Applications of Biotechnology in the Nursery Stock Industry©

G. C. Douglas
Teagasc, Agriculture and Food Development Authority, Kinsealy Research Centre, Malahide Rd. Dublin 17, Ireland

INTRODUCTION

This paper summarizes developments in plant biotechnology as applied to the nursery stock sector and reviews progress in developing methods to facilitate the production of new cultivars of nursery stock by embryo rescue, somatic embryogenesis, polyploidy, and genetic modification.

EMBRYO CULTURE AND SOMATIC EMBRYOGENESIS

The germination of seeds, which have complex requirements for breaking dormancy can be accelerated by excising the embryos from seeds and culturing them in vitro. Where seeds are scarce it may also be useful to germinate embryos in vitro to obtain seedlings more quickly (Meynet et al., 1994).

Embryos, which are not fully mature in the seeds, have the capacity to produce multiple secondary embryos in vitro. These can be matured in vitro and germinated as normal seedlings. The process of somatic embryogenesis is possible for a range of ornamentals and is a feasible way to vegetatively propagate plants on a large scale including: *Camellia* sp. (San Jose and Vieitez, 1993); *Cercis canadensis* (Geneve and Kester, 1990); *Cornus floridus* (Trigiano et al., 1998), *Ilex aquifolium* (Hu et al., 1978); *Magnolia* sp. (Merkle and Watson-Pauley 1994; Merkle, 1997); *Paulownia tomentosa* (Radojevic, 1979); *Aesculus hippocastanum* (Kiss et al.,1992); *Paeonia albiflora* (Kim et al.,1996a); *Feijoa sellowiana* (Vesco and Guerra 2001), and *Liriodendron* sp. (Merkle, 1997). In general, somatic embryogenesis is only possible by using immature or newly mature embryos as the starting tissue. Therefore, it is necessary to know the phenotype of the seed material before starting.

Somatic embryogenesis is very useful for producing plants, from scarce seed sources such as the yellow flowered *Magnolia cordata* (Merkle and Watson Pauley, 1994), and for bulking up large numbers of plants of conifer species derived from the seed material of high-yielding selected crosses.

Wide crosses often fail because the embryos abort before they are mature enough to germinate normally. Embryo rescue can make it possible to obtain hybrid plants by culturing the hybrid embryos to maturity in vitro. In such cases, the hybrid embryos can be proliferated via somatic embryogenesis, leading to a build up of stocks of scarce materials such as hybrids of *Liriodendron tulipifera* × *L. chinense* (Merkle, 1997). The method has also been developed for holly (Mattis et al., 1995) and rose (Marchant et al., 1993). Hybrids of scented *Cyclamen purpurascens* with *C. persicum* were produced by hybrid embryo rescue and progeny were scented (Ishizaka and Uematsu, 1995). Similarly, hybrids of scented *Viburnum carlesii* × *V. lantana* have been possible through embryo rescue (Hoch et al., 1995). New colours and plant forms are possible from crosses of the orange coloured *Ornithogalum dubium* with the white flowered *O. thyrsoides* when embryo rescue produced viable hybrids (Griesbach et al., 1993). New types of alstromeria have been produced by embryo rescue of *A. ligtu* hybrids × *A. pelegrina* (Ishikawa et al., 1997)

and also in *Zantedeschia* (Yao and Cohen, 1996) and *Limonium* (Morgan et al., 1998). Interspecific hybridisation was facilitated in *Helianthus* sp. and may lead to new garden cultivars (Espinasse et al., 1991).

POLYPLOIDY

Plants with extra sets of chromosomes can be produced by culturing buds in vitro with colchicine. Polyploid plants have larger cells, resulting in plants with larger leaves and flowers. Polyploidisation has been achieved effectively in vitro for rose (Ma et al., 2001), lilac (Rose et al., 2001) buddleia (Rose et al., 2000), salvia (Gao et al., 1996), and agapanthus (Nakano et al., 2003). Polyploid plants may have desirable characters for commercialisation. Alternatively they can be employed as parents in crosses with species, which are polyploid in nature. In the case of lilac, hybrids of S. *vulgaris* × *S. pinnatifolia* were sterile, but fertile hybrids may be possible following polyploidisation of the hybrid, leading to lilac selections with large flowers and pinnate leaves (Rose et al., 2001). In some polyploid crosses of *Hibiscus syriacus,* such as diploid crossed with tetraploid, embryo rescue has been used to obtain viable progeny (Kim et al., 1996b).

GENETIC MODIFICATION

In 2003, 68 million ha of genetically modified (GM) crops were grown globally, mainly maize, soybean, and cotton (FAO, 2004). The modified genes conferred resistance to insects and herbicides and benefited the crop grower. The next generation of GM plants will be targeted at adding value for the consumer so that public acceptance and profitability will be increased. For the nursery and flower industries, the genes of interest will be those conferring novel characters such as new flower colours, fragrance, early or retarded flowering, extended shelf-life, and disease resistance.

