Modern Methods for Breeding Ornamentals©

Trine Hvoslef-Eide and Nina Ingrid Vik
The Plant Cell Laboratory, Department of Horticulture and Crop Sciences, P.O. Box 5022, N-1432 Aas, Norway

INTRODUCTION

Breeding of ornamentals has traditionally been done by growers. Some growers had a special eye for picking up mutations, while others have done serious, planned crosses to improve plant material systematically. Breeding of ornamentals at the Department of Horticulture started with a small project on keeping quality in Christmas begonia (*Begonia* ×*cheimantha*) in 1989. Breeding of ornamentals does in principle not differ from breeding of any other crop, but the breeding goals may vary. In addition, most of the ornamentals are vegetatively propagated.

Modern methods for breeding often include in vitro techniques and/or molecular biology in one or more steps in the breeding process. The following have been included in this paper: embryo rescue, in vitro selection, somaclonal variation, double haploids and chromosome elimination, and transformation/gene technology. This is not a complete review on the subject, the aim is to illustrate the methods mentioned with some history as well as some of the more recent examples within ornamentals.

The use of modern methods is particularly intriguing in ornamentals, since the aesthetical value is so high, and new colours are so attractive. Biotechnological methods for breeding of ornamentals will definitely continue to be part of the toolbox for future ornamental plant breeders. Some of the arguments against genetically modified crops do not apply for ornamentals, since they are not eaten. Therefore, ornamentals may serve as model plants for testing or optimising methods, gene expression, etc. The public acceptance of these products is probably higher than of food, in which case more knowledge is required of the long-term effects on human health.

BIOTECHNOLOGICAL METHODS OF ORNAMENTAL BREEDING

Embryo Rescue. This is the oldest of the modern techniques involving in vitro culture and continues to be an important way of creating novel variation (Bridgen, 1994; Sharma et al., 1996). The foundation of modern hybrid lily cultivars was laid in the 1950s when crosses were made using species that naturally would have given nonviable embryos. Crossings are dissected out before the embryo aborts and placed on a culture medium in vitro to develop directly into plantlets.

In flower bulbs, interspecific hybridisation is the most important source of genetic variation (van Tuyl, 1997). Many of the cultivars have originated from complex species crosses which have given rise to a broad range of shapes and colours to plants and flowers in plants like *Alstroemeria* (Buitendijk et al., 1995; Ishikawa et al., 1997; Kamstra et al., 1999), *Allium*, (Dubouzet et al., 1994), *Gladiolus*, *Hippeastrum*, *Lilium* (Morimoto and Kohno, 1996; Roh et al., 1996; van Tuyl and van Holstein, 1996), *Narcissus*, and *Tulipa* (Custers et al., 1995; van Creij et al., 1997). At the Eucarpia symposium in 1998, van Tuyl et al. (2000) reported a breakthrough in lily breeding as they were able to make interspecific crosses even between European and

Asiatic cultivars overcoming pre-fertilisation barriers by the cut style method, the grafted style method, and the in vitro isolated-ovule pollination technique. The post-fertilisation barriers were circumvented by in vitro pollination and/or rescue methods as embryo-, ovary-slice- or ovule culture.

In a breeding programme for *Alstroemeria* Mercuri et al. (1998) and Burchi et al. (2000) used embryo rescue to obtain more than 120 new interspecific crosses, while the same combinations *in vivo* produced only a few viable seeds.

Roses are important ornamental plants and embryo rescue techniques are widely used. At Meilland, they include embryo rescue amongst the modern techniques for variety development (Gudin and Mouchotte, 1996). El Mokadem et al. (2000a) obtained interspecific hybrids through parthenogenesis and embryo rescue.

In *Dendranthema,* various techniques have been developed which might help breeders to meet the demand of the cut flower industry in the new century. These strategies are discussed in a review by Rout and Das (1997) and include embryo rescue together with somaclonal variation and transformation techniques.

