Breeding Strategies for Woody Ornamentals: Selection Towards Disease Resistance with Particular Reference to Powdery Mildew©

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In breeding programmes, including those for woody ornamentals, polyploidization and interspecific hybridisation offer potential to introduce new genetic variation. Some examples in *Buddleja* and *Hibiscus* breeding programs are presented. In roses, as in many other species, disease resistance breeding is currently one of the major challenges. To be able to select genotypes with enhanced disease resistance, appropriate bioassays are needed. Inoculation protocols to test powdery mildew resistance of both parent plants and large populations of progenies have been developed. By these methods an early selection towards powdery mildew resistance is possible in a cost-effective way.

INTRODUCTION

Currently, in woody ornamentals, most new introductions are the result of "lucky finds" in seedling populations or from spontaneously occurring mutations. Since most plants are vegetatively propagated, these mutations might result in a stable, new cultivar. Only in a few genera (such as *Rosa*, *Rhododendron*, *Clematis,* and *Hydrangea*) are progenies raised from controlled pollinations and a planned breeding and selection scheme followed. For most species, the market share is too low to justify costly breeding programs.

Objectives for new introductions vary with the genus and during time. Novelty itself (flower, growth habit, leaf colour, and fruit set for example) is today still the most important character to interest both the grower and the consumer. Besides morphological characteristics, physiological ones such as winter hardiness, growth vigour, flowering period, and multiplication rate may also be selection criteria. More recently, driven by a market desire to reduce the use of chemical fungicides, disease resistance has become a major criterion for many genera.

In breeding programmes for woody ornamentals, polyploidy induction and interspecific hybridisation can be used to introduce new genetic variation. At the Institute for Agricultural and Fisheries Research (ILVO, Belgium) research on interspecific breeding, ploidy breeding, and the development of appropriate disease-resistant selection protocols are performed. This research is done in close collaboration with BEST-select a cooperative association of 22 Flemish nursery companies <www.bestselect.be>.

PLOIDY BREEDING

Most organisms contain two copies of their chromosome sets (2n) in their cells, and single copies (1n) in their gametes or sex cells (pollen or egg in the case of plants). Polyploidy is the condition in which cells or organisms contain more than two copies

of their chromosome sets. Where an organism is normally diploid (2n), some spontaneous aberrations may occur that are usually caused by a hampered cell division. Polyploid types are termed corresponding to the number of chromosome sets in the nucleus: triploid (3n), tetraploid (4n), pentaploid (5n), and so on.

Polyploidy can be induced during cell division by chemicals such as colchicine, oryzalin, or trifluralin. Polyploid plants in general are more robust than diploids, often with larger flowers, bigger leaves, and more vigorous growth. Many ornamental plants show a higher level of ploidy either because they have been selected after spontaneous doubling or because polyploidy has been induced by breeders. Examples are roses (often tetraploid) (Leus, 2005) and chrysanthemums (often hexaploid, 6n) (Endo et al., 2004).

The induction of polyploidy is also a common technique in interspecific crosses to overcome the sterility in F1 progenies or to generate genotypes with equal ploidy level.

Use of Ploidy Breeding in *Hibiscus*. In *Hibiscus syriacus* most cultivars are tetraploid with 80 chromosomes per cell (Skovsted, 1941). However some hexaploid cultivars have been developed, including 'Diana'; 'Hélène'; 'Flogi', Pink Giant™ rose of Sharon; and 'Melrose'. Compared to most of the tetraploid cultivars, these hexaploids have bigger flowers, grow very vigourously, and produce few seeds. Because of this reduced seed production, flowering is never inhibited during the season and there is no spread of unwanted seedlings in the garden. The goal of our work was to generate a hexaploid *H. syriacus* cultivar with a deep blue flower colour and vigorous growth. Seedlings from *H. syriacus* 'Oiseau Bleu' were first chromosome doubled with colchicine. Then seedling populations were generated from crosses between the chromosome-doubled *H. syriacus* 'Oiseau Bleu' seedlings (octaploid) and other cultivars (tetraploid). Determination of the ploidy level showed that all seedlings from the interploidy cross were hexaploid, indicating that the F1 seedlings were true hybrids.

After two subsequent selection cycles, one blue-flowering seedling was finally selected. Morphological characteristics of this selection were compared to existing commercial cultivars. Growth vigour of the hexaploid selection was significantly better than the existing commercial tetraploid cultivars 'Oiseau Bleu' and 'Marina'. For example, the selection had put on 59 cm of shoot growth in 1 year, while 'Oiseau Bleu' and 'Marina' grew 25.8 cm and 50.7 cm, respectively. The leaf morphology of the hexaploid clones was similar to the tetraploid cultivars. Flower shape and flower colour were similar to 'Oiseau Bleu'. No fruit formation was observed on the hexaploid selection. As a consequence, flowering was not inhibited during the season and flowering period was significantly extended compared to the commercial cultivars. The new selection is being propagated, and market introduction is planned for 2008.

