MOLECULAR TOOLS FOR MODERN ORNAMENTAL PLANT BREEDING AND SELECTION

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Abstract

Though the development of sophisticated breeding strategies in ornamentals is lagging behind those for most of the agricultural crops, over the last years molecular methods have been quickly adopted. Apart from the use of molecular tools for the identification and verification of varieties two main areas are relevant for ornamental plant breeding. Marker assisted breeding utilises the information of markers linked to genes of interest to develop more efficient selection strategies. This is of particular importance where important traits are difficult to analyse or where simultaneous combinations of several genes are needed (e.g. resistance genes). In addition, the introgression of interesting target genes from wild species genomes may be more efficient with marker assisted selection against the genetic background of the wild donor species. The second area comprises techniques for genetic engineering of ornamental plants. The available gene pool for novel target genes is virtually unlimited in this area and reports on successful transformations are already available for *Dianthus, Rosa, Petunia, Dendrathema, Pelargonium* and many other ornamentals. For both areas the target traits are mainly centred around disease resistance, stress tolerances, delayed senescence, post harvest performance, novel colours and changed plant architecture. Of main importance for the future availability of genes both for marker assisted selection and for genetic engineering are the results from the ongoing genome projects in model organisms. These provide valuable information on the genetic architecture of flowering plants. The efforts undertaken in these projects also boosted technological developments (like e.g. microarrays, bioinformatic tools, transformation technologies) that will strongly influence ornamental plant breeding in the near future.

1. Introduction

Despite the tremendous economic importance of the floricultural industry worldwide with production values of several billion US dollars per year (Jain and De Klerk, 1998) strategies for breeding new cultivars lag behind those developed for many agricultural crops. Apart from the high diversity of ornamental species under cultivation which limit the input that can be afforded for the individual crop, reasons can be found in complex genetic systems of the major crops as e.g. roses, carnations, chrysanthemums and in the lack of genetic variability in the gene pools available. For a long time simple breeding schemes were sufficient to generate a large number of varieties with novel traits that could be marketed successfully. However, with increasing competition between breeders and producers, rising prices for energy and increasing limitations for the application of pesticides, more sophisticated strategies for breeding new ornamental varieties are necessary to meet the demands imposed by the market.

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2. Molecular techniques currently applied to ornamental plant breeding

The tremendous advances made in modern biology over the last five decades have led to the development of methods applicable not only to basic research but also to the field of plant breeding. Ornamental plant breeding greatly profited from advances made in the areas of breeding hybrid seed, the use of different mutagens for mutation breeding and various applications of plant tissue culture (De Jeu, 2000; Jain and De Klerk, 1998)

More recent developments were boosted by the fast progress made in the area of molecular genetics and led to the development of two major areas. The use of molecular markers and approaches to genetically modify plants via genetic engineering. Therefore, current applications in these two areas will be discussed in more detail below.

2.1. Molecular markers

As a tool to analyse genetic differences between genotypes at the level of the DNA, molecular markers were first applied in the field of human genetics (Botstein *et al*., 1980) but were quickly adapted by plant geneticists and breeders. Major advantages provided by molecular markers are their selective neutrality, their availability in practically unlimited numbers and their high resolution that may display genotypic differences down to single base pairs (Michelmore *et al*., 1991; Paterson *et al*., 1991). All types of molecular markers have been applied to ornamental crops (Arus, 2000; Debener, 2001) but due to their high reliability and the high information content AFLP- and microsatellite markers are used increasingly and are currently the markers of choice.

Markers can be used very effectively to distinguish genotypes and therefore provide novel tools for the protection of breeders rights (Lesur *et al*., 2000). Furthermore, information from marker profiles can be used to analyse genetic relationships between genotypes or to infer phylogenetic relationships between species and closely related genera (Weising *et al*., 1995). These data provide valuable information about the genetic diversity among relatives of ornamental plants and may subsequently be used to broaden the gene pool of a particular ornamental crop. This type of analyses it is the most widely applied among ornamental plants with species of more than 20 genera analysed so far (Table 1, Debener, 2001).