Pelargonium is another genus where embryo rescue has an enormous potential. Horn (1994) has put together a comprehensive review on breeding of *Pelargonium*. Novel flower colours in *Pelargonium* were obtained by Denis-Peixoto et al. (1997) by using the yellow-flowered species *P. quinquelobatum* as the female parent. Within these species, however, the regenerants frequently exhibit somaclonal variation in addition to the variation obtained by hybridisation (Cassells et al., 1995).

In Vitro Selection. This is a space- and labour-intensive method, which can be used for characteristics that are expressed and can be selected for in vitro. Successful examples can be found in work from Ahrendsburg, Germany, where in vitro selection was used to select a low-temperature-tolerant *Dendranthema* cultivar that could be produced at a much lower temperature in the same number of days in the greenhouse (Preil et al., 1991; Huitema et al., 1991). Another example from the same laboratory is the high pH tolerant *Rhododendron* rootstock selected on high pH medium (Preil, 1990). These rootstocks can be used to produce *Rhododendron* plants that will grow in normal garden soils alongside other garden plants. They are presently on the market. In our own laboratory we have developed a method for selection of ethylene tolerance in vitro (Hvoslef-Eide et al., 1992) based on differences in yellowing of shoots after sterile ethylene exposure on tissue culture plants of different cultivars. Similar methods have later been used with success in breeding *Ranunculus* for longevity (Mensuali-Sodi et al., 2000).

In vitro selection for improved disease resistance is a feasible approach if the toxin produced by the disease is readily available. Remotti et al. (1997) have successfully regenerated *Gladiolus* plants resistant to fusaric acid, which is the toxin produced by *Fusarium oxysporum*. The selected plants were to be tested further for *Fusarium* resistance once they had reached maturity.

Somaclonal Variation. Somaclonal variation was defined by Larkin and Scowcroft (1981) as the novel variation created by in vitro culture. It was launched as an exciting breeding method to create variation and includes the variation already existing in the tissue in the form of hidden chimeras as well as the variation created by the tissue culture method used. In our laboratory we have discovered several new cultivars in the disease-free plant programme. A novel flower colour may emerge and is placed on the market as a new cultivar. Recent examples are *Begonia* 'Karelsk

Jomfru', which first emerged as a cultivar with darker flowers and leaves, named 'Flamme' (Sivertsen and Skjeseth, pers. comm.). This cultivar has proved to be impossible to propagate through tissue culture. Out of such a culture came another cultivar with the same dark flowers, but with normal 'Karelsk Jomfru' leaves, not the dark leaves of 'Flamme'. Another example is *Kalanchoe* 'Charm' which also came out of culture with a darker flower colour. Breeders in the Philippines have also experienced that in vitro techniques have yielded variations in flower colour in *Kalanchoe*. This suggests that susceptibility to somaclonal variation was genotype-dependent (Zamora et al., 1998). Such novel variation can be desirable in a breeding programme, but not in a set up for vegetative propagation.

Other examples of published somaclonal variation in ornamentals are: Development of a chimeral, thornless rose (Rosu et al., 1995), variation in *Pelargonium* (Cassells et al., 1997), *Begonia* ×*hiemalis* and *Saintpaulia* (Jain, 1997), *Gladiolus* (Remotti et al., 1997), rose (Schum et al., 1996), *Alstroemeria* (Anastassopoulos and Keil, 1996), and *Rhododendron* (Mertens and Samyn, 1994).

A variant of the somaclonal variation is the deliberately induced mutations in vitro. This may be done with radiation or chemicals, often combined with in vitro selection. Examples of recent use of induced mutations in ornamental breeding are; gamma radiation in *Dendranthema* (Nagatomi et al., 2000), the use of ion beam for in vitro mutation in *Eustoma* (Ohki et al., 2000), and treatment with chemicals to induce variegated forms in *Saintpaulia* (Gaj and Gaj, 1996).

