PLANT BREEDING OF ORNAMENTAL CROPS : EVOLUTION TO A BRIGHT FUTURE ?!

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Summary

Plant breeding is evolution conducted by mankind. In ornamental crops breeding by hybridization has resulted in hundreds of new cultivars each year. Breeding and breeding research have contributed to the strong growth of the Dutch horticultural industry, but present sexual breeding has its limitations.

New techniques such as in vitro fertilization and methods to overcome crossing barriers, micropropagation and the use of molecular markers directly supply the convential breeding techniques.

The first results with genetic modification of ornamental crops are described. It is expected that the introduction of alien genes will attribute strongly to resistance breeding in the future.

For brussels sprouts, outdoor tomatoes and strawberries the importance of breeding and selection on mechanisation is demonstrated. In most ornamental crops this selection seems to happen in a indirect way. The relation between mechanisation and breeding of ornamentals is discussed.

1. Introduction

Plant breeding is evolution conducted by mankind. It is an activity which can be visualised as a cyclic process of searching for genetic variation, recombination of desired characters through sexual hybridization and selection of new genotypes.

Principally all heritable traits can be introduced in new cultivars, but the genetic variation that exists in plants is essential. The genetic recombination in the crossing process leads to new genetic variation. Sexual hybridization is only possible among plants belonging to the same or closely related species. Thus the use of genetic variation is limited. So far thousands of cultivars of ornamental crops are produced by the process of sexual breeding. For some crops, e.g. *chrysanthemum* and Begonia, mutation induction contributed also considerably to the development of new cultivars. This mutation breeding is mainly restricted to new colours and flower shapes.

For many years the Dutch industry for ornamental crops grew strongly. The yield used to increase with 5 to 10 % per year. The economic importance is demonstrated in table 1. The annual increase stagnated after 1991.

Table 1 : Production value of Dutch agriculture (excl. livestock)* x Dfl. 1.000.000

		1970	1988	1992
Horticulture	- vegetables, fruits	1364	3747	4343
	- ornamental crops	944	6043	7482
Arable crops		1922	3176	2613

* PVS.CBS

2. New techniques

Sexual breeding has led to many successes. In fact, all cultivated crops in the world are the result of this process. Still many limitations must be overcome. New techniques, usually mentioned under the general term of 'biotechnology' are emerging tools in breeding.

Biotechnology includes in vitro propagation, regeneration of cells, embryo rescue, ovule and ovary culture, isolation and fusion of protoplasts, production of haploid plants, genetic modification, isolation of genes, molecular fingerprinting and use of molecular markers.

Four techniques, which are important for floriculture, will be described below : procedures to overcome crossing barriers, micropropagation, genetic modification, and the use of molecular markers. The first two techniques already play a very important role in the enlargement of the assortment of ornamental crops and the introduction of new cultivars. The latter two are now being developed in The Netherlands, and other countries.

2.1. Overcoming crossing barriers

Techniques used to overcome crossing barriers can be illustrated by the success with lily (Van Tuyl et al., 1990, 1991). Interspecific hybridization proves to be a very useful method to widen the genetic variation and to renew the assortment. The genus *Lilium* comprises about 80 species, classified in seven sections. The existing cultivars, i.e. Asiatic and Oriental hybrids, originate from interspecific hybridization within two sections. The sexual barriers preventing new interspecific crosses can be divided in pre- and postfertilization barriers. The first type of barrier can be bypassed by using cut style techniques, where the pollen are brought into the stylar surface cutting the style with the stigma. An elegant addition to this technique is the grafted style method. Compatible pollen is deposited on a compatible stigma and after one day the style is cut 1 to 2 mm above the ovary and then attached to the ovary of the female parent.

After fertilization the growth of the hybrid embryo can be limited by postfertilization barriers. By using alternative routes of seed setting such as embryo rescue, ovary slice culture and ovule culture, Van Tuyl and associates (1991) succeeded in producing many new interspecific combinations (figure 1). This process was optimized by starting in vitro pollination and fertilizations. For combinations where incompatibility or incongruity are limiting factors those techniques resulted in seedsetting. In compatible combinations natural pollination and fertilization still give better results.