Many ornamental species are polyploids in which genetic analyses of horticulturally important characters are difficult to perform. Molecular markers may facilitate these analyses via whole chromosome maps or by linkage to individual target genes.

Ornamental crops for which genetic analyses have been performed with the aid of molecular markers are listed in table 1. Once linkage between a target locus and a set of molecular markers has been established, marker assisted selection (MAS) may be performed (Mohan *et al*., 1997, Visscher *et al*., 1996). The main advantages of MAS is evident for traits difficult to score in large populations (as e.g. QTL loci) due to the possibility to minimise the so called “linkage drag” e.g. the amount of unwanted genome from a donor genotype in introgression breeding programmes which is transferred along with the target genes (Gebhardt and Salamini, 1992). Minimising linkage drag is of particular importance if wild donor germplasm is used to introgress genes of interest into the genetic background of highly advanced crop genotypes. Furthermore, breeding for disease resistance to several pathogens or pathogenic races simultaneously can be supported by molecular markers (Kelly and Miklas, 1998).

2.2. Genetic engineering

After the first successful transformation of plants in the early 80ies, many attempts were made to modify the genetic architecture of plants by the introduction of foreign genes which are normally not present in the natural gene pool of the respective species. Meanwhile, a variety of techniques has been developed to deliver cloned DNA to the

nucleus of the target plant cell. The most commonly used are the so called agro transformation and particle bombardment (Birch, 1997). However, all published reports on the transformation of ornamental plants are based on methods to regenerate whole plants via tissue culture.

A number of economically important ornamentals as e.g. *Petunia*, *Dianthus*, *Dendrathema* and *Rosa* have been successfully transformed with different constructs (Table 2). Among the first genes transferred to ornamental plants other than marker genes were genes for the modification of flower colour, genes for the modification of the ethylene biosynthesis, genes for the biosynthesis of phytohormones and defence genes against fungal pathogens (Table 2). As examples petunias were modified in their flower pigment composition and several novel colours were created (Mol *et al*., 1999) whereas transgenic roses expressing a rice chitinase gene showed a reduced susceptibility to a fungal pathogen (Marchant *et al*., 1998). Furthermore several applications for the release of transgenic ornamentals were registered at national and international authorities (a link to different national lists for the release of transgenics can be accessed under: www.nbiap.vt.edu/cfdocs/globalfieldtests.cfm).

Although several positive examples for genetically modified ornamentals are available original expectations concerning the speed with which novel varieties can be produced for the market are not met. One reason for this is the occurrence of technical problems with many ornamental crops being “recalcitrant” to regeneration via plant tissue culture (Birch, 1997). Additionally, in some cases high rates of somaclonal variation due to the tissue culture conditions and small or unexpected effects of transgenes in foreign genetic backgrounds (for example, gene silencing via cosupression) occurred (Gallie, 1998). Another reason is the still growing public concern about the risks that may be caused by transgenic organisms released into the environment (Hails, 2000) which is a serious obstacle for marketing transgenic ornamentals on the major markets in Europe and the US.

Part of these problems may be overcome by new technical developments that allow in planta transformation for a growing number of plant species therefore avoiding the disadvantages of plant tissue culture. Other problems could be solved by the development of new vectors and selectable markers which avoid the use of antibiotic resistance genes and new promoters which will allow a more precise and coordinated expression of target genes (Birch, 1997; Hansen and Wright, 1999; Rohini and Raho, 2000).

3. New technologies for the next decades

Over the next decade ornamental plant breeding will be strongly influenced by two areas of modern biological research: One is the human genome project which has promoted and still is promoting the development of new technical tools for genome analysis (Schafer and Hawkins, 1998) which, after some lag period, will also be adapted by plant scientists and breeders. Among recent developments in this area are the so called microarrays, which allow the simultaneous screening of many thousand sequences for their expression profiles or for screening genetic differences among target genes within a very short time (Hoheisel, 1997). Another promising technology is based on the so called SNPs (single nucleotide polymorphisms) which in combination with high throughput separation systems as e.g. mass spectrometry, will open up new dimensions for the screening of DNA polymorphisms in large populations (Griffin and Smith, 2000). Once this technology becomes available to plant breeders at reasonable costs, it will speed up genotyping, linkage analyses and pathogen diagnostics tremendously.