Double Haploids and Chromosome Elimination. This method is most efficient for producing parent lines for hybrid seed production. Double haploids are produced from either male or female gametes and the term used is also gametic embryogenesis. Production from male gametes is preferred, as they are much more abundant than the female ovules. Male gametic embryogenesis develops either through anther cultures, or microspore cultures. The liquid cultures with microspores which are immature pollen are usually preferred since they give a more uniform development of embryos, as well as higher numbers from each anther. These methods are widely used in cereals and *Brassica*, but examples are scarce in ornamentals.

By using chromosome elimination through crossing incompatible species (also called the bulbosum technique), where the species in question retains its chromosomes and the foreign chromosomes are eliminated in the process, it is possible to obtain the same result — a plant with haploid chromosomes, ready for doubling and production of a parent line with identical genes on each chromosome set. El Mokadem et al. (2000b) irradiated *Rosa* pollen, pollinated plants, and got double parthenocarpic plants after embryo rescue. While Han et al. (1996) obtained haploid plants in hybrid lily by regeneration directly from anthers. The haploid plants produced through gametic embryogenesis or chromosome elimination may double either spontaneously in the developmental process, or with chemicals like colchicine or oryzalin.

Transformation/Gene Technology. One of the major limitations in traditional breeding of ornamental species is the size of the gene pool within a species, which a breeder may use in the search for promising combinations. Embryo rescue is one method used to achieve successful interspecific crosses, but far more powerful tools have arisen in the latest years. Gene technology allows the breeder to insert a specific gene from any species in the attempt to alter specific characteristics of the

plant. This may be done without the traditional need for backcrossing, giving new desirable traits without disturbing the original character of the species.

Methods of genetic transformation are numerous, and this paper will deal with three different techniques for transferring specific genes to ornamentals. Two of the most commonly used are: transfer by *Agrobacterium* and particle bombardment. The third method is the method of DNA electrophoresis. The three methods have been compared in Table 1.

Table 1. A comparison between three different methods for gene transfer into ornamental plants tested in our laboratory.

Transfer method	In vitro required	Feasible numbers	Transformation frequency	Costs
Agrobacterium	yes	XXX	X	XX
Particle gun	yes	XXX	X	0
DNA electrophoresis	no	X	XXX	XX

0=poor; XXX=excellent

The natural transformation process of the soil bacterium *Agrobacterium tumefaciens* is by far the method most frequently used in genetic transformation today and also for ornamentals. We take advantage of a system that allows for a fairly accurate insert of a specific DNA sequence in the host chromosome. One of the most important prerequisites for the use of this method is to have a well established in vitro regeneration protocol, to achieve transformed plants from the explants used. However, *Agrobacterium* is limited to a certain host range and a certain bacterial strain will not be able to give successful transformations in any given species. Many of the strains have been chosen because they are suited for transformation of the model plant tobacco, and they may be far less suitable for other species. This may be amended by either manipulating a certain *Agrobacterium* strain (Stanton and Liu, 1994) or by searching for a strain that is more suitable. Finding a virulent strain for transformation of monocotyledons is a much greater problem. Another common problem is to free the plant material completely from the bacteria after co-cultivation. There are numerous reports on *Agrobacterium*-mediated transfer of genes into ornamentals. Some recent examples are flower colours in *Petunia* where the *Lc* gene from the monocot maize increases the production of anthocyanins in the dicot (Bradley et al., 1998), or change in flower patterns, like in *Eustoma* where the antisense chalcone syntase gene gives novel flower patterns (Deroles et al., 1998). In addition to this, several ornamentals have been transformed with the antisense ACC oxidase gene, which regulates one of the later steps in the ethylene biosynthetic pathway. The goal was to improve keeping quality by reducing the climacteric ethylene production in the plant, as in Christmas begonia (*Begonia ×cheimantha*) where antisense ACC oxidase has given rise to transgenic plants with improved shelf life (Einset and Kopperud, 1995; Hvoslef-Eide et al., 1995). Transformed 'White Sim' flowers of carnation similarly showed a 3 to 4 times longer vase life

compared to 'White Sim' flowers from nontransformed plants (Savin et al., 1995).