Classical plant breeding has well-known limitations. Some of these can be overcome using genetic modification technology, for example to introduce individual genes or gene groups into plants. This technology is now well advanced for trees (Halpin and Boergan, 2003). Genetic modification has been demonstrated in principle for a wide range of ornamentals, including azaleas (Mertens et al., 2000); roses (Marchant et al., 1998); agapanthus, muscari, lily (Suzuki and Nakano, 2002), lavender (Nebaur et al., 2000); and gentians (Hosokawa et al., 2000). Useful genes, which have been successfully transferred include crown gall resistance to apple (Viss et al., 2003) and Basta resistance to pear rootstocks (Lebedev et al., 2002). Genetic modifications for virus resistance has been proven for almond (Raquel et al., 2002) as well as for resistance to plum pox and sharka in plums (Ravelonandro et al., 2000; Scorza et al., 2001) respectively.

Increased protection against pathogens such as *Xanthomonas* and mildew has been demonstrated in transgenic pelargonium (Renou et al., 2000) and roses (Li et al., 2003). Recent work on genetic modification of *Forsythia* has resulted in a novel bronze-orange petal colour by transferring two genes affecting pigment synthesis, one for anthocyanidin synthase from *Mathiola incana* and the other, the gene for dihydro-4- flavonol reductase from *Antirrhinum major*; only the expression of both genes resulted in a change in flower colour (Rosati et al., 2003). Flower longevity has been extended in *Torenia* by modification with genes which block ethylene production (Aida, 1998). Blocking the expression of pigment synthesis genes has resulted in *Eustoma* flowers with altered pigmentation (see, Davies and Schwinn,

1997), and roses with a reduced intensity in flower colour (Courtney-Gutterson, 1994). Genes coding for fragrance compounds have been transferred from *Clarkia* to carnation but did not lead to detectable scent improvement (Lavy et al., 2002). Carnations have been transformed with pigment genes from petunia resulting in blue-pigmented flowers. In addition, the same company, (Suntory Ltd.), announced the world's first "blue" rose by genetic modification with a gene from viola.

Future developments and applications in plant biotechnology will be in the use of molecular markers. These markers are expected to serve as indicators of physiological responses in breeding lines and will help the breeder to select combinations of desirable traits. For ornamental nursery stock, markers for freezing tolerance have been reported in subjects such as rhododendron (Lim et al., 1999).

Acknowledgements: The technical assistance of Mr. Sean Egan is gratefully appreciated and the greenhouse work of Ms. Donna Gregan.

LITERATURE CITED

Aida, R. 1998. Gene silencing in transgenic torenia and its applications for breeding. J. Jap. Soc. Hort. Sci. 67:1200-1202.

Courtney-Gutterson, N. 1994. The biologist's palette: Genetic engineering of anthocyanin biosynthesis and flower colour. p. 93-124. In: B.E. Ellis, G.W. Kuroki, and Stafford, H. (eds.), Genetic engineering of plant secondary metabolism. Recent Advances in Phytochemistry Vol. 28. Plenum Press, New York.

Davies, K.M. and **K.E. Schwinn.** 1997. Flower colour, p. 259-294**.** In: R.L. Geneve, J.E. Preece, and S.A. Merkle (eds.). Biotechnology of ornamentals plants, biotechnology in agriculture series, No. 16, CAB International.

Espinasse, A., J. Volin, C.D. Dybing, and **C. Lay.** 1991. Embryo rescue through in ovulo culture in Helianthus. Crop Sci. 31:102-108.

Food and Agriculture Organization. 2004. Agriculture Series 35: The state of food and agriculture 2003-2004, Agriculture Biotechnology meeting the needs of the poor. Food and Agriculture Organization , Viale delle Terme di Caracalla, 00100 Rome, Italy.

Gao, S.L., D.N.; Zhu, Z.H.; Cai, and **D.R. Xu.** 1996. Autotetraploid plants from colchicine-treated bud culture of *Salvia miltiorrhiza* Bge. Plant Cell Tissue and Organ Cult. 47: 73-77.

Geneve, R.L. and **S.T. Kester.** 1990. The initiation of somatic embryos and adventitious roots from developing zygotic embryo explants of *Cercis canadensis* L. cultured in vitro. Plant Cell Tissue and Organ Cult. 22:71-76.

Griesbach, R.J., F. Meyer, and **H. Koopowitz.** 1993. Creation of new flower colors in Ornithogalum via interspecific hybridization. Journal of the American Society for Hort Sci. 118:409-414.

