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Propagation and mutagenesis of several cultivars of the ornamental shrub *Hebe*

Key external stakeholders:
Commercial growers of nursery stock shrubs, micropropagation companies

Practical implications for stakeholders:

- Efficient vegetative propagation and the generation of novel plants is a highly desirable requirement for producers of ornamental shrubs and the foliage sector.
- For the ornamental shrub Hebe, we developed successful protocols for its micropropagation and also for the generation of novel morphological forms.
- Some of the novel variants generated may have commercial potential for the foliage and nursery stock industries after further testing.

Main results:

Methods were developed to micropropagate five cultivars of the ornamental flowering shrub Hebe under sterile conditions, with stability in the propagation rates over several years. Conditions to induce polyploids by chemical treatments were determined and resulted in some plants with thicker leaves, stems and fewer flowers. Treatment levels of gamma irradiation on buds in vitro were optimised and resulted in plants with new morphological traits such as leaf variegations. Photo above shows a variegated shoot produced on a green plant after a radiation treatment. Some mutants generated may have potential for commercial development as novel variants after further evaluation.

Opportunity / Benefit:

Methods developed to micropropagate Hebe under sterile conditions provide the opportunity to produce disease free plants for a range of commercial cultivars. Mutagenic treatments such as polyploidy and irradiation were successful for inducing various morphological forms, and some of these traits such as leaf variegations and altered flowering patterns may have the potential as novel cultivars after further testing in commercial nurseries.

Collaborating Institutions: UCD

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1. Project background:

The genus *Hebe* contains important evergreen flowering shrubs used for horticultural purposes. There are many 88 species and many cultivars, several of which have horticultural value as: pot plants, garden plants, foliage plants and as shrubs for landscaping. Hebe is usually propagated by cuttings, but more uniform disease free plants could be provided on a large scale by micropropagation, if efficient systems could be developed. Similarly, micropropagation systems can be adapted for inducing mutations as a useful tool to obtain novel varieties of Hebe with potential for commercial development.

The overall objective of the project was to develop methods for micropropagating five commercial cultivars of Hebe 'Pink Fantasy', 'Cobb', 'Wiri Mist', Nicola's Blush' and 'Oratia Beauty'. We also aimed to determine the effectiveness of various mutagenic treatments to generate novel variants; these included the application of chemical treatments for the induction of higher levels of ploidy and irradiation of cultured buds.

2. Questions addressed by the project:

- Can various commercial cultivars of Hebe be micropropagated efficiently over the long term?
- What levels of growth regulators sustain shoot production and rooting?
- Can mitosis halting chemicals such as colchicine and oryzalin be used effectively to generate plants with higher levels of chromosome ploidy?
- What is the radiosensitivity level for Hebe to generate variants with altered shoot morphology and recovery of viable plants after treatments?
- What is the range of morphological variants that can be produced from mutagenic treatments and is there any potential for generating novel cultivars of Hebe?

3. The experimental studies:

Shoot cultures of Hebe cultivars, 'Cobb', 'Wiri Mist', Nicola's Blush', 'Pink Fantasy'and 'Oratia Beauty' were established *in vitro* under sterile micropropagation conditions. Shoot tips and nodes of all cultivars were used as explants. The micropropagation rates were determined for various combinations of growth regulators. Buds were cultured on modified Woody Plant Medium containing BA, 0.2 mg l^{-1} and α-NAA, 0.001 mg l^{-1} for 'Wiri Mist' and 'Pink Fantasy'; BA, 0.2 mg l^{-1} and IBA, 0.01 mg l^{-1} for 'Cobb' and BA, 0.5 mg l^{-1}, IBA, 0.01 mg l^{-1} and GA_3, 2.0 mg l^{-1} for both 'Oratia Beauty' and 'Nicola's Blush'. TSB medium was used for detection of bacteria in the bud and stem explants. Nodal segments of Hebe 'Oratia Beauty' were treated *in vitro* with colchicine (500 and 1000 µM) and oryzalin (11.5, 58 and 289 µM) to induce polyploid plants. All treated explants were rooted, acclimatized and plants with different ploidy levels were compared in morphology and ploidy level was confirmed by flow cytometry.

The radiosensitivity of Hebe 'Oratia Beauty' and 'Wiri Mist' was determined using single nodal explants from micropropagated shoots in vitro. A source of gamma rays ($^{60}C_0$) was used to deliver doses of 20, 30, 40, 50 Gy for 'Oratia Beauty' and 15, 30, 45, 60 Gy for 'Wiri Mist'. Weight gain and survival after treatments and morphological characteristics were recorded.