The use of particle bombardment to literally shoot the DNA into plant cells is not widely used for transforming ornamentals, but it is used frequently and with success in rice, wheat, and maize. The big advantage of nonhost limitations will perhaps make particle bombardment more popular for transformation of ornamentals in the future. These methods also require in vitro regeneration protocols and if bought commercially the particle delivery systems are quite expensive. Similar systems may however be built at a low cost and are cheap to operate. One of the main problems using the particle gun is the high probability of obtaining chimeral plants rather than solid transformants. Results of particle bombardment may depend on choice of explant, at least that is what Kamo et al. (1997) found when transforming *Gladiolus* with a potyvirus coat protein gene. Particle gun bombardment of cormel slices gave rise to transformed plants showing a striped pattern of GUS expression, a clear indication of chimerism. When using the same procedure on suspension cells transgenic plants with a more uniform GUS expression throughout the leaves were obtained. More recently, we have had success with transient (temporary) expression of the GUS gene also in suspension cells of *Cyclamen* (Borgen, 1999).

The third method we have tested for poinsettia is gene transfer through electrophoresis. This method is not patented and thereby free to use by anyone. Ahokas (1989) first described it in barley. This method can be used on plant material *in vivo* and *in vitro*. By using the method on intact plant material protocols for in vitro culture are not necessary, which is a clear advantage.Table 2 shows that a current of 0.5 mA for 10 min is sufficient in poinsettia to transfer the negatively charged DNA into the meristems of small plants (Bakke and Gjerde, 1998).

Table 2. Regrowth of lateral meristems and lateral buds and number of positive GUS tests after electrophoresis with pJE101 using varying current and time of exposure. Regrowth was registered after 4 to 5 weeks in the greenhouse and expression of GUS after 8 to 9 weeks (Bakke and Gjerde, 1998).

Time (min)	0.2 mA	0.3 mA	0.4 mA	0.5 mA	0.6 mA	0.7 mA	0.8 mA	1.0 mA	1.1 mA	1.5 mA
3										1(0)(2)
5		1(0)(1)	1(0)(1)	1(1)*(0)	1(1)(1)*	1(0)(0)		1(0)(1)	1(1)*(1)*	
10		2(0)(2)	3(1)(2)	4(2)*(4)*	3(0)(2)*	1(0)(2)	1(0)(1)*			
15	1(1)(1)		1(1)*(1)	2(1)(2)	1(1)(1)					

(1): Number of lateral meristems treated at the given exposure time and current.
(2): Number of lateral meristems with regrowth.
(3): Number of lateral bud breaks.
*: GUS positive.

The negative electrode is placed on top of the exposed meristem through a 1-ml pipette tip with DNA cast in an agarose gel. The positive electrode is placed at the base of the plant, either in the soil or through the stem itself pierced by a silver thread.

We found that close to 25% of the treated meristems show GUS expression with blue sectors. This method requires more horticultural skills to get stable transformants than the previously described methods, but requires less expensive equipment and may be performed by nurseries that have co-operation with a molecular biology laboratory to provide the DNA cast in pipette tips. Recently, we have proved stable transformation by Southern blot (Nina Vik et al., unpublished results) in poinsettia plants.

CONCLUSIONS

Biotechnological methods for breeding ornamentals will definitely continue to be part of the future tool box for ornamental plant breeders. Some of the arguments against genetically modified crops do not apply for ornamentals, as these products are not eaten. Thus, the public acceptance for these products is probably higher than with food, where the requirements are much more knowledge of the long-term effects on health.

Acknowledgements. The authors wish to acknowledge the excellent work done by several undergraduate students over the years; Kristin Bakke, Håkon Gjerde, Sigrid Gåseidnes, Anders Borgen, and Melinda Gyorgy (Hungary). We also want to thank Tone I. Melby for exquisite technical assistance.

LITERATURE CITED

Ahokas, H. 1989. Transfection of germinating barley seeds electrophoretically with exogenous DNA. Theoret. Appl. Genet. 77:469-472.