Halpin, C and **W. Boerjan.** 2003. Stacking transgenes in forest trees TRENDS in Plant Sci. 8:363-365.

Hoch, W.A., E.L. Zeldin, J. Nienhuis, and **B. H. McCown.** 1995. Generation and identification of new viburnum hybrids. J. Environ. Hort. 13:193-195.

Hu, C.Y., J.D. Ochs and **F.M. Mancini.** 1978. Further observations on *Ilex* embryoid production. Zeitschrift fur Pflanzenphysiologie 89:41-49.

Hosokawa, K., R. Matsuki, Y. Oikawa, and **S. Yamamura.** 2000. Production of transgenic gentian plants by particle bombardment of suspension-culture cells. Plant Cell Rep. 19:454-458.

Ishizaka, H. and **J. Uematsu. 1995.** Interspecific hybrids of *Cyclamen persicum* Mill. and *C. purpurascens* Mill. produced by ovule culture. Euphytica 82:31-37.

Ishikawa, T., T. Takayama, I.H.shizaki, K. Ishikawa, and **M.Mii.** 1997. Production of 1997 interspecific hybrids between *Alstroemeria ligtu* L. hybrid and *A. pelegrina* L. var. *rosea* by ovule culture. Breed. Sci. 47:15-20.

Kim, Y.S., B.K. Lee, Y.S. Kim, and **B.K. Lee.** 1996a. Somatic embryogenesis and plant regeneration in cotyledon culture of *Paeonia albiflora*. J. Korean Soc. Hort. Sci. 37:6, 827-830

Kim, J.H., D. J. Ham, H.T. Lim, and **K.C. Lee.** 1996b. Cross compatibility, seed germinability, and embryo rescue following 2x times 4x cross in *Hibiscus syriacus*. J. Korean Soc. Hort. Sci. 37:713-718

Kiss J., L.E. Heszky, E. Kiss, and **G. Gyulai.** 1992. High efficiency adventive embryogenesis in somatic embryos anther, filament and immature proembryo origin in horsechestnut (*Aesculus hippocastanum* L.) tissue culture. Plant Cell Tissue and Organ Cult. 30:59-64.

Lavy, M., A. Zuker, E. Lewinsohn, O. Larkov, U.Ravid, A. Vainstein, and **D. Weiss.** 2002. Linalool and linalool oxide production in transgenic carnation flowers expressing the *Clarkia breweri* linalool synthase gene. Molec. Breed. 9:103-111.

Lebedev, V.G., S. V. Dolgov, and **K.G. Skryabin.** 2002. Transgenic pear clonal rootstocks resistant to herbicide "Basta". Acta Hortic. 596:193-197.

Li, X., G. Ksenjia, C. Bruno, B.W. Korban, and **S. Schuyler** 2003. Transgenic rose lines harboring an antimicrobial protein gene, Ace-AM P1, demonstrate enhanced resistance to powdery mildew (*Sphaerotheca pannosa*). Planta 218: 226-232

Lim, C.C., S.L. Krebs, and **R. Arora.** 1999. A 25-kDa dehydrin associated with genotype- and age-dependent leaf freezing-tolerance in Rhododendron: a genetic marker for cold hardiness? Theor. Appl. Genet. 99:912-920.

Ma, Y., D.H. Byrne, and **J. Chen** 1997. Amphiploid induction from diploid rose interspecific hybrids. HortScience 32:292-295.

Marchant, R., M.R. Davey, J.A. Lucas, C.J. Lamb, R.A. Dixon, and **J.B. Power.** 1998. Genetic engineering of rose for resistance to blackspot, P. 291-299. In: M.R. Davey, P.G. Alderson, K.C. Lowe, J.B. Power(eds.) Tree biotechnology: towards the millennium. Nottingham University Press, Nottingham, U.K.

Marchant, R., J.B. Power, M.R. Davey, and **J**. **Chartier-Hollis.** 1993. Embryo rescue, for the production of F_1 hybrids, in English rose. Euphytica 74:187-193.

Mattis, P.R., H.J. Swartz, and **G. Eisenbeiss.** 1995. Development of embryo rescue and shoot regeneration techniques in Ilex. J. Environ. Hort. 13:164-168.

Merkle S.A. and **B.A. Watson-Pauley.** 1994. In vitro conversion of pyramid magnolia somatic embryos. Hort. Sci. 29:1186-1188.

Merkle S.A. 1997. Somatic embryogenesis in ornamentals, p. 13-33 In: Geneve, R.L., J.E. Preece and S.A. Merkle (eds.). Biotechnology of ornamentals plants, Biotechnology in Agriculture Series No. 16, CAB International.