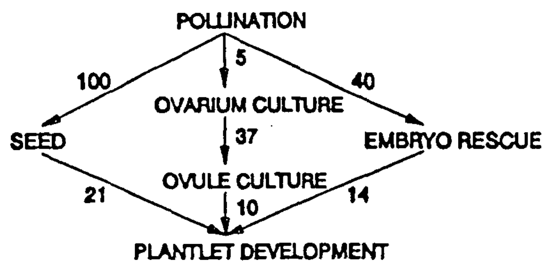

Figure 1 : Diagram of alternative routes for application of in vitro culture methods. Numbers are minimal days in a compatible intraspecific situation (crosses between different *L. Longiflorum* cvs.). After Van Tuyl et al., 1991.

There is a large assortment of thousands of species and cultivars, while the life span of many cultivars is very short. The success of Dutch flower industry and the strong international position are results of close cooperation between trade, production, breeding, research and extension services. During the last two decades the number of Dutch breeding companies has increased to about 50. More fundamental breeding research is executed by institutes such as CPRO-DLO and universities. Breeding strategies are focused more and more on resistance to diseases and pests, on product quality and on suitability to cultivation techniques.

Sexual breeding has some major limitations. As mentioned above the availability of new traits is restricted to related species, because of existing crossing barriers. Moreover, the breeding process sometimes takes many years, especially when backcrosses are needed and/or the juvenile phase is long e.g. for tulips and woody ornamentals. Five to fifteen years can be normal for the first crossing to the last selections. This breeding process is even more complicated for quantitative traits (yield, partial resistance, product quality), which are influenced by environmental factors.

A general problem is intrinsic to sexual hybridization : the process is not directed. Therefore the outcome is not predictable and large populations are needed for further selection.

2.2. Micropropagation

Micropropagation is the in vitro equivalent to cutting or other procedures of vegetative propagation.

For hundreds of species this technique is routine, executed in labs all over the world. In The Netherlands the production of in vitro plants increased from 7.4 million plants in 1980 to 94 million in 1990 (Pierik, 1990). The main species *lily, Gerbera, Nephrolepsis, Spathiphyllum* and *Anthurium* form 70 % of this number.

One bulb of a selected genotype of *lily* can be propagated to one million bulblets within 12 months. In the past this multiplication phase lasted 5-10 times longer.

New genotypes are introduced faster after use of micropropagation. In some crops the market is even deluged with new cultivars. Anyhow, the need for fast multiplication procedures still exists. For some main crops, e.g. tulip, techniques are still under investigation (Schoenmakers, 1993).

2.3. Genetic modification of ornamental crops

By genetic modification isolated genes are introduced into plants. This procedure is marked by two steps : the introduction of the genes in the plant genome and the regeneration of the transformed cell into a whole plant. By genetic modification completely new traits can be brought to expression, since the gene pool is no longer restricted to related species or even the plant kingdom. Crossing barriers can be bypassed and the genome of the plant remains unchanged after the introduction of a single gene.

The genetic modification of these plants is based on the natural infection by the soil borne bacterium *Agrobacterium tumefaciens* (Hooykaas and Schilperoort, 1992). Most dicotyledons are susceptible for this infection, but monocotyledons are much less sensitive.

Transgenic plants of horticultural crops have been produced, some of them with economic value, like herbicide-resistance in tomato (De Block et al., 1987). For ornamental crops transformation and regeneration procedures are available for chrysanthemum for stem explants (Ledger et al., 1991, Lemieux et al., 1990), for carnation (Lu et al., 1991), rose (no publication) and a number of minor crops such as Petunia (Horsch et al., 1985) and Anthurium (Kuehnle and Chen, 1993). Van Wordragen (1991) showed in her thesis that chrysanthemum calli became resistant to larvae of the tobacco bud worms after introduction of a *Bacillus thuringiensis* crystal toxin protein gene.

In The Netherlands the *Agrobacterium* mediated gene transfer in the monocot tulip was successfully investigated, but because of the low sensitivity of tulip (and lily) to this transformation procedure alternative methods have been developed (Wilmink et al., 1992). The particle bombardment technique seems very promising for these recalcitrant crops and was also used for the introduction of gene constructs in pollen grains of lily in order to create a new route to the production of transgenic lilies (Van der Leede-Plegt et al., 1991).

Fungi, insects, bacteria, nematodes and viruses cause important diseases in many crops. Until recent years the resistance breeding in ornamentals was limited (Dons et al., 1991). Genetic modification will attribute strongly to the process of resistance breeding in the future, when genes for desirable traits are available. So far, successes have been reported mainly for arable and vegetable crops.