Anastassopoulos, E. and **M. Keil.** 1996. Assessment of natural and induced genetic variation in *Alstroemeria* using random amplified polymorphic DNA (RAPD) markers. Euphytica 90(2):235-244.

Bakke, K. and **H. Gjerde.** 1998. Transformation of poinsettia (*Euphorbia pulcherrima* Willd. & Klotzsch. Cand. Scient. Thesis, pp. 105. Agricultural University of Norway, 1998, Dept. of Horticulture and Crop Sciences.

Borgen, A. 1999. Transformation of *Cyclamen persicum* and *Begonia* ×*cheimantha* using a gene particle gun. Extended essay for International Baccalaureate, Ås videregående skole, Ås, Norway 1999.

Bradley, J.M., K.M. Davies, S.C. Deroles, S.J. Bloor, and **D.H. Lewis.** 1998. The maize Lc regulatory gene up-regulates the flavonoid biosynthetic pathway of *Petunia*. Plant J. 13(3):381-392.

Bridgen, M.P. 1994. A review of plant embryo culture. HortScience 29:1243-1246.

Buitendijk, J.H., N. Pinsonneaux, A.C. van Donk, M.S. Ramanna, and **A.A.M. van Lammeren.** 1995. Embryo rescue by half-ovule culture for the production of interspecific hybrids in *Alstroemeria*. Scientia Hortic. 64:65-67.

Burchi, G., A. Mercuri, C. Bianchini, R. Bregliano, and **T. Schiva.** 2000. New interspecific hybrids of *Alstroemeria* obtained through in vitro embryo rescue. Acta Hort. 508:233-235.

Cassells, A.C., C.J. Barett and **B.F. Carney.** 1995. Creation of variability in *Pelargonium* ×*domesticum* by hybridisation of stable with unstable genotypes and enhancement of expression instability in the hybrids by adventitious regeneration *in vitro*. Acta Hort. 420:81-84.

Cassells, A.C., J.T. Croke, and **B.M. Doyle.** 1997. Evaluation of image analysis, flow cytometry, and RAPD analysis for the assessment of somaclonal variation and induced mutation in tissue culture-derived *Pelargonium* plants. J. Appl. Bot. 71(3-4):125-130.

Creij, M.G.M. van, J.L. van Went, and **D.M.F.J. Kerckhoffs.** 1997. The progamic phase, embryo and endosperm development in an intraspecific *Tulipa gesneriana* L. cross and in the incongruent interspecific cross *T. gesneriana* × *T. agenensis* DC. Sexual Plant Reproduction 10(4):241-249.

Custers, J.B.M., W. Eikenboom, J.H.W. Bergervoet, and **J.P. van Eijk.** 1995. Embryo-rescue in the genus *Tulipa* L.; successful direct transfer of *T. kaufmanniana* Regel germplasm into *T. gesneriana* L. Euphytica 82(3):253-261.

Denis-Peixoto, L., A. Cadic, and **J.P. Renou.** 1997. Interspecific crosses between *Pelargonium* ×*hortorum* and *P. quinquelobatum* using embryo rescue and molecular characterization of hybrids by an endogenous *chs* probe. Plant Breeding 116(2):177-180.

Deroles, S.C., J.M. Bradley, K.E. Schwinn, K.R. Markham, S. Bloor, D.G. Manson and **K.D. Davies.** 1998. An antisense chalcone synthase cDNA leads to novel colour patterns in lisianthus (*Eustoma grandiflorum)* flowers. Mol. Breeding 4:59-66.

Dubouzet, J.G., K. Arisumi, T. Etoh, M. Maeda and **Y. Sakata.** 1994. Studies on the development of new ornamental *Allium* through interspecific hybridisation, 3: Hybridisation of autumn-flowering species through pull-style pollination, cutflower culture and embryo rescue. Memoirs of the Faculty of Agriculture, Kagoshima Univ. (Japan) 30:35-42.