INTERSPECIFIC HYBRIDISATION

Interspecific hybridisation offers the potential to introduce new genetic variation such as different growth habits, new colours, and improved cold hardiness and disease resistance. But interspecific hybridisation becomes more difficult the less closely related the parents are. Frequently multiple barriers are observed. Growth and development of "alien" pollen tubes can be impeded in the female style (prezygotic incongruity). After fertilization, embryo development can be arrested by malformation of nurse tissue, mostly endosperm (postzygotic incongruity). Heavy chlo-

rosis occurring after interspecific hybridisation may cause inviability of the hybrid seedlings. Finally lack of growth vigour and hybrid sterility may hamper further use of the hybrids.

In vitro protocols and polyploidization strategies can play an important part in overcoming these barriers in interspecific crosses (Hogenboom, 1973; Eeckhaut et al., 2006). Some examples in *Hibiscus* and *Buddleja* breeding programs are described here, with the aims of more vigorous plants (in *Hibiscus*) and altered flower colour (*Buddleja*).

Hibiscus. Crosses between winter-hardy *Hibiscus* species were only successful when *H. syriacus* was used as the seed parent (Table 1). Both in vitro and in vivo sowing resulted in plantlets for *H. syriacus* × *H. paramutabilis* crosses. However, for the cross *H. syriacus* × *H. sinosyriacus* in vitro embryo rescue yielded more seedlings. Unfortunately, a lot of these in vitro seedlings were lost during acclimatisation due to total and variegated albinism and growth aberrations. The F1 progeny from *H. syriacus* × *H. paramutabilis* grew very vigorously, and leaf morphology was always intermediate compared to the parent plants. The hybrid seedlings had bigger flowers compared to both parent species, and flower colour was intermediate. Although the F1 plants from *H. syriacus* × *H. paramutabilis* crosses had low pollen fertility, some F2 hybrids were generated.

Table 1. Overview of interspecific *Hibiscus* crosses and obtained hybrids.

Parentage				
♀	♂	Fruits	F_1-hybrids	F_2
H. syriacus	*H. paramutabilis*	x	x	x
H. paramutabilis	*H. syriacus*	0	-	-
H. syriacus	*H. sinosyriacus*	x	x	-
H. sinosyriacus	*H. syriacus*	0	-	-

Buddleja. In *Buddleja*, the efficiency of interspecific crosses depends on the species combination (Table 2). Failure of some cross combinations, such as *B. davidii* × *B. globosa,* could be explained by difference in ploidy level. Polyploidization of one parent species might help to overcome this barrier. Crossing compatibility with *B. lindleyana* was unilateral and only successful when it was used as pollen donor. The progenies from *B. davidii* × *B. lindleyana* were all triploid, indicating their hybrid nature. The plantlets had intermediate morphological characteristics and had reduced fertility. In *Buddleja* this sterility can be an advantage for commercial cultivars since it prohibits the uncontrolled spread of seedlings in cultivation. In spite of the low fertility of *B.* × *weyeriana* 'Sungold', which is a selection from a *B. globosa* × *B. davidii*, F1 and F2 hybrids could be obtained from crosses with *B. davidii* in both directions using embryo rescue.

DISEASE RESISTANCE BREEDING

In roses, disease resistance is generally low, because in the past aesthetic properties and productivity were the main breeding goals. The major fungal pathogen of roses grown in greenhouses and also an important disease on field-grown roses is powdery mildew (*Podosphaera pannosa*). To be able to select genotypes with enhanced disease resistance, four steps are necessary: understand the pathogen, understand pathogen-host interactions, screen parent plants, and establish resistance-screening protocols to test progenies.

Pathotypes. Knowledge about the population structure of a pathogen can aid resistance-breeding strategies. Results on the occurrence of different pathotypes are important for a better understanding of resistance in plant genotypes, as well as to interpret disease resistance screening. Variation in pathotypes might also explain observations of different resistance levels of the same cultivar at different locations or in subsequent years. A number of different pathotypes have been isolated from roses (Linde and Debener, 2003; Leus et al., 2006). The most virulent of these are the most interesting for plant resistance screening in a breeding program.

Pathogen–Host Interactions. Two rose species (*R. wichurana* and *R. laevigata*) and two cultivars ('Excelsa' and 'Gomery') were selected to examine microscopically the interaction between fungal development and plant resistance mechanisms. On the different rose genotypes tested, different resistance mechanisms towards powdery mildew development were found. These mechanisms influence both mycelium formation and sporulation. Resistance reactions depended on the proportion between normal and abnormal haustoria, papillae formation, physiological

Table 2. Overview of interspecific *Buddleja* crosses and obtained hybrids.