Genes which give resistance to insects, viruses and bacteria are already available. The first effective transformation of tomato to the fungus *Fusarium oxysporum* has been reported (oral presentations by Mogen International B.V., 1993). Other genes for traits such as flower colour, flower morphology, flower induction or the extension of vase life of cut flowers carnations are under investigation.

Vase life of cut flowers, e.g. carnation, is shortened due to the sensitivity of those flowers to ethylene. The pathway and enzymes involved in the ethylene biosynthesis are known. The enzymes ACC-synthase and ACC-oxidase, which play a role in last steps of the biosynthesis, have been identified and cloned.

By anti-sense transformation the expression of those genes can be inhibited (Hamilton et al., 1990). By this approach carnations with a longer vase life can be produced.

2.4. Genetic markers

Molecular genetic techniques offer possibilities to study the variation in DNA of plant cells. The DNA can be isolated and with restriction enzymes it can be digested into small DNA-fragments. Plants with different DNA give fragments which vary in length and this can be made visible with specific techniques in so called RFLD or RAPD patterns. Certain molecular markers, such as RFLPs and RAPDs can be linked to a gene for a useful trait, e.g. for disease resistance. In this way populations of plants can be studied which vary for this desirable trait. The molecular marker which co-varies with the presence or absence of a trait can be used for indirect selection in populations. Molecular markers are a fast and strong tool for conventional breeding, even for genetically complex characters as resistances and yield. This technique will increase the efficiency of the breeding process. Thereabove, selection with a small amount of plant material and in the juvenile phase of seedlings becomes possible. So, molecular markers are a perfect tool for the selection of vegetatively propagated ornamental crops.

These techniques are developed for many crops e.g. tomato (Tanksley et al., 1988) and apple, usually in combination with the construction of a genetic map of this crop. In 1989 the research on molecular markers has been started for lily. At the moment a genetic map is constructed using RAPD's. In The Netherlands the use of molecular markers is also studied for insect resistance genes in chrysanthemum.

3. Plant breeding and mechanisation and cultivation techniques

The main topics of the Agribex-symposium 1994 are the mechanisation and cultivation techniques in agriculture. Mechanisation and automation play an important role in present agriculture and horticulture. Suitability for mechanisation, as one time over mechanical harvest is a determining selection criterion for many crops.

Although mechanisation and automation determine cultivation handlings in glasshouse culture, in breeding programs for glasshouse crops they play a minor role in the selection process. So far, in ornamental crops, the techniques have to fit those crops.

Presently I will illustrate the relation between plant breeding and mechanisation with four examples : brussels sprouts, tomatoes, strawberries and ornamentals. Important characteristics in those crops are uniformity, plant habitus and the use of F_1-hybrids. Almost all ornamental crops are propagated vegetatively. The uniformity of these clones exceeds that of F_1-hybrids.

3.1. Brussels sprouts

Until the sixties the cultivation of brussels sprouts in The Netherlands was labour intensive. Nieuwhof (1969) gave in his standard work Cole Crops an overview of the revolutionary changes in the cultivation of this crop, caused by the use of new cultivation techniques and appropriate cultivars.

Sprouts were picked by hand three to five times, as the lower sprouts grew more rapidly than those higher up the stem. By decreasing the plant distance and topping the plants the growth of the lower sprouts was delayed and the growth of the upper sprouts was hastened. New F_1-hybrids were uniform and suitable for those cultivation techniques. Moreover the hybrids were better than normal cultivars as earliness and winter hardiness improved.

The measures mentioned above made sprouts particularly suitable for one-time picking and mechanical harvesting and contributed strongly to the success of this crop.

3.2. Tomatoes

In many countries breeding programs for mechanical harvesting of tomatoes have been executed, be it always for outdoor cultivation (Lukyanenko, 1991). Requirements of tomato cultivars, especially processing tomatoes, are uniform setting and ripening, resistance to over-ripening and cracking, fruit firmness and shape, high soluble content, compact plant habitus, good foliage development and tolerance to stress conditions. The whole process of developing machine harvestable processing tomatoes took more than twenty years of research. The use of new cultivars changed the cultivation and harvesting of tomatoes and contributed to a higher tomato production.

In recent years trangenic tomato plants have been developed the fruits from which have a longer shelf life, since fruit ripening and cell softening are delayed (Sheehy et al., 1988, Hamilton et al., 1990).