Einset, J.W. and **C. Kopperud.** 1995. Antisense ethylene genes for *Begonia* flowers. Acta Hort. 405:190-196.

El Mokadem, H., Y. Jacob, and **J. Meynet.** 2000a. Utilisation of partenogenetic diploid plants of *Rosa hybrida* for interspecific hybridisation. Acta Hort. 508:185-190.

El Mokadem, H., C. Martineau, and **J. Meynet.** 2000b. Genotypic ability of *Rosa hybrida* to produce partenogenetic plants after pollination with irradiated pollen. Acta Hort. 508:243-245.

Gaj, M. and **M.D. Gaj.** 1996. The high frequency of variegated forms after *in vitro* mutagenesis in *Saintpaulia ionantha* Wendl. Acta Soc. Bot. Poloniae 65(3-4):339-343.

Gudin, S. and **J. Mouchotte.** 1996. Intergrated research in rose improvement – A breeder's experience. Acta Hort. 424:285-291.

Han, D.S., Y. Niimi, and **M. Nakano.** 1996. Regeneration of haploid plants from anther culture of the Asiatic hybrid lily 'Conneticut King'. Plant Cell Tiss. Org. Cult. 47:153-158.

Horn, W. 1994. Interspecific crossability and inheritance in *Pelargonium*. Plant Breeding 113:3-17.

Huitema, J.B.M., W. Preil, and **J. de Jong.** 1991. Methods for the selection of low-temperature tolerant mutants of *Chrysanthemum morifolium* Ramat. by using irradiated cell suspension cultures. III. Comparison of mutants selected with or without preselection *in vitro* at low-temperature. Plant Breeding 107:135-140.

Hvoslef-Eide, A.K., K. Boger, M. Olsen, and **T. Fjeld.** 1992. Breeding for keeping quality in Christmas begonia (*Begonia* ×*cheimantha* Everett) using *in vitro* selection. Acta Hort. 336:101-107.

Hvoslef-Eide, A.K., J.W. Einset, and **T. Fjeld.** 1995. Breeding for keeping quality in Christmas begonia (*Begonia* ×*cheimantha* Everett) with traditional breeding and biotechnology. Acta Hort. 405:197-204.

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

Jain, S.M. 1997. Micropropagation of selected somaclones of *Begonia* and *Saintpaulia*. J. Biosci. (Bangalore) 22(5):585-592.

Kamstra, S.A., M.S. Ramanna, M.J. de Jeu, A.G.J. Kuipers, and **E. Jacobsen.** 1999. Homologous chromosome pairing in the distant hybrid *Alstroemeria aurea* × *A. inodora* and the genome composition of its backcross derivates determined by fluorescence *in situ* hybridisation with species-specific probes. Heredity 82:69-78.

Kamo, K., A. Altman, and **M. Ziv.** 1997. Bean yellow mosaic virus coat protein and gusA gene expression in transgenic *Gladiolus* plants. Acta Hort. 447:393-399.

Larkin, P.J. and **W.R. Scowcroft.** 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Mensuali-Sodi, A., G. Serra, and **F. Tognoni.** 2000. In vitro culture for evaluation of flowering longevity of potted *Ranunculus* hybrids. Acta Hort. 508:315-317.

Mercuri, A., G. Burchi, C. Bianchini, R. Bregliano, and **T. Shiva.** 1998. Interspecific *Alstroemeria* hybrids. Colture Protette, 27(2):79-81.

Mertens, M. and **G. Samyn.** 1994. Regeneration and somaclonal variation of *Rhododendron simsii* offer prospects to the obtainment of commercial valuable sports. Med. Fac. Landb. Toeg. Biol. Wet. Uni. (Belgium) 59(4b):2495-2497.

Morimoto, Y. and **S. Kohno.** 1996. Pollen-less lilies (*Lilium longiflorum*) were bred by embryo rescue and their use. Res. J. Food Agric. (Japan) 19(4):16-20.

