse from shoot cultures. At least two different bacterial isolates were detected for each cultivar.

Viable shoot cultures and weaned plants were obtained from cultured apical Meristems of 10 Buddleia cultivars giving viabilities of 32-72%. The number of shoots produced, the micropropagation rate and the root number produced in vitro was higher in Meristem derived shoots compared to those derived from shoot-tips. The subsequent growth rate of Meristem derived plants, in the greenhouse, was also greater. The number of roots produced by conventional cuttings collected from Meristem derived plants was significantly higher than in cuttings which were collected from plants derived from shoot-tips or from the original stock plants.

Endogenous bacteria were not detected in either shoot cultures derived from Meristems or in 10-week-old weaned plants derived from Meristems whereas those derived from shoot-tips showed the presence of endogenous bacteria when sterilised explants were cultured on nutrient agar or on TSB. Whole plants regenerated from Meristems proceeded to the flowering stage and the resulting flowers were true to type for each variety; see figure on page 1 for cultivars, left to right, 'Ile de France', 'Empire Blue', White Ball, and 'Lochinch'.

Factors affecting adventitious bud and shoot production in leaf and internode explants were determined for 'Lochinch', 'Border Beauty', 'Ile de France' and 'Pink Delight' using explants collected on Meristem derived shoot cultures. Adventitious shoots appeared on leaf and internode explants of every cultivar when cultured on MS supplemented with 0.5-5.0 mM TDZ. The highest percentage regeneration was achieved from bisected internode explants cultured on 0.5 mM TDZ, with 93-100% regeneration among the cultivars whereas BA was less effective. The best response was obtained using 5.0 mM TDZ which gave over 10-11 shoots per explant in all bisected explants for all cultivars.

Shoot cultures of *Tulbaghia* were established using shoot offsets and flower-derived explants; the latter were the most efficient and produced the greatest number of variegated shoots. Micropropagation rates increased significantly after subculture, however, only 32–35% of explants retained the variegated phenotype and there was no significant difference between the media tested. The effects of wounding were investigated on shoot production and on plant variegation. Explants were wounded by two methods, a) a transverse cut at the base of the shoot and b) a longitudinal cut at the base of the shoot at the start of the culture period. Longitudinal cuts gave best results in liquid culture as well as in agar cultures. The overall micropropagation rate was 2.4 for the shoots treated with a transverse basal cut and 5.2 for shoots cut longitudinally; production of variegated shoots increased significantly from 35% to 71% in explants wounded by a longitudinal cut.

With *Erysimum*, shoot tip and nodal explants were readily established as micropropagating cultures, however, only 53.3% retained the variegated phenotype for both explant types. MS medium with + 0.88 µM BAP produced the highest micropropagation rates (4.18) and shoot tips were superior to double node explants for the propagation rate and also for retaining the variegated phenotype in 79% of the explants. Rooting, survival of plantlets after weaning and retention of the variegated phenotype was 100% using activated charcoal (3.0g/L). Paclobutrazol drenches on greenhouse grown plants of Erysimum significantly reduced both plant height and internode length even at the lowest level of 2.0 mg a.i. per 2 L of compost, however, it resulted in undesirable leaf distortions and could not be recommended as a commercial application.

Shoot cultures of *Salvia* established easily and produced an average of six shoots per jar per sub-culture period. Rooting was 100% with 1.0 mg·L-1 IBA and survival at weaning was 93-100%; the maximum loss of variegation was 19%. Paclobutrazol applied in vitro (2.0mg/L) significantly reduced both the length of the internodes and the number of nodes produced but the effect disappeared in weaned plants. When applied to weaned plants in the greenhouse, paclobutrazol significantly reduced plant height even at the lowest dose (0.5 mg a.i.) and at 8.0 mg a.i. plants were 70% shorter than controls. A side effect was to induce bud break, with shoot numbers increasing at doses above 0.5 mg a.i. resulting in compact plants. There was no loss in variegation among plants treated with the two highest levels of paclobutrazol.

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5. Opportunity/Benefit:

- Nursery stock propagators who received bacteria free *Buddleias* have healthy stock plants for further commercial propagation.
- Micropropagating companies and conventional propagators will use the results obtained to propagate and grow the cultivars studied and the results will be applicable to a range of related species.
- Specialist propagators may be commissioned by growers to undertake Meristem culture and the detection of bacteria, and the protocols described will shortcut much developmental work and should reduce the costs of the service to the growers.

6. Dissemination:

Main publications:

S. Phelan, A. Hunter and G.C. Douglas (2009)- -
Micropropagation and growth traits in ten *Buddleia* cultivars derived from Meristems and adventitious shoot regeneration Scientia Horticulturae 120, (4), 518-524.
S. Phelan, A. Hunter and G.C. Douglas (2008)- -
Micropropagation and growth regulation of *Erysimum linifolium* 'Variegatum' Propagation of Ornamental Plants 8(2): 87-92, 2008
S. Phelan, A. Hunter and G.C. Douglas. (2007)- -
Micropropagation and growth regulation of *Tulbaghia violacea* 'Silver Lace'. Proc. XXVII IHC-S10 Plant Biotechnology Ed.-in Chief: P. E. Reid. Acta Horticulturae (ISHS) 764 : 113-118.
Phelan S. Hunter A. and Douglas G. C. (2005)- -
Bacteria detection and micropropagation of ten *Buddleia* cultivars. Propagation of Ornamental Plants 5: 164-169.
Phelan S, Hunter A & Douglas GC (2005) - -
Effects of Paclobutrazol on *Salvia greggii* 'Variegata' *in vitro*. In: G. Libiaková & A. Gajdosova (eds) Final Meeting of COST Action 843: Quality enhancement of plant production through tissue culture. Stará Lesná (Slovak Republic), June 28-July 3, 2005. ISBN 80-89088-41-4, pp. 23-24.

Popular publications:

Producing bacteria-free Buddlejas T-Research 1: 1 30-31

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