These transgenic plants produce antisense-RNA which down-regulates the expression of ripening enzymes. Ripening now fully depends on exogenous supplied ethylene. This quality trait offers great opportunities for once over mechanical harvesting. In 1992 approval was given to Calgene Inc. in the USA to commercialize a transgenic tomato with delayed cell wall softening. Apart from this tomatoes with a longer shelf life have produced by breeding companies by ways of conventional breeding. These cultivars contain special genes, like the nor and rin genes, which retard ripening.

In glasshouse cultivation tomatoes are picked continuously from March to November. Breeding for mechanized harvest is insignificant, although on open plant habitus contributes to easier handling and will save labour costs. Introduction of sideshootless cultivars has been hampered by undesirable pleiotropic effects on reduction of fruit yield and quality.

3.3. Strawberry

Mechanisation in strawberry harvesting is regarded as a necessity when labour costs are too high, when there is a labour shortage exists or when the prices are low. Requirements for strawberry fruits for machine harvesting and mechanical decapping are long pedicels, firmness and calyx removal (Gooding et al., 1983). Concentrated ripening and field resistance to fungal diseases are important traits as well (Fisher and Ulrich, 1989). It is remarkable that these requirements are not always selection criterions in an early phase of the selection process. Already developed selections or cultivars are being selected for machine harvesting on the very end of the selection process (Gooding et al., 1983). This is sometimes also the case for other crops (Kinchina, 1976).

Gooding and co-workers, however, stated that specific breeding programs are required in order to obtain a suitable combination of plant and fruit characters. Fisher and Ulrich (1989) and Thuesen (1989) developed such programs. And although variation between years was found, genotypes could be recognized with a consistently high expression of desirable traits.

3.4. Ornamental crops

No literature is known about breeding programs directly aimed at mechanisation or automation in ornamental crops as such. The new magazine Hort Technology, published by the American Society for Horticultural Science, focuses on aspects of mechanisation, automation and computerization, also for glasshouse production. However breeding for mechanisation in glasshouse crops is not taken into consideration (Ting, 1992).

Reflection on the relation between mechanisation and its requirements is needed, since labour costs are high. It cannot be expected that the manipulation of the physical environment will solve all problems. Adapted cultivars will come into play.

So far selection on mechanisation happens in an indirect way. Uniformity is a first prerequisite for ornamental crops and therefore a crucial selection in the vegetatively propagated crops.

Chrysanthemum, but also lilies or tulips show this uniformity in height, plant size, flowering time and flower size. Cropping time is already an economically important trait for most crops. For example, for the year round cultivation of chrysanthemum genotypes are selected which react predictably on day length making once over harvest possible. In The Netherlands experiments are planned for the development of the once over harvest in roses, knowing that the cultivation of potroses is fully automatised. The architecture of a plant determines the suitability for packing and transport handlings. Chrysanthemum cultivars with brittle leaves that cannot withstand harvest and transport are eliminated. For tulips erect leaf shapes are desired.

Clones of the potplant Hedera were selected by a Danish company for once over harvest, thus making mechanisation of packing and transport possible. For many ornamental crops like garden plants the process of sowing, potting and internal transport is mechanized. Transplants in plugs are suitable for automation. The requirements for those are high : uniform germination and growth, high percentage of germination, compact growth, etc. Container cultivation of other ornamentals requires an uniform growth and habitus as well. According to Langton (1991) some attempt has been done to manipulate uniformity, although this character is partly determined genetically. Image analyzing techniques are available for the automatized selection on plant habitus. For all those traits genetic aspects are involved.

4. Conclusions

Ornamental crops have a bright past. In The Netherlands the production and auction turnover increased strongly until 1992. New cultivation techniques and new cultivars contributed to this expansion. The wide assortment of ornamentals seems a unique selling point for Dutch breeders and trade companies. Although breeding by hybridization resulted in hundreds of new cultivars each year, the breeding techniques have been developed to a moderate extent compared to other crops. Breeding and breeding research started quite recently ; breeding for mechanisation and automation is almost absent.

The genetic variation in ornamental plant kingdom is rich and not fully exploited. Methods for overcoming crossing barriers in hybridization and for in vitro multiplication are commonly in practice now. In addition to conventional breeding biotechnological techniques such as genetic modification and the use of molecular markers will open new ways in the breeding process. The faster introduction and selection of new genotypes give opportunities to react adequately to the requirements of society and consumers. The availability of efficient procedures of genes for desirable traits are prerequisites for that.

Breeding and breeding research will be needed more and more in the coming decades. If realized a bright past will be continued in a bright future.

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