If we compare the U.K. with mainland Europe we see an enormous gap in attitude towards the market. In the U.K. there is no daily auction system, so buyers are forced to plan ahead. They have to rely on producers. New lines tend to be instigated by the breeder, while in Holland they are instigated by demand at the auction. In the U.K. breeders and propagators have to plan in conjunction with foliage producers and the floristry trade to ensure success. In other words there have to be very close links between breeders, propagators, and the trade. This trend is much more advanced in the U.K. than in other countries.

Review of Techniques used at Angers to Increase Genetic Diversity

Alain Cadic
INRA-CR Angers, Unité d'amélioration des espéces fruitiéres et ornementales, BP57, 49071 Beaucouzé Cedex, France

This paper surveys various techniques to develop and select new woody ornamental cultivars. Mutagenic treatments have proved valuable in modification of characteristics such as plant habit, flower colour, earliness of flowering, or to modify chromosome number in species such as *Forsythia, Weigela, Malus, Lonicera, Clematis, Hydrangea*, and *Pelargonium* [*P.* ×*hortorum* and *P. peltatum* (syn. *P.* ×*hederaefolium*)]. Intra- or inter-specific hybridization is preferred if existing genetic diversity is broad enough and plant biology makes it possible. Combinations of these techniques have also been successfully used in *Forsythia* and *Weigela*. Genetic transformation has been developed using *Agrobacterium tumefaciens* as a vector to introduce disease resistance in *Pelargonium* and to change flower colour of *Pelargonium* and *Forsythia*.

INTRODUCTION

Whatever the plant species may be, a breeding scheme always starts with a full description of breeding objectives and the gathering of a good collection of appropriate species and cultivars; this allows us to estimate existing and available genetic diversity.

Crosses are made within the collection to create new populations of seedlings from which individuals not expressing desired traits will be weeded out. Selection is based on criteria such as frost resistance, disease resistance, plant habit, flower colour, etc. We aim to select from a population with wide genetic diversity; this diversity has to be targeted to genes which can contribute to achieve our stated breeding objectives.

To incorporate the desired genes, breeders benefit from a range of tools from conventional hybridization to the advanced genetic transformation through mutagenesis. Any selected genotype should be capable of being the mother plant of a new cultivar reproduced by cuttings or grafting.

EXISTING GENETIC RESOURCES

The existing genetic resources or genetic "raw materials" for breeding programmes consist of species and selected cultivars kept in botanical or horticultural collections; or of plants found in their natural habitat. Working collections are established to detect the most valuable genotypes almost immediately usable by growers or to be used latter on in a breeding program. For example, we have tested members of the Rosaceae against fire blight (*Erwinia amylovora*) in this way. Pretty good resistance has been found in several *Crataegus* and *Sorbus* species (e.g., *C. canadensis, C. delosii*, *S.* ×*arnoldiana*, and *S. decora*) which are now under evaluation for their ornamental qualities and possible use.

Natural genetic resources have long been exploited, but it is still possible to use them to broaden the genetic variability of cultivated plants because often only a small sample of the natural gene pool has been used to provide the stock of all plants in cultivation. New fire-blight-resistant genotypes of *Sorbus* have been introduced from natural sources in Yunnan (China), for example. Natural sources can also be used to introduce species which are scarcely known or even unknown in cultivation. We have been trialling collections of *Leptospermum*, *Coprosma,* and *Corokia*, introduced in 1987 from the coldest parts of the South Island New Zealand to select for frost resistance. Many have survived winter temperatures down to -12C. During the winter of 1996-97 temperatures reached -15C at ground level so we were able to select the hardiest individuals to provide clones for use in areas were these species are not usually considered hardy (Cadic and Harris, 1997; Decourtye and Harris, 1992; Decourtye et al, 1991; Lecomte and Cadic, 1993; Paulin et al, 1993)

HYBRIDIZATION

Controlled hybridization is the oldest but still the most common breeding tool. Crossing two complementary parents will produce a population of seedlings, which is a random sample of all possible combinations of the parental genes. The breeder then selects seedlings to retain the most desirable combinations. The limitations are that it must be biologically possible to cross the parents containing the desired genes, and the genes must be present in the working collection.

Hybridization has been used to obtain cultivars of *Pyracantha* resistant to scab (*Spilocaea pyracanthae*) and fire blight (*Erwinia amylovora*). The technique is also being used as part of the breeding program on *Pelargonium* ×*hortorum* and *P. peltatum* (syn. *P.* ×*hederaefolium*). Events linked to floral biology may interfere with breeding scheme. For example, in *Cotoneaster*, several polyploid species are also apomicts (producing seed "vegetatively" so that progenies are absolutely identical to the mother parent).

Ornamental horticulture has long made use of hybridization between different species. However, some species are so genetically distinct that interspecific crosses are not readily achieved. With *Berberis*, a solution has been to increase the number of combinations between red deciduous species and evergreen ones. Interspecific hybrids had green deciduous leaves and it was necessary to get a second generation by selfing those whose fertility had not been altered. In shrubby *Lonicera*, the number of possible crosses to combine early flowering as found in *L. fragrantissima* with the red-purple flower colour of *L. tatarica* 'Arnold's Red' is small. All crosses made by using fresh or preserved pollen failed

but recent observations have shown that this is because of a failure of embryo development rather than of fertilisation. We are developing "embryo rescue" techniques whereby the embryo can be removed from the seed and grown in vitro, to circumvent this difficulty (Cadic, 1987; 1992).