Parentage						
♀	♂	Fruits	Isolated ovules	Germinating ovules	F_1-hybrids	F_2
B. davidii	*B. globosa*	0				
B. davidii	*B.* ×*weyeriana*	x	x	x	x	x
B. davidii	*B. lindleyana*	x	x	x	x	-
B. lindleyana	*B. davidii*	0				
B. lindleyana	*B.* ×*weyeriana*	0				
B. lindleyana	*B. alternifolia*	0				
B. alternifolia	*B. lindleyana*	x	x	-		
B. ×*weyeriana*	*B. davidii*	x	x	x	x	x

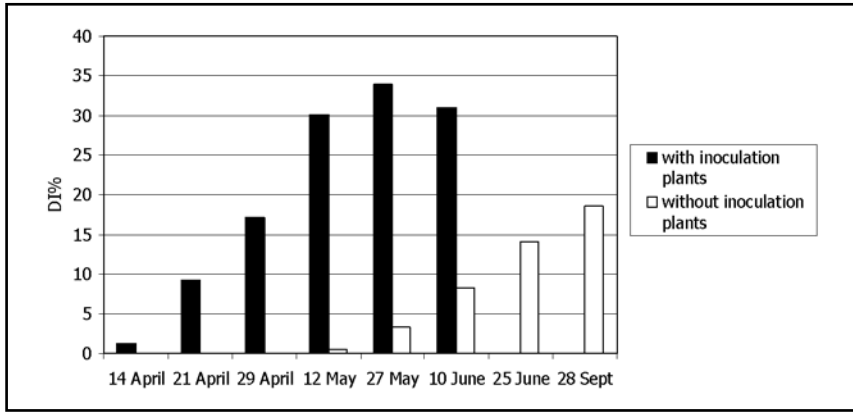

Figure 1. Disease index (DI%) for seedlings scored on different dates in 2004 in two different greenhouses, with and without inoculation plants. In the greenhouse with inoculation plants the disease is present earlier in the season and reaches also higher levels compared to the control without inoculation plants.

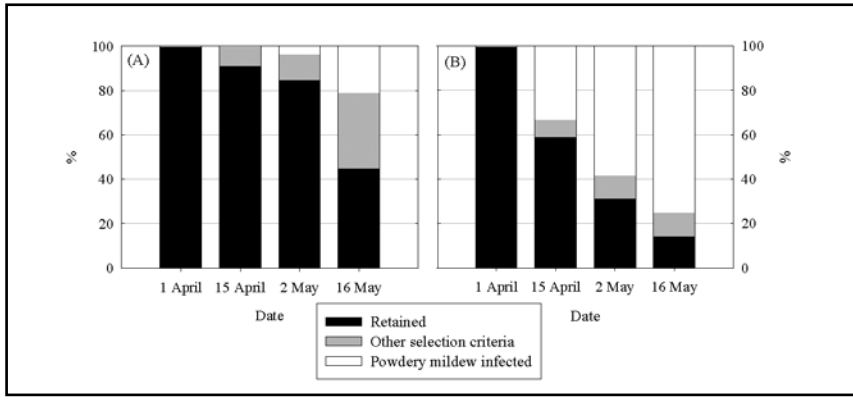

Figure 2. Selection pressure in a commercial rose breeding programme in 2002. Rose seedling populations were grown in either a greenhouse without (A) or with (B) powdery mildew inoculation. Negative selection was done on different dates

responses, and formation of antifungal phenolic compounds (phytoalexins). Two different forms of physiological responses could be observed, with and without cell collapse. The most important resistance mechanism was, however, the inhibition of normal haustoria formation.

Screening of Parent Plants. To test disease resistance in individual rose genotypes an inoculation tower can be used (Leus et al., 2003). The use of this tower allows infections with characterized powdery mildew isolates under standardized test conditions on detached rose leaves. Factors that need to be controlled are the conidia density dispersed and the age of the leaves used in the test. This method is more suitable for use with characterized monoconidial isolates to test resistance in candidate parent plants or on small numbers of promising cultivars. The method is

not suited for large populations. Since, disease-resistance sources are very rare in the today's gene pool of cultivated roses, this screening method is also valuable for the detection of resistant genotypes in wild rose species (Leus, 2005).

Resistance Screening of Progenies. In a practical breeding programme it is important to have an easy, cheap, and fast screening method to test disease resistance on large seedling populations. The earlier in the selection process resistance is screened, the more resistant genotypes can be selected for further evaluation in a cost-effective way. In the rose breeding program at ILVO inoculation plants were used to evaluate powdery mildew resistance on seedling populations in the greenhouse. In this method artificial inoculation was performed by placing very susceptible genotypes (*R.* 'Pfänder's Canina') at regular spacings among rose seedling populations. These susceptible plants were then artificially inoculated by dusting a conidial mixture of powdery mildew very early in the growing season. It was shown that inoculation plants introduced the pathogen homogenously with a higher infection pressure and earlier in the season when compared to natural infection (Fig. 1) (Leus et al., 2003). By this method an early selection towards powdery mildew resistance is possible as is also shown in Figure 2. When comparing this artificial infection screening with field resistance positive correlations were found.

CONCLUSIONS

The results presented here demonstrate that in woody ornamentals new cultivars with improved characteristics can be developed by using a range of techniques. In most cases an integrated approach is necessary to obtain results. Close collaboration between research and industry helps ensure that new introductions will be commercially successful.

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