The second area is the increasing knowledge about the structure and function of plant genomes gained within the framework of several plant genome projects (Sommerville and Sommerville, 1999; The Arabidopsis genome initiative, 2000). With the completion of the Arabidopsis genome sequence and approaches to analyse the degree of genome synteny in many other crops, gene identification will be facilitated in crop

species with less developed resources. Especially genes with general functions in plants e.g. hormone, stress or defence metabolism can already be isolated from almost any species via sequence homologies. Furthermore, for related species gene orders may also be conserved to a certain extend (the so called "synteny" of genomes) so that genes might be isolated via positional cloning from different species even in the absence of sufficient sequence conservation of the original target gene (Bennetzen, 2000; Ku *et al*., 2000).

As the genome structure alone does not provide sufficient data on gene function many projects on functional genomics in model plants intend to unravel gene functions on a large scale (Sommerville and Sommerville, 1999). For many characters the genetic architecture of plants is similar over a large range of taxa. For example, genetic networks responsible for the development of angiosperm flowers and certain components of strain specific disease resistance are widely conserved among higher plants (Ellis *et al*., 2000; Theißen, 2001). This information has already been used to isolate homologous genes from a large number of taxa and could be an interesting general strategy to modify important traits in ornamental crops. Limitations to these strategies are only met in those cases where components of the secondary metabolism (e.g. pigment composition) are unique to certain taxa and have to be analysed in detail before the metabolic pathways can be redirected via genetic engineering.

4. Conclusions

According to the growing number of publications on the application of molecular methods in ornamental plant genetics and breeding a change in strategies has already taken place that opens up new perspectives for the creation, selection and use of genetic variability. It can be expected that ornamental plant breeding will strongly profit from the enormous progress made in projects on plant genomics and also from non plant organisms like (e.g.) the human genome project. Therefore the speed with which strategies in ornamental plant breeding will change over the next decades is steadily increasing.

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Table 1. examples of ornamental genera for which marker analyses were performed. V = variety identification, P = genetic or phylogenetic distances, G = genetic analyses or chromosome maps

Genus	**V**	**P**	**G**
Alstroemeria	X	X	X
Calladium	X		
Cephalotaxus	X		
Cymbidium	X		
Dahlia	X		
Dendrathema	X	X	
Dianthus	X		X
Euphorbia (Poinsettia)	X	X	
Geranium		X	
Gerbera	X		
Heliconia	X		
Juniperus	X	X	
Lilium	X		X
Osteospermum	X		
Ozothamnus	X		
Pelargonium	X		
Petunia	X	X	X
Rhododendron	X	X	X
Rosa	X	X	X
Scaevola	X		
Syringa	X		
Viola		X	

Table 2. Ornamental plants that have been altered successfully in horticulturally important traits via genetic engineering.

Trait	Genus
plant architecture	*Pelargonium*, *Begonia*, *Rhododendron*, *Rosa*
prolonged vase life	*Dendranthema, Dianthus, Pelargonium*
flower morphology/colour	*Begonia*, *Dendranthema*, *Dianthus*, *Gerbera*, *Petunia*, *Rosa*, *Antirrhinum*
Resistance to viral or fungal pathogens	*Dendrathema, Petunia, Cyclamen, Rosa*

Table 3. Ornamental plants for which applications for field trials have been filed in different countries.

Genus	Modified traits	Country
Petunia	flower colour	Germany
Saintpaulia	flower clour	Netherlands
Dianthus	vase life	Japan
Dianthus	flower colour	Japan
Dianthus	resistance to fungal pathogens	Japan
Dendranthema	flower colour	Japan
Petunia	virus resistance	Japan
Torenia	flower colour	Japan
Dendranthema	v irus resistance	Japan
Calendula	plant architecture	Italy
Pelargonium	plant architecture	Italy
Rosa	flower colour	Australia
Rosa	resistance to fungal pathogens	Australia