MUTAGENESIS AND POLYPLOIDY

Mutagenic treatments are developed to modify permanently the genetic constitution of a given genotype — in other words to produce mutant strains. This is achieved by using chemicals such as EMS or physical agents such as X-rays and gamma rays. With vegetatively propagated species treatments can be applied to meristems and mutants (sports) have to be selected from corresponding growing shoots. The hundreds of cells of a treated meristem are randomly affected by the applied treatment and the sprouting new shoot is usually a chimera (i.e., a mixture of two or more genetically distinct tissues). In this situation it is difficult to identify the mutant parts, to protect them from strong competition by non mutant cells and to regenerate and select homogeneous plants.

In-vitro culture, however, allows mutagenic treatments to be combined with regeneration from callus, internodes, or leaves. Here regeneration starts from one or a very few cells so mutants are more easily detected and selected. In vitro culture has allowed this research station to undertake gamma ray treatments in nearby Angers hospital. Mutagenic treatments have to be made on the best existing cultivars and are commonly used to induce slight modifications, for instance in the length of internodes (*Forsythia, Lonicera*), precocity of flowering (*Forsythia*), flower colour (*Weigela*), and leaf variegation.

Inducing polyploidy (multiple sets of chromosomes) is a special mutagenic treatment. For example, chemicals such as colchicine have been used to double chromosome numbers. Cultivated types of *Pelargonium* and their wild relatives have 18 chromosomes in their nonsexual cells but colchicine can induce tetraploidy (36 chromosomes per cell). Such treatments are used to increase the variability allowing crosses between more distantly related species.

A tetraploid *P. quinquelobatum* has been successfully crossed with tetraploid cultivars of *P.* ×*hortorum*. Several triploid *Weigela* cultivars have been selected by using first in vitro culture and colchicine to get tetraploid forms then by crossing tetraploid with normal cultivars. The resulting triploid cultivars do not produce seeds and do not need to be pruned each year. Such cultivars have an advantage in amenity planting schemes (Cadic et al., 1980; Cambecedes et al., 1992; Duron and Decourtye, 1977).

GENETIC TRANSFORMATION

Genetic transformation consists of the introduction of a specific gene into cells which are then induced to regenerate full plants. Modified strains of *Agrobacterium tumefaciens*, the bacterium responsible for crown-gall disease, are used to transfer genes. More recently particle guns have been developed to transform monocot species which are not susceptible to *Agrobacterium*. This technique, in theory, allows transfer of any gene from any living organism irrespective of sexual or evolutionary barriers.

Transformation can only be carried out on plants which can be regenerated in vitro from calluses, internodes, or leaf-blade explants. You also have to know exactly

where to find the gene you wish to transfer, you must be able to clone it and you must have been able to research its mode of expression. Laboratories which are developing genetic transformation need to master all the tools of molecular biology and they have to follow rules enacted by ethic committees and by specialised commissions.

At Angers, our first work on genetic transformation was on *Forsythia* in 1991 and on *Pelargonium* in 1993, involving flower colour and disease resistance. The very popular red flowering ivy pelargonium 'Roi des Balcons' is completely sterile and thus cannot be hybridized. It looks possible to block the biosynthetic pathway which produces anthocyanin pigment so that a white flowering cultivar could be produced.

Pelargonium cultivars used as pot plants or bedding plants are rather susceptible to a bacterium (*Xanthomonas campestris* pv. *pelargonii*) and to several viruses including the pelargonium flower break virus (PFBV) and the pelargonium leaf curl virus (PLCV). Resistance has not been found among known cultivars. Cecropin, a protein produced by an insect, has proved effective against the bacterium and can be introduced into pelargoniums.

We are also experimenting with genes from rat and yeast which can be introduced into pelargoniums to give resistance to viruses. (Robichon et al, 1995; Rosati et al, 1996)

CONCLUSION

Horticultural enterprise depends to a large extent on developing new crops. Hybridization remains one of the commonest ways to produce new cultivars from crossing parental lines with desirable traits. Vegetatively propagated woody plants have a juvenile period from 2 to 5 years and, with exception of a small number of species such as rose, rhododendron, and lilac, few have been systematically bred and selected so that a noticeable progress will be seen after just one or two selection cycles.

Mutagenic treatments are successful for vegetatively propagated ornamentals because resulting mutants can be cloned readily. The technique is ideal in cases where genetic variability is lacking and when only a slight modification of a good cultivar is required. Results are in part a matter of chance since treated cells are randomly affected by the applied treatment. More or less sophisticated strategies can be developed to increase the probability of detecting variants and recovering them as solid mutants. About half the number of cultivars issuing from Angers has been produced using mutagenic treatments. Among the so-called biotechnologies, only genetic transformation has been used in the Angers breeding program and, so far, no new cultivar has been produced. However, this technique is promising. Limitations might come from gene availability, gene patenting, consumer resistance, and costs.

Acknowledgements. To Laurence Arene, Veronique Bellenot, Luc Decourtye, Michel Duron, Jean-Pierre Renou, Carlo Rosati, whose results have been used in this paper.

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