Nagatomi, S., E. Miyahira, and **K. Deg.** 2000. Induction of flower mutation comparing with chronic and acute gamma irradiation using tissue culture techniques in *Chrysanthemum morifolium* Ramat. Acta Hort. 508:69-73.

Ohki, S., K. Chantani, Y. Sakai, and **K. Koreeda.** 2000. Use of ion beam for in vitro mutation breeding of ornamental plants. Acta Hort. 508:75-82.

Preil, W. 1990. Zur Auslese kalktoleranter Veredelungsunterlagen. pp. 95-103. In: Rhododendron und immergrüne Gehölze. Jahrbuch Dtsch. Rhododendron-Gesellschaft, Bremen.

Preil, W., J.B.M. Huitema, and **J. de Jong.** 1991. Methods for the selection of low-temperature tolerant mutants of *Chrysanthemum morifolium* Ramat. by using irradiated cell suspension cultures. II. Preselection in vitro under low-temperature stress. Plant Breeding 107:131-134.

Remotti, P.C., H.J.M. Löffler, and **L. Vloten-Doting.** 1997. Selection of cell-lines and regeneration of plants resistant to fusaric acid from *Gladiolus* ×*grandiflorus* cv. 'Peter Pears'. Euphytica 96:237-245.

Roh, M.S., R.J. Griesbach, R.H. Lawson, and **K.C. Gross.** 1996. Production and identification of interspecific hybrids of *Lilium longiflorum* and *L. elegans*. Acta Hortic. 414:93-100.

Rosu, A., R.M. Skirvin, A. Bein, M.A. Norton, M. Kushad, and **A.G. Otterbacher.** 1995. The development of putative adventitious shoots from a chimeral thornless rose *(Rosa multiflora* Thunb. Ex J. Murr.) *in vitro.* J. Hort. Sci. 70:901-907.

Rout, G.R. and **P. Das.** 1997. Recent trends in the biotechnology of *Chrysanthemum*: A critical review. Scientia Hort. 69:239-257.

Savin, K.W., S.C. Baudinette, M.W. Graham, M.Z. Michael, G.D. Nugent, C.-Y. Li, S.F. Chandler and **E.C. Cornish.** 1995. Antisense ACC Oxidase RNA delays Carnation petal senescence. HortScience 30:970-972.

Schum, A., K. Hofmann, and **N. Ghalib.** 1996. In vitro Tekniken – Protoplastkulturen zur Erweiterung der genetischen Variabilität bei *Rosa* spec. pp. 30-31, Jahresbericht der Budesanstalt für Züchtungsforschung an Kulturpflanzen, Germany.

Sharma, D.R., R. Kaur, and **K. Kumar.** 1996. Embryo rescue in plants: A review. Euphytica 89(3):325-337.

Songstad, D.D., D.A. Somers, and **R.J. Griesbach.** 1995. Recent advances in alternate DNA delivery techniques. Plant Cell Tiss. Org. Cult. 40:1-15.

Stanton, G.B. and **C.-N. Liu.** 1994. Genetic manipulation of *Agrobacterium tumefaciens* strains to improve transformation of recalcitrant plant species. Plant Molecular Biology Manual. Kluwer Academic Publishers.

Tyul, J.M. van and **H.C.M. Holstein.** 1996. Lily breeding in the Netherlands. Acta Hort. 414:35-45.

Tyul, J.M. van 1997. Interspecific hybridization of flower bulbs: a review. Acta Hort. 430:465-476.

Tuyl, J.M. van, A. van Dijken, H.-S. Chi, K.-B. Lim, S. Villemoes, and **B.C.E. van Kronenburg.** 2000. Breakthroughs in interspecific hybridisation of Lily. Acta Hort. 508:83-88.

Zamora, A.B., S.S. Gruezo, and **S.V. Siar.** 1998. Somaclonal variation: Breeder's tools for recovering potential cultivars in ornamentals (Kalanchoe). Philippine J. Crop. Sci. 23 (Suppl. no1):71.