Mertens, M., J. Heursel, E.van Bockstaele, and **M. de Loose**. 2000. Inheritance of foreign genes in transgenic azalea plants generated by Agrobacterium-mediated transformation. Acta Hortic. 521:127-132.

Meynet, J., R. Barrade, A. Duclos, and **R. Siadous.** 1994. Dihaploid plants of roses (Rosa ×hybrida, cv. 'Sonia') obtained by parthenogenesis induced using irradiated pollen and in vitro culture of immature seeds. Agronomie 14:169-175.

Morgan, E.R., G.K.Burge, J.F. Seelye, M.E. Hopping, and **J.E. Grant**. 1998. Production of inter-specific hybrids between *Limonium perezii* (Stapf) Hubb. and *Limonium sinuatum* (L.) Mill Euphytica, 102:109-115.

Nakano M., S. Tanuka, M. Oata, E. Oakawa, S. Suzuki, and **H. Saito.** 2003 Regeneration of diploid and tetraploid plants from callus-derived protoplasts of *Agapanthus pracecox* ssp. orientalis (Leighton) Leighton. Plant Cell Tissue and Organ Cult. 72:63-69.

Nebauer, S. G., I. Arrillaga, L. del Castillo-Agudo, and **J. Segura.** 2000. Agrobacterium tumefaciens-mediated transformation of the aromatic shrub *Lavandula latifolia*. Molec. Breed. 6:539-552.

Radojevic L. 1979. Somatic embryos and plantlets from callus cultures of *Paulownia tomentosa* Steud. Zeitschrift fur Pflanzenphysiologie 91:57-62.

Raquel, H. C. Silva, R. Batista, A. M. Santos, T. Lourenco, M. Mascarenhas, G. Nolasco, and **M.M. Oliveira.** 2002. Virus diseases in Portuguese almond trees: diagnosis, in situ detection and genetic engineering for virus resistance. Acta Hortic. 591: 581-584.

Ravelonandro, M., R. Scorza, A. Callahan, L.Levy, C. Jacquet, M. Monsion, and **V. Damsteegt.** 2000. The use of transgenic fruit trees as a resistance strategy for virus epidemics: the plum pox (sharka) model. Virus Res. 71:63-69.

Renou, J. P., I. Mary, S. Hanteville, J. P. Narcy, A. Diolez, and **D. Florack.** 2000. Evaluation of the protection against Xanthomonas in transgenic Pelargonium containing a chimaeric cecropin gene. Acta Hortic. 508:323-325

Rosati, C., P. Simoneau, D. Treutter, P. Poupard, Y. Cadot, A. Cadic, and **M. Duron.** 2003. Engineering of flower color in forsythia by expression of two independently-transformed dihydroflavonol 4-reductase and anthocyanidin synthase genes of flavonoid pathway. Molec. Breed. 12:197-208

Rose J. B., J. Kubba and **K. R. Tobutt.** 2000. Induction of tetraploidy in *Buddleia globosa*. Plant Cell Tissue and Organ Cult. 63:121-125.

Rose, J.B., J. Kubba, and **K.R. Tobutt.** 2001 Induction of tetraploids for breeding hardy ornamentals Acta Hortic. 560:109-112.

San-Jose, M. C. and **A. M. Vieitez.** 1993. Rgeneration of Camellia plantlets from leaf explant cultures by somatic embryogenesis and caulogenesis. Scientia Hortic. 54:303-315.

Scorza, R., A. M. Callahan, V. Damsteegt, L. Levy, and **M. Ravelonandro.** 2001. Resistance to plum pox potyvirus in a transgenic woody perennial fruit tree, European plum (*Prunus domestica* L.) results from post-transcriptional gene silencing. Acta Hortic. 550:425-430.

Suzuki, S. and **M. Nakano.** 2002. Agrobacterium-mediated production of transgenic plants of *Muscari armeniacum* Leichtl. ex Bak. Plant Cell Rep. 20:835-841

Trigiano, R. N., R. M. Beaty and **J. T. Dietrich.** 1989. Somatic embryogenesis and plant regeneration in *Cornus florida*. Plant Cell Rep. 8:193-218.

Vesco, dal L. L., and **M. P.Guerra.** 2001. The effectiveness of nitrogen sources in *Feijoa* somatic embryogenesis. Plant Cell Tissue and Organ Cult. 64:19-25.

Viss, W.J., J. Pitrak, J. Humann, M.Cook, J. Driver, and **W. Ream.** 2003. Crown-gall-resistant transgenic apple trees that silence Agrobacterium tumefaciens oncogenes. Molec. Breed. 12:283-295.

Yao J.L. and **D. Cohen.** 1996. Production of triploid *Zantedeschia* hybrids using embryo rescue. N.Z. J. Crop Hort. Sci. 24:297-301.