4. Main results:

Shoot cultures free of culturable bacteria were established for all cultivars. Micropropagation rates were recorded over two culture periods, in the first year and subsequently after a prolonged phase of subculturing, i.e. in the second year. There was no significant difference in the micropropagation

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rates obtained for first year compared to the second year for each cultivar. The mean micropropagation values were 2.7, 3.2, 6.5 5.5 and 7.9 in year 1 for 'Cobb', 'Wiri Mist', Nicola's Blush', 'Pink Fantasy' and 'Oratia Beauty' respectively and the values for year 2 were 3.9, 3.9, 6.0, 5.5 and 7.9 respectively. This indicates the stability of cultures and the suitability of the media used for all cultivars. When activated charcoal was added to the media, 100 % of the explants rooted for all cultivars and when rooted shoots were acclimatized to the greenhouse, more than 96 % of the plants survived.

Polyploids were induced by treating buds with colchicine and oryzalin and overall survival rates from treatments were greater than 73%. Some shoots exhibited an altered phyllotaxy of leaves in vitro as the first manifestation of higher ploidy. All treated explants were rooted, acclimatized and plants with different ploidy levels were detected. Flow cytometry was used to identify plants as diploids, mixoploids and polyploids. The highest frequency of tetraploids was 45.7% using oryzalin (289 µM) and 28.6% using colchicine (1000 µM). Leaves of diploids and tetraploids were not significantly different for length and width, while some tetraploids showed altered phyllotaxis. Stomatal density was significantly lower and stomatal length was significantly higher in tetraploid plants when compared to diploids. Principal component analysis (PCA) was performed and revealed that stomatal traits accounted for a large proportion of variability. There was no difference in the rooting percentage in cuttings from tetraploid (4x) and control (2x) plants. Tetraploid and diploid plants were established in the field and one tetraploid plant produced very few flowers compared to diploid controls. Production of flowers is undesirable in plants used for foliage production so this variant may have potential value as a foliage cultivar.

After irradiation, the fresh weight gain/loss of the single nodal explants was measured over two consecutive culture periods and the radiation dose resulting in a 50% loss in fresh weight was computed as the LD 50 value. With 'Oratia Beauty', the LD 50 was determined as 37 Gy from the fist culture period and 33 Gy from the second. The cultivar 'Wiri Mist' was the least sensitive to radiation: its LD 50 was determined at a dose of 48 Gy from the fist culture period and 56 Gy in the second. We recovered plants with altered leaf distributions on stems and also with altered morphology of individual leaves and shoots suich as leaf variegations (see photo above). The recovery of putative mutants such as plants with altered leaf morphologies was higher in the more radiosensitive cultivar, 'Oratia Beauty' (7.1%), than in 'Wiri Mist' (2.2%).

5. Opportunity/Benefit:

This is the first study to show that several cultivars of Hebe can be successfully micropropagated and that buds of the micropropagated shoots can be mutated by chemical treatments to produce plants with higher ploidy levels. Furthermore, these buds can also be mutated by irradiation to give novel morphological forms including leaf variegations and all of these variants may have potential for commercial development.

6. Dissemination:

Presentations at Irish Plant Scientists Association Meeting and Agricultural Research Forum

Main publications:

Gallone Angelo, Alan Hunter and Gerry C. Douglas (2014) Efficient Polyploid Induction *in vitro* using Colchicine and Oryzalin on *Hebe* 'Oratia Beauty' Scientia Horticulturae 179:59-66.

Gallone A., A. Hunter and G.C. Douglas (2012) Radiosensitivity of *Hebe* 'Oratia Beauty' and 'Wiri Mist' irradiated with γ-rays from ^{60}Co . Scientia Horticulturae, 138, 36-42. http://www.sciencedirect.com/science/article/pii/S0304423812000635

Gallone, A. A. Hunter, G. C. Douglas (2009) Micropropagation of *Hebe* 'Pink Fantasy' Agricultural Research Forum 2009, p132, *ISBN 1-84170-538-1* http://www.agresearchforum.com/publicationsarf/2009/proceedings2009.pdf

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Gallone A., (2012) Micropropagation and induction of variation in *Hebe* cultivars by polyploidy induction and γ-irradiation, Ph. D thesis, UCD

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